CHARACTERIZATION OF BACTERIOPHAGE INSENSITIVE *ESCHERICHIA COLI* MUTANTS AND THEIR SUBSEQUENT FITNESS CHANGES USING GENOMIC AND PHENOTYPIC METHODS

BY

Zeyan Zhong

Department of Food Science and Agricultural Chemistry

McGill University, Montréal, Canada

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Dedicated to my parents

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ABBREVIATION

ABC	Adenosine triphosphate (ATP)-binding cassette
ADP	Adenosine diphosphate
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
AV	Activity value
BIM	Bacteriophage insensitive mutant
CA	California
CAD	Canadian dollar
CC	Creative Commons
CDC	Center of Disease Control and Prevention
CDT	Cytolethal distending toxin
CFR	Code of Federal Regulations
CFU	Colony-forming unit
CNDSS	Canadian Notifiable Disease Surveillance System
CRISPR	Clustered regularly interspaced short palindromic repeats
DBP	Dipeptide-binding protein
DL	Detection limit
DNA	Deoxyribonucleic acid
DOC	Deoxycholate
EAEC	Enteroaggregative *Escherichia coli*
ECDC	European Center of Disease Control and Prevention
ECOR	*Escherichia coli* reference collection
EDTA	Ethylenediaminetetraacetic acid
SA	European Food Safety Authority
EHAEC	Enterohemorrhagic-aggregative *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.
ETEC	Enterotoxigenic *Escherichia coli*
FDA	Food and Drug Administration
FL	Florida
FSIS	Food Safety and Inspection Service
GA	Georgia

GC-MS	Gas Chromatography and Mass Spectrometry
GLASS	Global Antimicrobial Resistance Surveillance System
GRAS	Generally considered as safe
HC	Hemorrhagic Colitis
HMW	High molecular weight
HUS	Hemolytic Uremic Syndrome
IAFP	International Association of Food Protection
IBIS	Institute de Biologie Intergrative et des System
ID	Identity
KDO	3-Deoxy-D-manno-oct-2-ulosonic acid
KEGG	Kyoto Encyclopedia of Genes and Genomes
KY	Kentucky
LB	Luria-Bertani broth
LEE	Locus of effacement enterocyte
LMW	Low molecular weight
LPS	Lipopolysaccharide
MA	Massachusetts
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MIR	Membrane-interacting region
MOI	Multiplicity of infection
MWCO	Molecular weight cut-off
NESP	National Enteric Surveillance Program
NM	Non-motile
NSERC	National Science and Engineering Research Council
OD	Optical density
OM	Outer membrane
OMP	Outer membrane protein
OMV	Outer membrane vesicle
ORF	Open reading frame
PAGE	Polyacrylamide Gel Electrophoresis
PAS	Phage-antibiotic synergy
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
PFGE	Pulse-field Gel Electrophoresis
PFU	Plaque-forming unit
PHAC	Public Health Agency of Canada
PM	Phenotype Microarray
PTS	Phosphotransferase
QC	Québec

RAST	Rapid Annotations using Subsystems Technology
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SNP	Single Nucleotide Polymorphism
STEC	Shiga toxin producing *Escherichia coli*
TMS	Trimethylsilyl
UCD	University College Dublin
UDP-Glc	Uridine diphosphate glucose
UK	United Kingdom
USA	United States of America
USD	US dollar
USDA	United States Department of Agriculture
UT	Utah
UTI	Urine Tract Infection
VA	Virginia
VT	Vermont
VTEC	Verotoxigenic *Escherichia coli*
WHO	World Health Organization
WT	Wildtype

ABSTRACT

Foodborne disease continues to be a global health and economic burden that affects everyone on this planet. Each year, one in ten people becomes ill after ingesting contaminated foods. This high prevalence of foodborne cases also causes the loss of hundreds of billions of dollars due to hospitalization, physician care and pharmaceutical costs. Among common foodborne disease-causing agents, Shiga-toxin producing *Escherichia coli* (STEC) strains, represented by serotype O157:H7, are one of the leading causes of foodborne illness-related hospitalization. These strains are characterized by their ability to produce Shiga-toxin and cause symptoms including bloody diarrhea and kidney failure. Traditionally, exposure to this diarrheal agent was often associated with consuming contaminated beef products. Recently, STEC incidence has been increasingly linked to contaminated fresh produce such as romaine lettuce. The reoccurring cases of STEC highlight the fact that current safety biocontrols are insufficient to suppress this pathogen in foods, and novel antimicrobial approaches are in great demand.

Bacteriophages, or phages, are a promising antimicrobial alternative to control food borne pathogens. These microorganisms are viruses that naturally infect bacteria. Bacteriophage research in recent decades have successfully demonstrated the phage antimicrobial effects in reducing bacterial pathogens in foods. Since the first phage-based biocontrol agent ListShield was approved by the Food and Drug Administration (FDA) for usage in ready-to-eat meat products as a "Generally Considered as Safe (GRAS)" processing aid in 2015, more and more phage-based antimicrobials have been developed for inhibiting microbial growth in agricultural settings.

However, one problem of using phage-based biocontrol agents as a food safety mitigation strategy is the emergence of bacteriophage insensitive mutants (BIMs). Upon phage predation,

bacteria are able to develop phage resistance through a variety of mechanisms. One of, if not the most, common way is by altering membrane components which the infecting phages use as receptors. Given that these components have essential physiological functions for bacterial growth, phage-induced modifications in these structures may consequently lead to fitness changes that affect the bacterial well-being in dynamic environments like foods.

In this work, we characterized BIMs of *E. coli* B and *E. coli* O157:H7 challenged by phage T4 and the T4-like phage AR1, respectively, and delineated their physiological changes using genomic and phenotypic approaches. The results in this study are delivered in three chapters (III, IV and V) following a general introduction (Chapter I) and a comprehensive review (Chapter II).

In the sequences of six *E. coli* B BIMs (ZZa0, ZZa1, ZZa2, ZZa3, ZZa4 and ZZa5), a six-amino acid deletion in the glucosyltransferase WaaG, which catalyzes the addition of glucose residues to the outer core of lipopolysaccharide (LPS), was consistently identified. Bacteriophage T4 and T4-like phages employ glucose as a receptor and the putative loss of glucose in LPS was confirmed by the lack of T4 adsorption and LPS glycosyl composition analysis. As LPS molecules play an active role in maintaining outer membrane integrity and permeability, the abbreviated LPS consequently led to outer membrane destabilization and sensitized the BIMs to various compounds, especially to sodium dodecyl sulfate (SDS). The hypersensitivity to SDS was further exploited to develop a T4-SDS synergic treatment which successfully prevented the phage resistance and reduced the bacterial concentration in broth by 5-log. Together, the findings in this chapter suggest that the altered LPSs of BIMs provide a mechanistic basis for increased membrane permeability. Taking advantage of this trade-off of phage resistance, we designed a novel T4-SDS synergic treatment which prevented the emergence of BIMs as well as achieved a 5-log reduction of *E. coli* B in broth culture.

Genomic characterization of *E. coli* O157:H7 BIMs showed that three (ZZb1, ZZb3, ZZb4) out of four isolated mutants developed resistance to phage AR1 by modifying their outer membrane protein C (OmpC), while the last mutant ZZb2 conferred AR1 resistance via mutation of protein HldE, which is involved in building the LPS inner core. As expected, modifications in outer membrane components resulted in varied permeabilities to different substrates. In particular, the deep rough mutant ZZb2 became more sensitive to sodium cholate and displayed hypersensitivity to SDS, consistent to the finding of *E. coli* B mutants. Furthermore, biofilm formation assays suggested that all *E. coli* B BIMs generally have higher tendency to produce biofilm than the wildtypes. When grown at 37°C, both *E. coli* O157:H7 BIMs ZZb2 and ZZb4 had higher absorbance values than the wildtype in the biofilm formation assay, suggesting these mutants tend to produce more biofilm. Phenotypic characterization with calcofluor also showed supportive results, collectively showing enhanced biofilm formation as a fitness change in exchange of phage resistance.

Lastly, the metabolic profiles of BIMs and the wildtypes were characterized by BIOLOG Phenotypic Microarrays (PM) and compared using an integrated genomic-phenomic program called DuctApe. Compared to the wildtype *E. coli* B, BIM ZZa3 was sensitized to osmotic and pH variations but showed increased activities in metabolizing carbon sources. For the two *E. coli* O157:H7 mutants ZZb2 and ZZb4, metabolism of dipeptides, N-acetyl-neuraminic acid (Neu5Ac) as a carbon source, D-glucose 6-phosphate as a phosphate source, D-serine as a nitrogen source were constantly different from that of the wildtype. Among other metabolic changes, BIM ZZb2 with severely truncated LPS exhibited elevated tolerance to food preservatives sodium lactate and sodium chloride, as well as acidic pressure, possibly associated with the enhanced biofilm production.

Collectively, the genomic and phenotypic characterizations of T4-like phage insensitive *E. coli* mutants demonstrate physiological changes induced by phage predation. Revelation of these modifications provides insights towards developing novel phage-based biocontrol agents and reveals how microbial interactions between phages and bacteria in foods impact the survivability of foodborne bacterial pathogens and pathogenicity in animal hosts.

RÉSUMÉ

Les maladies d'origine alimentaire continuent d'être un fardeau sanitaire et économique mondial qui affecte tout le monde sur la planète. Chaque année, une personne sur dix tombe malade après avoir ingéré des aliments contaminés. Cette forte prévalence des cas d'origine alimentaire se traduit également par la perte de centaines de milliards de dollars couvrant l'hospitalisation, les soins médicaux et les frais pharmaceutiques. Parmi les agents pathogènes d'origine alimentaire courants, les souches d'*Escherichia coli* productrices de shigatoxines (STEC), représentées par le sérotype O157: H7, sont l'une des principales causes d'hospitalisation liée à des maladies d'origine alimentaire. Ces souches se caractérisent par leur capacité à produire de la Shiga-toxine et à développer des symptômes allant de la diarrhée sanglante à l'insuffisance rénale. Traditionnellement, l'exposition à cet agent diarrhéique était souvent associée à la consommation de produits à base de viande bovine, à condition que le tractus intestinal des bovins soit le principal réservoir de ce pathogène. Récemment, l'incidence des STEC a été de plus en plus liée aux produits frais contaminés comme la laitue romaine. Les cas récurrents de STEC soulignent également le fait que les agents de lutte biologique actuels sont insuffisants pour éliminer ce pathogène dans les aliments, et de nouvelles approches antimicrobiennes sont très demandées.

Les bactériophages, ou phages, sont des alternatives antimicrobiennes prometteuses pour le contrôle des pathogènes d'origines alimentaires. Ces micro-organismes sont des virus qui infectent naturellement les bactéries. La recherche sur les phages ressuscitée au cours des dernières décennies a démontré avec succès les effets antimicrobiens des phages dans la réduction des bactéries pathogènes dans les aliments. Depuis que le premier agent de lutte biologique à base de phages, ListShield a été approuvé par la Food and Drug Administration (FDA) pour une utilisation dans les produits de viande prêts à consommer en tant qu'auxiliaires technologiques

«généralement considérés comme sécuritaire (GRAS)» en 2015, de plus en plus d'antimicrobien à base de phages ont été développés pour inhiber la croissance microbienne dans les pratiques agricoles.

Cependant, l'émergence de mutants insensibles aux bactériophages (MIB) constitue un problème lié à l'utilisation d'agents de lutte biologique à base de phages pour atténuer la sécurité alimentaire. Lors de la prédation des phages, les bactéries sont capables de développer une résistance aux phages grâce à une variété de mécanismes. L'un des moyens, sinon le plus courant, consiste à modifier les composants de la membrane que les phages infectants utilisent comme récepteurs. Étant donné que ces composants ont des fonctions physiologiques essentielles pour la croissance bactérienne, les modifications induites par les phages dans ces structures peuvent par conséquent conduire à des changements de forme physique qui affectent le bien-être bactérien dans des environnements dynamiques comme les aliments.

Dans ce travail, nous avons caractérisé les MIB d'*E. coli* B et *E. coli* O157: H7 contestés par les phages T4 et AR1, respectivement, et délimité leurs changements physiologiques à l'aide d'approches génomiques et phénotypiques. Les résultats de cette étude sont présentés en trois chapitres (III, IV et V) après une introduction générale (chapitre I) et un examen complet (chapitre II).

Dans les séquences de six MIB d'*E. coli* B (ZZa0, ZZa1, ZZa2, ZZa3, ZZa4 et ZZa5), une délétion de six acides aminés dans la glucosyltransférase WaaG, qui catalyse l'ajout de résidus de glucose de liaison T4 au noyau externe de lipopolysaccharide (LPS), a été systématiquement identifié. Les bactériophages T4 et de type-T4 engage glucose comme récepteur et la perte putative de glucose dans le LPS a été confirmée par le manque d'adsorption de T4 et l'analyse de la composition glycosylée du LPS. Puisque les molécules de LPS jouent un rôle actif dans le maintien

de l'intégrité et de la perméabilité de la membrane externe, les LPS abrégés ont par conséquent conduit à une déstabilisation de la membrane externe et sensibilisé les MIBs à divers composés, notamment au dodécyl sulfate de sodium (SDS). L'hypersensibilité au SDS a été davantage exploitée pour développer un traitement synergique T4-SDS qui a réussi à empêcher la résistance aux phages et à réduire la charge bactérienne en bouillon de 5 log. Ensemble, les résultats de ce chapitre suggèrent que les LPS modifiés par les MIBs fournissent une base mécaniste pour l'augmentation des perméabilités des membranes. Profitant de ce compromis entre la résistance aux phages, nous avons conçu un nouveau traitement synergique T4-SDS qui a empêché l'émergence des MIB et obtenu une réduction de 5 log d'*E. coli* B en bouillon de culture.

Les caractérisations génomiques des MIBs d'*E. coli* O157: H7 ont montré que trois (ZZb1, ZZb3, ZZb4) sur quatre mutants isolés ont développé une résistance au phage AR1 en modifiant leur protéine de membrane externe C (OmpC), tandis que le dernier mutant ZZb2 a conféré une résistance à l'AR1 via protéine mutante HldE, impliquée dans la construction du noyau interne du LPS. Comme prévu, les modifications des composants de la membrane externe ont entraîné des perméabilités variées à différents substrats. En particulier, le mutant rugueux profond ZZb2 est devenu plus sensible au cholate de sodium de sel biliaire et s'est montré hypersensible au SDS, conformément à la découverte de mutants *E. coli* B. En outre, les tests de formation de biofilm suggèrent que tous les MIBs d'*E. coli* ont généralement une tendance plus élevée à produire du biofilm que les types sauvages. Le mutant insensible aux bactériophages ZZb2 a produit beaucoup plus de biofilm que le type sauvage et un autre mutant ZZb4 à 37 ° C, ce qui suggère que ce mutant pourrait avoir une capacité de colonisation et une tolérance élevées chez l'homme.

Enfin, les profils métaboliques des MIB et des types sauvages ont été caractérisés par BIOLOG Phenotypic Microarray (PM) et comparés à l'aide d'un programme génomique-

phénomique intégré DuctApe. Comparé à *E. coli* B de type sauvage, le MIB ZZa3 a été sensibilisé aux variations osmotiques et de pH, mais a montré une activité accrue dans la métabolisation des sources de carbone. Pour les deux mutants *E. coli* O157: H7 ZZb2 et *E. coli* O157: H7 ZZb4, leurs métabolismes des dipeptides, l'acide N-acétyl-neuraminique (Neu5Ac) comme source de carbone, le D-glucose 6-phosphate comme source de phosphate, la D-sérine comme azote la source était constamment différente de celle du type sauvage. Parmi les autres changements métaboliques, le MIB ZZb2 avec un LPS sévèrement tronqué a montré de manière unique une tolérance élevée aux conservateurs alimentaires courants et à la pression acide, probablement associée à la production accrue de biofilm.

Collectivement, les caractérisations génomiques et phénotypiques des mutants d'*E. coli* insensibles aux phages de type T4 décrivent la vue d'ensemble des changements physiologiques induits par la prédation des phages. La révélation de ces modifications fournit des informations sur le développement de nouveaux agents de lutte biologique à base de phages et révèle comment les interactions microbiennes entre les phages et les bactéries dans les aliments ont un impact sur la survie des bactéries pathogènes d'origine alimentaire et la pathogénicité chez les animaux hôtes.

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CONTRIBUTION OF AUTHORS

The work reported in all chapters of this study was conducted by the author Zeyan Zhong, PhD candidate, in consultation with his supervisor Dr. Lawrence Goodridge. Zhong planned and performed the experiments, collected and analyzed results, and drafted this thesis and other published, submitted and prepared manuscripts. Dr. Goodridge, who secured the grant to initiate this project, guided and supervised the PhD candidate in performing the research, as well as in reviewing and editing manuscripts and scientific presentation materials. Post-doc fellow Dr. Sudhakar Bhandare also provided guidance and reviewed manuscripts for publication. Most of the work in this study was conducted at the Diagnostic, Enrichment, Testing and Characterization (D.E.Te.CT) laboratories, previously under Dr. Goodridge's supervision, in the Department of Food Science and Agriculture Chemistry at Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, Canada.

The whole genomes of 13 *Escherichia coli* strains used in this work were sequenced and assembled under the supervision of Dr. Roger C. Léveque at the EcoGenomics Analysis Platform at the Institute de Biologie Intergrative et des System (IBIS), Université Laval, Québec City, Québec, Canada. Research assistant Jean-Guillaume Emond-Rheault at IBIS assisted in bioinformatic analysis of the bacterial genomes.

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Biofilm formation assays for wildtype *E. coli* O157:H7, BIMs ZZb2 and ZZb4 were performed by research assistant James Bannister under Dr. Joel Weadge's supervision in Wilfrid Laurier University, Waterloo, Ontario, Canada.

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PUBLICATIONS

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Zhong, Z. et al., (2020). Bacteriophage-induced Lipopolysaccharide Truncations of Foodborne pathogen *Escherichia coli* O157:H7 Mutants Increase Bacterial Cell Membrane and Enhance Biofilm Formation. (manuscript in preparation)

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Zhong, Z. et al., (2020). Bacteriophage resistance-associated physiological changes: a review (manuscript in preparation)

CONFERENCE PRESENTATIONS

Zhong, Z., Jeukens, J., Emond-Rheault, J-G., Freschi, L., Kukavica-lbrulj, I., Levesques, R., Hu, Y., Fanning, S., Goodridge, L. Characterization of Bacteriophage Insensitive *Escherichia coli* via Comparative Correlation of Genomic and Phenotypic Microarray Data. Oral presentations in International Association for Food Protection Annual Meeting in Louisville, KY (Jul 23rd, 2019) and L'Association Québécoise pour L'innocuité Alimentaire Annual Meeting in Quebec City, QC (Sep 19th, 2019)

Zhong, Z., Jeukens, J., Emond-Rheault, J-G., Freschi, L., Kukavica-lbrulj, I., Levesques, R., Goodridge, L. Rational Design of Bacteriophage-Based Antimicrobials Eliminates the Formation of Bacteriophage Insensitive Mutants. Poster Presented at International Association for Food Protection Annual Meeting in Salt Lake City, UT (July 9th to 11th, 2018)

Zhong, Z., Mba, O., Oludare, T., Bhandare, T., Ngadi, M., Goodridge, L. A high-level and consistent inoculation method for *Escherichia coli* O157:H7 and *Listeria monocytogenes* on broccoli, potato and chicken at room and frozen temperature. Poster Presented at Le Conseil de la transformation alimentaire du Québec workshop in Saint-Hyacinthe, QC (Jun 5th, 2018)

Zhong, Z., Colavecchio, A., Jeukens, J., Emond-Rheault, J-G., Freschi, L., Kukavica-lbrulj, I., Levesques, R., Goodridge, L. T4 Bacteriophage Insensitive Mutants *Escherichia coli* Display Altered Antibiotic Resistance and Ability to Ferment Glucose. Poster Presented at International Association for Food Protection Annual Meeting in Tempa Bay, FL (Jul 7th to 12th, 2017)

CHAPTER I

A GENERAL INTRODUCTION

Foodborne diseases are a reoccurring food safety issue that endangers the well-being of the human race and pose a significant burden on the global economy. In the latest review, the World Health Organization (WHO) estimated that 600 million people in the world, mostly infants and young children, are sickened every year and 420,000 of them lost their lives owning to the consumption of contaminated foods (Havelaar et al., 2015). In Canada and the United States (U.S.), despite the tremendous investment in food safety control and prevention, foodborne diseases still cause a total of 52 million illness and 3,200 deaths annually, along with substantial financial costs (Buzby & Roberts, 2009). An economic report published by United States Department of Agriculture (USDA) points out that the 9.4 million illnesses caused by 15 major pathogens in the U.S. (20% of the total cases) are imposing a financial cost over $15.5 billion USD every year (Hoffman et al., 2015), and the total cost for all cases can cost the country up to 83 billion USD (Nyachuba, 2010). Although similar cost estimations in Canada are lacking, one study suggested that one single listeriosis outbreak in Canada would cost around 4.24 million CAD, which is a sum of healthcare, federal response outbreak costs and costs to the implicated facilities, highlighting the considerable burden of foodborne diseases and outbreaks for both the individual and the public (Thomas et al., 2015).

A variety of antimicrobial agents have been incorporated in food industries in order to minimize the risk of foodborne pathogens. For example, organic acid is approved for application as a pre- and post-chill decontamination treatment to remove foodborne pathogens in beef carcasses and subsequent beef products (Food Safety Inspection Service [FSIS] Notice 49-94). In

addition, salts of lactic acid are permitted for use in ready-to-eat meat products (21 CFR 184.1768) to inhibit Gram-positive bacteria. Cost-effective chlorine-based products are commonly employed for cleaning equipment in food processing plants (21 CFR Part 178) and sanitizing raw fruits and vegetable (21 CFR Part 173). In the recent years, with increased health awareness of consumers, natural occurring antimicrobial agents sourced from plants, animals and microorganisms are in great demand (Saeed et al., 2019). Particularly, the potential development of natural bacteriophages (phages) as a safety intervention against foodborne pathogens has been attracting tremendous attention (Sillankorva et al., 2012; Moye et al., 2018). Numerous phage-based commercial products are already available on the market, targeting common pathogenic bacteria in foods such as *Campylobacter jejuni* (*C. jejuni*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella* spp. and *Escherichia coli* (*E. coli*) O157:H7, and a large number of studies have been conducted to demonstrate the promising effect of phage-based antimicrobial agents in controlling pathogens in foods (Kazi & Annapure, 2016; Moye et al., 2018).

Apart from the recognized antimicrobial effects, there are several unique attributes that phages possess as an ideal biocontrol agent in foods. First of all, unlike indiscriminate antimicrobial compounds, phages only infect a narrow range of bacterial hosts so that they have negligible impact on the microbiota as well as on the human cells upon ingestion. These microorganisms also populate in a size that outnumber bacteria by ten times and are distributed widely in natural habitats, e.g., different waterbodies, soil and foods, providing an immense arsenal needed for combating foodborne pathogens (Whitman et al., 1998; Rohwer & Edwards, 2002). Phages can be isolated for any bacterial isolates, even for the multidrug resistant superbugs that are causing the global antimicrobial resistance crisis (El Haddad et al., 2019). Last but not least,

the use of phage as a food processing aid may not need extra labeling (depending on how they are approved), clearly answering the cumulative demand for clean label foods (Lewis & Hill, 2020).

Given that phages and bacteria are constantly in an antagonistic relation, bacterial hosts are able to develop resistance towards the infecting predators by exerting a variety of anti-phage mechanisms such as blocking phage DNA entry, restriction-modification (R-M) systems, CRISPR, superinfection exclusion systems (Sie) and abortive infection systems (Abi) in an attempt to evade phage predation (Labrie et al., 2010). These phage-resistant variants are termed bacteriophage insensitive mutants (BIMs). Among all these mechanisms, blocking phage attachment by modifying surface receptors is one of, if not the most, common and efficient strategy for bacteria to confer phage resistance (Oechslin, 2018).

In the case of Gram-negative bacteria like *E. coli*, for instance, the main outer membrane components lipopolysaccharides (LPS) and outer membrane proteins (OMPs) contain receptor sites to which phages can recognize and attach (Rakhuba et al., 2010; Bertozzi Silva et al., 2016), and there is copious evidence suggesting that BIMs of *E. coli* are generated via modifications in LPS and outer membrane protein C (ompC) (F. Yu & Mizushima, 1982; Chung et al., 2005; Washizaki et al., 2016). Of note, these structures are involved in important cellular functions such as regulating cross-membrane permeation, maintaining membrane integrity and acting as virulence factors. As a result, alterations in LPS and OMPs upon phage infection may result in a series of physiological changes which have an impact on survivability and pathogenicity of BIMs.

1.1 GENERAL HYPOTHESIS

Phage interactions with bacterial membrane components OMPs and LPS, as well as the emergence of bacterial mutants resistant to T4-like phages due to these altered receptors can lead to physiological changes that affect the fitness of the foodborne pathogen *E. coli* O157:H7 to survive in foods and the virulence of BIMs upon ingestion.

1.2 MAIN OBJECTIVE

This research project delineates how phage and bacterium interactions in complex environments, such as foods, affect bacterial survivability and pathogenicity in animal hosts.

1.2.1 SPECIFIC OBJECTIVES

1.2.1.1 Isolation and genotypic characterization of T4-like phage resistant *E. coli* mutants with modified LPS and/or OMPs

1.2.1.2 Phenotypic characterizations of T4-like phage resistant *E. coli* mutants by means of phage adsorption assay, LPS sugar composition analysis, minimum inhibitory concentration (MIC) assay and biofilm formation assay

1.2.1.3 Metabolic profile characterizations of T4-like phage resistant *E. coli* mutant using BIOLOG Phenotypic Microarray assay

1.3 REFERENCES

Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host receptors for bacteriophage adsorption. *FEMS microbiology letters, 363*(4).

Chung, W., Siu, J., & Tanner, K. (2005). Roles of lipopolysaccharide and the outer membrane protein OmpC on bacteriophage T4 infectivity in *Escherichia coli*. *J. Exp. Microbiol. Immunol, 7*, 62-67.

El Haddad, L., Harb, C. P., Gebara, M. A., Stibich, M. A., & Chemaly, R. F. (2019). A systematic and critical review of bacteriophage therapy against multidrug-resistant ESKAPE organisms in humans. *Clinical Infectious Diseases, 69*(1), 167-178.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., Praet, N., Bellinger, D. C., De Silva, N. R., & Gargouri, N. (2015). World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS medicine, 12*(12), e1001923.

Hoffman, S., Maculloch, B., & Batz, M. (2015). *Economic burden of major foodborne illnesses acquired in the United States*. Retrieved from

Kazi, M., & Annapure, U. S. (2016). Bacteriophage biocontrol of foodborne pathogens. *Journal of food science and technology, 53*(3), 1355-1362.

Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat Rev Microbiol, 8*(5), 317-327. doi:10.1038/nrmicro2315

Lewis, R., & Hill, C. (2020). Overcoming barriers to phage application in food and feed. *Current Opinion in Biotechnology, 61*, 38-44.

Moye, Z., Woolston, J., & Sulakvelidze, A. J. V. (2018). Bacteriophage applications for food production and processing. *10*(4), 205.

Nyachuba, D. G. (2010). Foodborne illness: is it on the rise? *Nutrition reviews, 68*(5), 257-269.

Oechslin, F. (2018). Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses, 10*(7), 351.

Rakhuba, D., Kolomiets, E., Dey, E. S., & Novik, G. (2010). Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol. J. Microbiol, 59*(3), 145-155.

Rohwer, F., & Edwards, R. (2002). The Phage Proteomic Tree: a genome-based taxonomy for phage. *Journal of bacteriology, 184*(16), 4529-4535.

Saeed, F., Afzaal, M., Tufail, T., & Ahmad, A. (2019). Use of Natural Antimicrobial Agents: A Safe Preservation Approach. *Act. Antimicrob. Food Packag*.

Sillankorva, S. M., Oliveira, H., & Azeredo, J. (2012). Bacteriophages and their role in food safety. *International journal of microbiology, 2012*.

Thomas, M. K., Vriezen, R., Farber, J. M., Currie, A., Schlech, W., & Fazil, A. (2015). Economic cost of a Listeria monocytogenes outbreak in Canada, 2008. *Foodborne pathogens and disease, 12*(12), 966-971.

Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the interactions between *Escherichia coli* receptors, LPS and OmpC, and bacteriophage T4 long tail fibers. *Microbiologyopen, 5*(6), 1003-1015. doi:10.1002/mbo3.384

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences, 95*(12), 6578-6583.

Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. *Journal of bacteriology, 151*(2), 718-722.

Chapter II

A comprehensive literature review

2.1. Foodborne diarrheal pathogens

Currently, the 31 disease-causing agents known to be transmitted via foods can be categorized into chemical and toxin hazards, parasitic disease hazards and enteric disease hazards (WHO, 2015b). Among these groups, enteric disease hazards composed of 11 key microbiological agents are the most frequent causes of foodborne illnesses worldwide, leading to 548 million diarrheal cases and 200,000 deaths every year (WHO, 2015b; Kirk et al., 2017). Although these adulterants are present universally, exposure to these pathogens occurs more frequently in low-income developing regions where preventive food safety measures are lacking (Kirk et al., 2017).

Foodborne enteric disease agents are dominated by bacterial pathogens, namely *Campylobacter*, *L. monocytogenes*, *Salmonella*, *Shigella*, and several types of pathogenic *E. coli*. The latter three genera belong to the family of *Enterobacteriaceae*, and are considered highly infectious contaminants in food establishments due to have a low infectious dose and being relatively readily transmitted from sick food handlers to consumers via the fecal-oral route (FDA, 2017b). In fact, these genera are the most prominent contributors to the global foodborne disease burden. In the European Union, non-typhoid *Salmonella* is the second most common cause of gastrointestinal tract infection (EFSA & ECDC, 2019). In the USA, a diverse array of foods contaminated with non-typhoid *Salmonella* are estimated to sicken 1.35 million people and cause 450 deaths annually (CDC, 2020b). Meanwhile, the genus *Shigella* (mostly *Shigella sonnei*) accounts for 500,000 shigellosis cases in the USA each year (CDC, 2020c), and shigellosis caused

by *Shigella dysenteriae* Type 1 is the most important cause of bloody diarrhea in developing countries (Todd, 2014).

Another foodborne pathogen that frequently results in hospitalization is pathogenic *E. coli*. Although most *E. coli* strains are harmless commensals living in the guts of endothermic animals and humans, several types of pathogenic *E. coli* have been commonly implicated in foodborne enteric diseases. These strains are classified based on their virulence mechanisms into at least six pathotypes: diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteroinvasive (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and Shiga-toxin producing *E. coli* (STEC) (Croxen et al, 2013). EAEC is commonly found in South America and other less-developed regions worldwide; it has been linked to persistent diarrhea in children after ingestion of contaminated foods like desserts and salsa (Koo et al., 2008; Vigil et al., 2009), which may explain the incidence of EAEC-related acute diarrhea in travelers. Another major cause of traveler's disease is ETEC; these strains cause mild to severe watery diarrhea by attaching to the mucosa of the small intestine and producing characteristic enterotoxins, either heat-labile, heat-stable, or both (Croxen et al., 2013). In developing countries, enteric infection of children by ETEC is exacerbated by the pre-existing malnutrition and immunocompromised condition, leading to this pathotype having become one of the main etiologic agents for acute infectious diarrhea and contributing to 20% of all childhood deaths worldwide (Qadri et al., 2005). Another diarrheagenic pathotype mostly prevalent in developing countries is EPEC, a group known for its ability to attach to the intestinal epithelial cells, efface the brush border microvilli (A/E lesion), and form actin pedestals at the site of bacterial attachment (Frankel et al., 1998). The typical localized and localized-like adherence patterns are absent in the less-studied DAEC group which is characterized by an unique diffuse adherence (Scaletsky et al., 1984). In contrast with other pathotypes, EIEC

demonstrates distinct invasiveness, being able to penetrate the intestinal epithelial barrier and to spread intracellularly and intercellularly, causing macrophage cell death and ultimately a massive inflammatory response (Pasqua et al., 2017). Although the incidence of and outbreaks caused by EIEC are not frequently reported, possibly due to having less severe clinical manifestation (Croxen et al., 2013), a large number of diarrheal cases have been associated with *Shigella*, which has a nucleotide identity of 80% to 90% with EIEC (D. J. Brenner et al., 1972) and applies the same invasive mechanism when infecting a host (Pasqua et al., 2017). The similarities between *Shigella* and the EIEC pathotype may lead to misclassifications during outbreak investigations, partially explaining the low incidence rate of EIEC.

In North America, the STEC pathotype is responsible for the majority of foodborne *E. coli* outbreaks. Recent investigations have shown a pattern that STEC is commonly found to be a contaminant in beef products and fresh produce (PHAC, 2017; CDC, 2020a). As the main reservoir for this pathogen is the intestinal tract of cattle, beef products can be easily compromised during the processing of raw meat in the slaughterhouses. Meanwhile, fresh produce like Romaine lettuce is grown outdoors and constantly exposed to various environmental factors such as manure, irrigation water, and nearby livestock in which STEC may be present and thus accidentally introduced to the produce. Since fresh produce is minimally processed and usually consumed raw, it suffers from a lack of effective bactericidal steps that prevent the contaminant from being transmitted to the consumer.

The STEC pathotype features a few distinctive characteristics that make these strains major threats to public health. First, it is equipped with a variety of virulence factors that contribute to severe symptoms such as bloody diarrhea (hemorrhagic colitis [HC]) and the life-threatening complication known as hemolytic-uremic syndrome (HUS) (Karmali et al., 2010). In addition,

STEC infection can occur at a very low infectious dose; concurrent studies following outbreaks associated with dry fermented sausages in Australia and the USA showed that less than 100 cells in those products were sufficient to cause diseases (Paton et al., 1996; Tilden Jr et al., 1996). All told, the prevalence of STEC in largely consumed foods, its high pathogenicity in humans and its low infectious dose account for the fact that STEC is one of the leading causes of foodborne gastroenteritis (Scallan et al., 2011).

2.2. Overview of Shiga-toxin producing *E. coli* (STEC)

2.2.1. Microbial characteristics, classifications and definitions

Shiga-toxin producing *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium which possesses the characteristic genes (*stx*$_1$ and/or *stx*$_2$) that encode Shiga toxin (Stx). More than 200 known serotypes of STEC with various somatic (O) antigens have been identified, while only a subset of strains have been linked to human illnesses (Blanco et al., 2004). Another classification scheme has also been suggested, grouping STEC strains into five seropathotypes (A to E) based on their relative incidence, outbreak frequency and association with severe complications (HC and HUS) (Karmali et al., 2003). Among these pathotypes, a subset of strains is enterohemorrhagic *E. coli* (EHEC) which causes severe human diseases. The most common disease-causing serotype EHEC O157:H7 belonging to seropathotype A has been implicated in numerous foodborne outbreaks. In addition to O157, six Non-O157 serogroups O26, O45, O103, O111, O121 and O145 (known as the "Big 6") categorized in seropathotype B have also been declared as adulterants in foods by the U.S Department of Agriculture (USDA) (USDA), because of their ability to cause severe human illnesses, especially to the young and the elderly, as well as the increasing presence in food products (Coombes et al., 2008; Bai et al., 2016).

2.2.2. Disease-causing mechanisms of STEC

2.2.2.1 Shiga toxin.

In 1897, this toxin was first discovered in *Shigella dysenteriae* (*S. dysenteriae*) by Dr. Kiyoshi Shiga (Trofa et al., 1999) and identified in a group of *E. coli* isolates 80 years later (Konowalchuk et al., 1977). As the key and distinct virulence factor of STEC, the presence of Stx, typically encoded by lambdoid prophages integrated in the bacterial chromosome (O'Brien et al., 1984), plays a crucial role in developing complications i.e. bloody diarrhea and serious sequelae HC and HUS. Shiga toxin expressed in STEC can be antigenically divided into two main groups: Stx1 (classified to subtypes a, c and d), which is identical to the Stx produced by *S. dysenteriae*, and Stx2 (classified to subtypes a to g), which is more often identified in patients with severe complications possibly owing to the synergetic interaction with the adhesin intimin eae (Boerlin et al., 1999). It should be noted that STEC strains can carry Stx1 or Stx2 alone, both Stx1 and Stx2, or even Stx2 with different subtypes. As a result, the Stx profiles of STEC isolates determine the disease-causing potency in humans. For example, strains producing Stx1a, Stx2a, Stx2c or Stx2d have been frequently associated with more severe human disease (Melton-Celsa, 2015), while subtypes Stx1c and Stx1d have been linked only to diseases with low severity (Kumar et al., 2012).

The mature Stx molecule is structurally made up of a subunit A and a pentamer subunit B in an AB_5 molecular configuration. Upon bacterial colonization, the subunit B of Stx recognizes and binds to the major receptor glycolipid globotriaosylceramide (Gb3) on the renal cell surface, followed by the internalization of Stx and retrograde trafficking from the Golgi to endoplasmic reticulum. In this intracellular process, the subunit A is cleaved by a serine protease furin anchored in the Golgi network (van Deurs & Sandvig, 1995). The processed subunit A subsequently acts as a glycosidase which removes an adenine from 28S rRNA, and thus suppressing renal cell protein

synthesis and contributing to the development of HUS. However, human intestinal epithelium do not express Gb3 or other Stx binding sties (Miyamoto et al., 2006; Zumbrun et al., 2010), further research is necessary to elucidate the impact of Stx production and release on the Gb3-negative environment.

2.2.2.2 The locus of enterocyte effacement (LEE).

Many disease related STEC serogroups possess a 35kb cluster of virulence genes on a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). This locus encodes the Type 3 Secretion System (T3SS) responsible for the translocation of a group of virulence factors in the host cells leading to the formation of an A/E lesion similar to that of EPEC. At first, these bacteria intimately attach to epithelial cells using different types of adhesins like type IV pili and long polar fimbriae (Xicohtencatl-Cortes et al., 2007; Farfan et al., 2011), as well as proteins with high affinity (Stevens et al., 2004). After localized adherence, the T3SS secretes channel-forming translocons and drives different effectors including a translocated intimin-binding receptor (Tir) into the epithelial cells lining in the small intestine. Tir subsequently anchors into host cell membranes and recognizes and binds to the intimin produced on the bacterial surface. This interaction between Tir and intimin generates signal transduction, leading to the subversion of host cellular pathways to promote massive actin polymerization beneath the attachment sites. This alteration forms a pedestal-like structure and efface microvilli on the epithelium, thereby at least partially contributing to diarrhea due to the decrease of absorptive surfaces (Croxen et al., 2013).

It is noteworthy that LEE-negative isolates are also capable of resulting in severe complications by exerting fundamentally different mechanisms from LEE-positive strains. For instance, while lacking the intimin binding modulated by T3SS, LEE-negative serotype O113:H21

encodes other adhesins such as fimbriae lfp_{O113} by the pathogenic island O113 (Doughty et al., 2002) and auto-agglutinating protein Saa (Paton et al., 2001) as essential elements to colonize the host cells. Following colonization, this strain is even able to internalize the epithelial cells through an invasion process possibly contributed by the flagellar antigen H21 (Luck et al., 2005; Luck et al., 2006). From the clinical EHEC isolates, Doughty et al. (2002) noticed a typical pattern that LEE-negative strains carries the 92-kb plasmid encoding EHEC hemolysin and produces the most cytotoxic Stx_2 variants (Fuller et al., 2011). The disease severity of LEE-negative *E. coli* was once demonstrated by a serious outbreak sickening almost four thousand people in northern Germany in 2011. Among these 800 individuals developed HUS and 53 died because of the ingestion of sprouts contaminated by *E. coli* O104:H4 (Frank et al., 2011), which was later identified to be a hybrid isolate that displays the EAEC pathotype as well as the acquired ability to produce Stx through horizontal gene transfer (termed as EHAEC) (Qin et al., 2011). All these examples have proven that LEE-negative STEC are as capable of causing severe human diseases as the LEE-positive strains.

2.2.2.3 Plasmid pO157.

A highly conserved plasmid pO157 required for full pathogenesis of *E. coli* O157:H7 was found in most clinical isolates (Levine et al., 1987; Ratnam et al., 1988). As the prototype of pO157-like plasmids, the pO157 displays a dynamic structure and consists of various genetic mobile elements associated with bacterial virulence.

Hemolysin ehx (EHEC-Hly). The plasmid-encoded hemolysin ehx in EHEC isolates is a virulence factor characterized by pore-forming property on sheep erythrocytes (Schmidt et al., 1995) and has been linked to the development of HUS (Aldick et al., 2007). This toxin was found to be secreted extracellularly in both a free and soluble form and a more biologically active form

that associates with the outer membrane vesicles (OMVs) (Aldick et al., 2009). The role of OMV-associated EHEC-Hly in the pathogenesis has been proposed. Bielaszewska et al. (2013) tested this toxin on human cell lines and discovered that EHEC-Hly is internalized via the endocytosis of OMVs, trafficked into endo-lysosomal compartments and eventually released from OMVs to target mitochondria. This subsequently leads to the disruption of mitochondrial membrane and the activation of caspase-9 and caspase-3 apoptotic pathways, fragmenting the DNA of human microvascular endothelial and intestinal epithelial cells (Bielaszewska et al., 2013). This finding of ehx-mediated cytotoxicity of microvascular endothelial cells is also mentioned in another study in which Aldick et al. (2007) also proposed that EHEC-Hly produced by *E. coli* O26 likely acts as a Stx-independent virulence factor contributing to the development of HUS.

Type II secretion system (T2SS). Adjacent to the hemolysin locus, Schmidt et al. (1997) revealed 13 open reading frames (ORFs) named *etpC* to *etpO* which show high similarities to genes that encode T2SS of other Gram-negative bacteria. This secretion pathway is known to deliver a number of protein effectors, including toxins and proteases, across the outer membrane to the extracellular space, acting as a virulence determinant. In a study conducted by Ho et al. (2008), the researchers evaluated the role of T2SS and its secreted protein YodA in the adherence and colonization of EHEC *in vivo*. The results showed that both T2SS and YodA are essential for EHEC binding to HeLa cells and in the infant rabbit infection model, the intestinal colonization of an *etpC* mutant was outcompeted by the wildtype for 5-fold and the *yodA* mutant showed even more severe (10- to 100-fold) decreased colonization in ileum, mid-colon and cecum (Ho et al., 2008). These findings indicate that pO157-encoded T2SS and the secreted substrates are important to EHEC pathogenesis by mediating both binding and colonization of EHEC in the gut environment.

Metalloprotease stcE. Another virulence factor stcE is also secreted by T2SS under the regulation of the LEE-encoded regulator (Ler) (Lathem et al., 2002). This StcE is a large metalloprotease that cleaves the C1 esterase inhibitor which is an anti-inflammatory agent regulating the classical complement, intrinsic coagulation and contact activation (Caliezi et al., 2000). As a result, the StcE-mediated cleavage of C1 esterase inhibitor possibly links to localized pro-inflammatory and coagulation responses. The stcE-mediated virulence may also promote the intimate adherence of *E. coli* O157:H7 to host cells by reducing mucous glycocalyx cell surface barrier and enhancing the interaction between the pathogen and host cells (Grys et al., 2005).

Catalase-peroxidase katP. Sanitizer hydrogen peroxide is commonly used in the sanitation procedure in food processing plants, killing harmful bacteria by oxidative stress. However, this effect might be neutralized by the bifunctional catalase-peroxidase katP produced specifically by *E. coli* O157:H7. Brunder et al. (1996) first identified this pO157-encoded enzyme and showed its catalytic activity in both cytoplasmic and periplasmic domains. Further investigation by Uhlich (2009) identified that katP, along with other factors (katG, katE, katP and ahpC), forms a complex network to protect *E. coli* O157:H7 from peroxide-mediated oxidative damage in an OxyR-dependent manner and promotes colonization using the produced oxygen.

Serine protease espP. The 104 kDa extracellular autotransporter espP is a widespread virulence factor produced by STEC serotypes O157 and O26. Genetic analysis showed that the deduced amino acid sequence of espP possesses a characteristic site of serine protease and has a high identity to that of IgA1 protease (Brunder et al., 1997). Although IgA protease activity was not detected in the same study, instead the authors observed that espP was capable of cleaving pepsin A, a digestive endopeptidase in gastric juice (Wolfe & Soll, 1988), and leading to human coagulation factor V degradation which could result in prolonged bleeding and increased mucosal

hemorrhage in the gastrointestinal tract of HC patients (Brunder et al., 1997). In addition, Orth et al. (2010) pointed out that this virulence factor of EHEC also negatively affect the human innate immune system by cleaving complement proteins and downregulating complement activation, suggesting that espP may be involved in the pathogenesis of EHEC-induced HUS.

Other pO157-encoded virulence elements such as putative adhesin toxB and lymphocyte inhibitory factor lifA/efa have been characterized in previous studies (Tatsuno et al., 2001; Janka et al., 2002). Of note that among the 100 ORFs in the sequence of pO157 (Burland et al., 1998), there are 35 proteins presumptively involved in pathogenesis and only a subset of those have been characterized (Lim et al., 2010). As a consequence, the biological importance of pO157 has not yet been fully understood. Other pO157-like plasmids such as pO26, pO103 were also identified in non-O157 STEC isolates, conferring similar virulence potential to each strain (Ogura et al., 2009).

2.2.2.4 Cytolethal distending toxins (CDTs).

Commonly produced by diarrheagenic *E. coli* and other Gram-negative bacteria, CDT generally consists of CdtB, the bifunctional site acting as both DNase and phosphatase, CdtA and CdtC, both of which are responsible for toxin binding to host cell surface and the delivery of CdtB into intracellular compartments. This heterotrimeric toxin is capable of affecting various cells types using diverse virulence mechanisms, i.e. inducing cell cycle arrest and apoptosis in epithelial cells, arresting cell cycle in lymphocytes and altering macrophage function which leads to pro-inflammatory responses (Scuron et al., 2016). Particularly, the gene cluster encoding this toxin is not common in STEC O157:H7 strains, but the toxin variant CDT-V is frequently found in closely-related sorbitol-fermenting STEC O157:NM (non-motile) strains, because of which 17.3% of EHEC patients in Germany and Austria between 1996 and 2006 developed HUS (Bielaszewska et

al., 2007). The association of CDT production with clinical symptoms like diarrhea and HUS was also supported by a number of studies (Clarke, 2001; Bielaszewska et al., 2004; Meza-Segura et al., 2017), emphasizing the significant role of CDT in causing human disease.

Through successive horizontal gene transfer, the acquisition of virulence factors from genetic mobile elements, i.e., plasmids and prophages, differentiate pathogenic *E. coli* isolates from other commensals. For instance, the pO157-like plasmids found in most clinical *E. coli* O157:H7 and non-O157 STEC isolates encodes a large number of virulence factors as described above. In addition to the diverse virulence factors acquired from genetic mobile elements in STEC, the interactions between known or putative virulence elements also designate the varied severity levels of resulting disease. For example, a significant statistical association in both univariate and multivariate analysis was found between the *stx*$_2$ gene and severe disease, supporting the putative synergetic effect between the adhesin intimin eae and Stx2 (Boerlin et al., 1999). This finding might at least partially explain why LEE-negative strains are less virulent than the LEE-positive isolates such as *E. coli* O157:H7. On the other hand, an antagonistic interaction was found between virulence factors. Brockmeyer et al. (2011) reported that serine protease espP breaks down hemolysin EHEC-Hly in a serotype-independent manner, suggesting that the interplay among virulence factors might be an additional way to determine the outcome of infection.

2.2.3. Symptoms, outbreaks and surveillance

The symptoms of STEC infection vary for each individual. Depending on the serotype, EHEC infected patients often experience severe stomach cramps and vomiting, along with mild diarrhea or bloody diarrhea (HC) and about 5 to 10% of them develop a serious complication HUS (CDC, 2020a). Typically, STEC infection has an incubation period for 3 to 4 days before showing initial symptoms like diarrhea, cramping and vomiting. In the following days after the onset of

symptoms, some patients may develop HC. The HUS symptom may appear approximately 7 days onset of diarrhea starts and is characterized by decreased frequency of urination, fatigue, pale color in cheeks and inside lower eyelids (CDC, 2020a). Other studies regarding Stx-mediated complications have also evidenced its association with brain damage (acute encephalopathy) (Tzipori et al., 1988; Kita et al., 2000) and a life-threatening illness known as thrombotic thrombocytopenic purpura (Motto et al., 2005). Most people diagnosed with STEC show improvements within 5 to 7 days, while others develop further complications and suffer from chronic damage or disease.

When two or more people from different households are sickened by the same contaminated food or drink, the instance is reported as a foodborne outbreak. In the most recent study (Majowicz et al., 2014), it was estimated that various STEC annually cause 2.8 million acute illnesses and 230 deaths worldwide and the North America sub-region has one of the highest instance rates (89 per 100,000 person-years). Although there are different modes of transmission for these pathogens, such as from contact with infected animals and other human, most cases can be attributed to the ingestion of fecal matter-contaminated food and drink. In the United States, ingestion of contaminated foods accounted for 52% of *E. coli* O157:H7 outbreaks from 1982 to 2002 (Rangel et al., 2005). As a transmission vehicle, several types of foods have been frequently associated with STEC outbreaks. From a total of 957 STEC outbreaks from 27 countries over the period from 1998 to 2007, a global report published by WHO (2019) indicates that besides the outbreaks with unknown food sources, the attributable foodborne STEC cases in region of the Americas largely result from the consumption of tainted beef products (40%), fresh produce (35%) and dairy (12%). These findings are also supported by two studies conducted domestically in the USA (Heiman et al., 2015) and Canada (Bach et al., 2002). In the past decades, beef and their

products remain a main causative source of STEC infection. A global assessment of STEC prevalence in beef products over the past 30 years has found O157 and non-O157 isolates in ground beef, sausage, retail cuts and whole carcasses at high rates (Hussein, 2007), emphasizing the importance of control measures i.e. proper heat treatment (cooking) to assure food safety. Recently, a notable association of STEC outbreaks with consuming fresh produce, especially Romaine lettuce, has been repeatedly identified (CDC, 2020a), leading to multistate and multi-provincial recalls and hospitalizing hundreds of people in both USA and Canada. As mentioned above, fresh produce grown in the field can easily become contaminated by soil, irrigation water, improper composted manure or animal faeces. In addition, the prevalence of STEC in fresh produce is also an indicative outcome of improper handling, storing and transporting from farm to fork after the harvesting process. Last but not least, since fresh produce like lettuce and sprouts are largely consumed raw, the lack of effective killing step also increases the risk of exposure to STEC.

To monitor the outbreaks and further analyze the distribution pattern, public health authorities have developed active surveillance systems aiming at reducing foodborne illnesses and deaths caused by STEC. Since 1996, the Foodborne Diseases Active Surveillance Network (FoodNet) has been implementing comprehensive sentinel surveillance systems and collecting case information of reported enteric diseases in North America through local and provincial public health laboratories (Allos et al., 2004). At the same time, the molecular subtyping network PulseNet characterizes and collects the DNA fingerprints of foodborne pathogens like STEC O157:H7 using pulsed-field gel electrophoresis (PFGE) into an electronic database, facilitating the rapid comparison of dispersed outbreak-related strains to the previously identified pathogens (Swaminathan et al., 2001). Other surveillance platforms in Canada include the Canadian Notifiable Disease Surveillance System (CNDSS) (Totten et al., 2019) and the National Enteric

Surveillance Program (NESP) (Public Health Agency of Canada, 2012), which collectively identify and monitor foodborne outbreaks as well as provide data for epidemiological analysis.

2.2.4. Antimicrobial interventions against STEC in foods

As the biggest reservoir for STEC and one of the major food stables, beef produced by Canadian registered establishments has a series of processing steps that involve a combination of physical and chemical mitigations to ensure microbiological safety (Beef Cattle Research Council, 2014). For instance, cattle are mechanically scrubbed and treated with chlorine-based sanitizers before skinning to remove visible contamination and reduce bacterial loads. After skinning, the carcasses are washed with hot water and sprayed with acid-based antimicrobial solutions. Although these extensive antimicrobial steps implemented in Canadian slaughterhouses have been shown to reduce the *E. coli* O157:H7/NM growth in ground beef (Pollari et al., 2017), these treatments cannot be applied on foods that are heat sensitive or minimally processed like fresh produce, another major vehicle causing STEC infection. These attributes may also account for the reoccurring STEC outbreaks associated with ready-to-eat salads. Because of low cost, chlorine-based sanitizers are commonly applied in washing processes as an bactericidal agent to decontaminate fresh produce (Gil et al., 2009). However, the antimicrobial effect of this type of sanitizers is significantly limited by the accumulation of organic particles which neutralize the active free chlorine in the wash water (Toivonen & Lu, 2013). Even worse, the chlorinated disinfection by-products remained on fresh products may pose a secondary health concern to the customers (International Agency for Research on Cancer, 1999). These limitations of current antimicrobial treatments and reoccurring STEC outbreaks collectively increase demand for food safety alternatives that are more applicable, affordable and effective in different food matrices.

One such method with increasing popularity is the use of phage-based biocontrol agents for eliminating foodborne pathogens present in the food chain (Moye et al., 2018).

2.3. Bacteriophages

2.3.1. Background

Phages are viruses that can infect and kill bacteria. In 1915, British scientist Frederick Twort first discovered "a small agent" that could kill bacteria. Two years later, French-Canadian scientist Felix d'Herelle observed that *Shigella* cultures incubated with fecal filtrates from a dysentery patient did not cause bacterial infection (dysentery) in guinea pigs and rabbits (Duckworth, 1976; Salmond & Fineran, 2015). D'herelle later termed these bacterial parasites as "bacteriophages" and his findings signified the beginning of phage therapy. However, limited knowledge of phage physiology, unawareness of lysogeny and, most importantly, the discovery of efficacious board-spectrum antibiotics in 1930s greatly impeded the development of phage therapy in most of the world (Carlton, 1999; Sulakvelidze et al., 2001), except for some east European countries where phage research was sustained and expanded to clinical implementation, veterinary and food safety applications (Kutateladze, 2015). In addition to the accumulating clinical evidence for the success of phage therapy in Russia, Georgia and Poland (Sulakvelidze et al., 2001; Weber-Dąbrowska et al., 2001), the vigorous animal studies conducted in the Western countries since 1980s (Smith & Huggins, 1982; O'Brien et al., 1984; Strockbine et al., 1986) revived phages as a promising antimicrobial agent and an ideal substitution for antibiotics in the crisis of multidrug resistance (Matsuzaki et al., 2005).

2.3.2. Phage structures

The structure of all phage virion particles universally consists of a protein-based capsid surrounding the viral genome ranged in size from 3.4 to 500 kb (Hatfull & Hendrix, 2011). The

essential roles of this major component in phage life cycle has been summarized by Gill and Abedon (2003): (i) preventing phage genome from physical damage as phages spend most of their life roaming extracellularly to search for susceptible hosts; (ii) functioning as a receptor binding protein with high affinity to bacteria surface during phage adsorption and (iii) the delivery of phage genome into the host cytoplasm.

Over 96% of isolated phages belong to the order of *Caudovirales* and distinctively possess a tail structure (Ackermann, 2003) connected with its capsid by a portal system formed by connector proteins (Lander et al., 2009; Lhuillier et al., 2009). Phage tails are sophisticated nanomachines interacting with particular proteinaceous or carbohydrate receptors on the bacterial host surface upon phage adsorption, imparting host specificity to phages and facilitating phage genome delivery. Because phages are ubiquitously present in every ecological milieu on this planet, adaptation to accessible hosts and to the surrounding environments evolutionally drive the variation of phage tail structures. Based on the tail morphology under transmission electron microscopy, *Caudovirales* phages are conventionally divided into at least three families: *Myoviridae*, distinguished by the contractile tails (i.e. phage AR1 and T4) (Figure 2.1); *Siphoviridae*, bearing their long non-contractile tails (i.e. phage HK97 and T5) and *Podoviridae* with short non-contractile tails (i.e. phage IME-EC2 and T7). The construction of long phage tails is rather similar and mainly based on three proteins: the tail tube protein for tube structure, the tape measure protein regulating the tube length and the tail terminator protein signaling the end of tail tube polymerization. In addition, the tail sheath protein that provides the tail contractile ability is required for *Myoviridae* phage tail assembly.

At the distal tail end, an organelle varying in size and morphology is responsible for host recognition. This could be a simple tail tip, i.e. Dit-like protein of *Siphoviridae* infecting Gram-

positive bacteria (Dunne et al., 2018), or a complex baseplate that undergoes a large conformational change, along with the tail sheath contraction, upon *Myoviridae* phage infection (Leiman et al., 2010). A needle-like lysozyme-associated complex extended from the baseplate or tail tip is also conserved among many phages (Kondou et al., 2005; Leiman et al., 2010; Sciara et al., 2010) and required for cell wall penetration during infection. It is important to note that host specific recognition is directly conferred by the unique receptor binding proteins on protruding baseplate proteins or tail fibers. For example, the distal tip of long tail fibers of the *Myoviridae* phage T4 recognizes and attaches to the receptors LPS and OmpC on the surface of *E. coli* K-12, and thus initiating phage adsorption (Washizaki et al., 2016).

2.3.3. Lifestyles

Once the viral nucleic acid is introduced to the host, phages will most likely enter either a lysogenic or lytic life cycle (Figure 2.2). After phage nucleic acid is introduced into the host cytoplasm, temperate phages exclusively undertake the lysogenic cycle, integrating their genome into the bacterial host genome as a prophage. It is believed that prophages can occupy as much as 20% of bacterial genomes (Casjens, 2003). These acquired foreign elements replicate normally with the host chromosome and are passed to the daughter cells during cell division. Of note, prophages also contribute to bacterial fitness and encode a variety of virulence factors such as cholera toxin secreted by *Vibrio cholera*, Stx produced by STEC and antibiotic resistance genes (O'Brien et al., 1984; Faruque & Mekalanos, 2012; Bondy-Denomy & Davidson, 2014). However, upon exposure to inducing signals such as DNA damaging agents or unfavorable environments (Otsuji et al., 1959; Ubeda et al., 2005; Goerke et al., 2006), temperate phages may excise themselves from the host genome and irreversibly switch from lysogenic cycle into lytic cycles, and eventually lyse the host cells for the release of phage progenies.

Upon the introduction of the viral chromosome, strictly virulent (obligatory lytic) phages, such as T4-like phages, immediately follow the lytic pathway where they directly hijack and redirect the host machinery for phage genome replication and viral protein synthesis, followed by the assembly of phage progenies. At the end of the lytic cycle, under favorable conditions, phage-encoded proteins such as holin and endolysin rupture the bacterial cell wall, causing host cell lysis and releasing phage progeny into the environment (Mathews, 1983). This immediate lytic growth of virulent phages upon infection renders them a preferred vehicle for developing phage-based biocontrol methods over temperate phages, which may introduce virulence genes to the hosts and transform non-pathogenic bacteria into pathogens.

Other less common alternative lifestyles were also reported and summarized (Hobbs & Abedon, 2016). Besides the well-documented classic lysogenic and lytic cycles, phages may also adopt an alternative lifestyle named pseudolysogeny, which is a form of stalled host-phage interaction where the infecting phage enters neither lysogenic or lytic cycle (Ripp & Miller, 1997). This state may be a consequence of cell starvation since this phenomenon is often observed in natural habitats where bacteria are usually deprived of nutrients.

2.3.4. T4-like bacteriophage infection process

T4-like phages are a diverse group of virulent, double stranded DNA tailed phages which share morphological similarities and genetic homologies to the well studied architype phage T4 (Figure 2.1). Since the 1940s, phage T4 has been a great contributor to the development of genetics and biochemistry as one of the major model systems (Karam & Drake, 1994). In addition to the fact that the whole genome sequence of phage T4 has also been completely decoded (Miller et al., 2003), the process of phage T4 infecting the host *E. coli* has been well-studied (Mathews, 1983; Karam & Drake, 1994; Hu et al., 2015; Washizaki et al., 2016), representing the typical infection

process of virulent tailed phages. There are four major steps in the T4-like phage infection process: adsorption, penetration, assembly and release of phage progenies (Mathews, 1983). Once a susceptible target has been located, the tips of long tail fiber extend from the tail structure and reversibly adhere to the receptors such as LPS and OmpC on the host surface. This is followed by short tail fibers unfolding from baseplate and irreversibly binding to the inner core heptose of LPS (Riede, 1987). Next, the intimate attachment to the host transmits a recognition signal to the baseplate, triggering a conformational change of this device from a hexagon to a six-pointed star with a large central hole from which the internal tail tube with a needle-like complex and a puncturing tip elongates. Following is the digestion of peptidoglycan linkages on the host surface by the lysozyme protein and the tail sheath contraction, pushing the tube core through the inner membrane and rapid delivery of the viral DNA into the host cytoplasm. Immediately after DNA injection, phage early transcribed genes hijack the host DNA replication machineries, degrade the bacterial chromosome and start replicating the viral DNA. The substructures e.g. heads, tails and tail fibers of progenies are subsequently synthesized and fully assembled. At the final step, the phage-encoded holin, which is an integral membrane protein, disrupts the bacterial inner membrane, allowing lysozyme to reach the outer membrane and attack the glycosidic bonds in the LPS layer. As a consequence, these disruptions in the bacterial membrane structure lead to cell lysis and release phage progenies to the environment.

2.3.4.1 Bacteriophage adsorption to *E. coli* outer membrane components

To initiate phage infection, phages must bind to the specific membrane components and securely anchor the phage particle onto the host surface (phage adsorption). The outer membrane of Gram-negative bacteria such as *E. coli* is constructed by a lipid asymmetry bilayer ornamented by protruding structures like LPS and OMPs. These components initially play essential roles in

maintaining the cell integrity, nutrient uptake and other essential activities for bacterial growth and survival (Delcour, 2009). Upon phage infection, high affinity phage proteins such as the tip of long tail fibers interact with these particular proteinaceous and/or carbohydrate receptor sites (Letarov & Kulikov, 2017) and trigger a series of events to secure the virion particle before the penetration step.

LPS is the most abundant element on the *E. coli* outer membrane and typically consists of O-antigen, the outer and inner core area and lipid A. Depending on the presence of O-antigen, there are two structural forms of LPS in *E. coli* and other Gram-negative bacteria: the smooth form LPS containing all three major components like that of *E. coli* O157:H7 and the rough form LPS lacking O-antigen like that of *E. coli* B and K-12 (Figure 2.3). The immunogenic virulence factor O-antigen contains 1 to 40 repeating oligosaccharide units with high variability among strains. It was found that phage T5 adsorbs to the surface of host *E. coli* F860 by binding its L-shaped tail fibers to the polymannose units in the O8 and O9 antigen (Heller & Braun, 1982). More recently, a *Myoviridae* phage Φ241 isolated from a high acidity and salinity environment successfully lysed 48 *E. coli* O157:H7 strains but not the other 18 non-O157 strains and two O antigen-negative mutants, implying that phage Φ241 specifically uses the O-antigen units of *E. coli* O157:H7 as the receptor during phage adsorption (Lu & Breidt, 2015). Interestingly, Van der Ley et al. (1986) noticed that O-antigens shielded the adsorption of twelve tested coliphages to proteinaceous receptors on the *E. coli* outer membrane, suggesting that complex interactions occur between superficial receptors upon phage attachment. Using the representative *E. coli* strains from the ECOR collection, Amor et al. (2000) has categorized five generally recognized core types: R1 (69.4%), R2 (11.1%), R3 (11.1%), R4 (2.8%) and K-12 (5.6%). *E. coli* O157:H7 strain was specifically found to have the R3 core type (Currie & Poxton, 1999), while *E. coli* B possesses a R1

core type (Prehm et al., 1976; Jeong et al., 2009). While targeting *E. coli* B, phage T4 uses the terminal glucose residues of LPS as receptor (Prehm et al., 1976). Similarly, T4-like phage AR1 binds to the analogous terminus glucose of *E. coli* O157:H7 LPS, in addition to OmpC, as receptor sites (S. Yu et al., 2000; Goodridge et al., 2003). Galactose is another sugar residue in LPS exploited by *E. coli* phages, mostly *Microviridae*, as receptors (Feige & Stirm, 1976; Picken & Beacham, 1977). Although sugar residues in the LPS inner core are rarely involved in phage binding to *E. coli*, T-even phage efficient attachment requires short tail fibers unravelled from the baseplate to irreversibly and securely bind to heptose of the inner core (Riede, 1987). Lastly, lipid A is a phosphorylated glucosamine disaccharide linked to six fatty acid chains. Not only does lipid A anchor the base of phage receptors in the LPS of the outer membrane, but it is also indispensable for viability of *E. coli* and other *Enterobacteriaceae* (Tan & Darby, 2005).

Based on their functions, OMPs are categorized into six classes, including slow porins (OmpA); OmpX; general porins (OmpC, OmpF and PhoE); substrate-specific proteins (LamB, ScrY); phospholipase A and TonB-dependent iron siderophore transporters (FhuA and FepA) (Koebnik et al., 2000). Numerous studies have shown the association of OMPs with phage infection to *E. coli*. T-even *E. coli* phage Ox2 was originally found to use the OmpA for adsorption, while its host range mutant phage 2h12h and 2h12.1 managed to infect *E. coli* using OmpC and OmpX, respectively, as receptors (Kaufmann et al., 1994). The substrate-specific protein LamB of *E. coli* has been known for its interaction with the protein J of classic temperate phage model lambda for decades (Randall-Hazelbauer & Schwartz, 1973), this interaction was recently revisited and advanced by Chatterjee and Rothenberg (2012) using fluorescently labelled lambda phages. In the light of X-ray crystallography, the three-dimensional structure of OMPs was also unveiled and summarized by Nikaido (2003). One should note that these transmembrane barrel proteins

formed by beta-strands are connected by periplasmic short “turns” and extracellular long “loops”. During adsorption, the tips of phage tail fibers bind to the variable loops on the extracellular side and snugly fit in the central cavity of OMPs (Bartual et al., 2010; Washizaki et al., 2016). Braun’s group in Germany studied the interactive binding of coliphages T1, T5 and phi80 to the specific receptor sites of protein FhuA on the *E. coli* cell surface (Killmann et al., 1995; Endriss & Braun, 2004). Their publications pointed out that a conserved a 34-amino acid sequence located at the transmembrane gating loop of FhuA might contain the binding sites of three tested phages (Killmann et al., 1995). In the follow-up study, they evaluated the role of each loop of receptor FhuA in phage binding using the single loop deletion mutants and concluded that FhuA loop 8 mutant was immune to infection caused by phages T1, T5 and phi80, while loop 4 deleted mutants merely developed resistance to TonB dependent phages T1 and phi80 but remained fully susceptible to TonB-independent phage T5 (Endriss & Braun, 2004). Washizaki et al. (2016) also briefly mentioned that mutations in loop 4 of OmpC could inactivate the adsorption of phage T4. Interestingly, although both phage T4 and phage AR1 are using OmpC as a receptor (F. Yu & Mizushima, 1982; Mathews, 1983; S. Yu et al., 2000), T4 can only infect *E. coli* K-12 but not *E. coli* O157:H7, while AR1 exclusively infects *E. coli* O157:H7. The reason is that the sequence of OmpC in *E. coli* O157:H7 has a fifteen amino acid substitution, one insertion and one deletion compared to that of *E. coli* K-12 (S. Yu et al., 2000). It is likely that these differences lead to structural modifications in the presumptive receptor site loop 4 of OmpC on *E. coli* O157:H7 surface, hence, avoiding recognition by phage T4 tail fibers. These findings collectively suggest that the success of phage attachment is determined by the accessibility of recognizable receptors with particular binding sites (sugar residues or amino acids) on the bacterial membrane.

Other protruding structures such as capsular lipopolysaccharide, pili, flagellar etc. can also be used as binding sites for *E. coli* phages. Two recent reviews (Bertozzi Silva et al., 2016; Letarov & Kulikov, 2017) have summarized the common phage receptors in extensive detail. The great diversity of phage receptors on the *E. coli* cell surface increases the likelihood of identifying diverse phages for use in controlling pathogenic bacteria.

2.3.5. Advantages of using bacteriophage applications for food safety purpose

The concept of using phage biocontrol to inactivate bacterial pathogens in various foods at different stages of the food chain has been supported by a large number of studies as summarized by Moye et al. (2018). As a biocontrol agent, phages offer unique advantages for suppressing pathogenic bacteria in the context of food safety. First of all, phages are the most abundant and diversified organisms on this planet. It is estimated that there are 10^{31} phage particles (ten times more than bacteria) populating with bacterial hosts in almost all ecological niche in the biosphere (Bergh et al., 1989; Rohwer & Edwards, 2002). The abundance of phages therefore forms an immense reservoir for selecting appropriate phages to target any specific bacteria, even the superbugs that show little response to antibiotics. In addition, the ability of constantly adapting and evolving with the bacterial hosts further diversifies the phage communities (Koskella & Brockhurst, 2014) and drives these viruses to continuously overcome the altered host systems as bacteria develop phage resistance for survival over time (Samson et al., 2013). Another property favoring the use of phages over other biocontrol agents is their high target specificity. Unlike other indiscriminative antimicrobial agents such as chemicals and antibiotics, phages recognize specific receptors on the bacterial cell wall and infect only a limited range of hosts. This high specificity feature allows phage-based agents to inactivate bacterial pathogens without causing collateral disruption to the natural microbiota in foods and in intestinal environments. Not only do phages

have these distinctive features that can improve food safety, but these microorganisms also address demands from consumer for natural ingredients in food products. Most currently available phage-based biocontrol products are formulated by phages that are naturally present in foods and other natural habitats (Moye et al., 2018). Together with other benefits such as limited impact on organoleptic properties of foods (Perera et al., 2015), these characteristics therefore account for the growing recognition of phage-based biocontrol products as an effective and natural tool to eliminate foodborne pathogenic bacteria.

2.3.6. Bacteriophage resistance mechanisms: blocking phage adsorption

Although the antimicrobial effect of phages on bacteria has been proved repeatedly, one problem of phage application lies on the circumstance that upon prolonged exposure, the hosts tend to develop resistance against the infecting phage using diverse strategies. These mutated bacteria are known as bacteriophage insensitive mutants (BIMs). The entire phage life cycle is subject to the challenge of various anti-phage mechanisms, such as blocking initial attachment, superinfection exclusion systems, restriction-modification systems, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (cas) proteins systems, toxin-antitoxin systems and abortive infection systems (Labrie et al., 2010). Among these mechanisms preventing phage attachment is the first line of defence and one of, if not the most, primary ways for developing phage resistance (Oechslin, 2018). This anti-phage defense can be achieved through genetic mutations which result in the loss of phage binding receptor sites (Nordstrom & Forsgren, 1974; Chart et al., 1989; Pedruzzi et al., 1998; Capparelli et al., 2010; Kim & Ryu, 2012). One example is that a number of genes that encode glycosyltransferases in the operon waa are involved in LPS core biosynthesis (Yethon et al., 1998; Yethon et al., 2000). Upon phage selective pressure, spontaneous mutations in these genes frequently leads to alterations in the LPS structure, denying

access to the specific phage binding sugar moieties in the receptor (Zhong et al., 2020). Other strategies including secretion of superficial extracellular matrices (Stirm, 1968; Hammad, 1998; Hanlon et al., 2001) and production competitive inhibitors (Destoumieux-Garzón et al., 2005; Scholl et al., 2005) were also reported. As a result, bacteria avoid phage attachment by the modified or blocked superficial phage receptors, terminating the infection cycle at the very beginning.

2.3.7. Fitness changes of bacteriophage insensitive *E. coli* mutants

Approximately every 48 hours, the global bacterial population is reduced by half due to phage infection (Deresinski, 2009; Gilmore, 2012). It is obvious that the inactivation of phage adsorption by altering phage receptors enable BIMs to survive and continue propagating. However, these modifications of phage receptors may impact bacterial physiological fitness, given that they are primarily involved in various essential functions for cell growth.

One of the most frequently studied fitness changes in phage-resistant mutants is attenuated virulence (Leon & Bastias, 2015). This reduction can be most likely attributed to the fact that bacteria shun away from phage recognition by modifying their membrane components which serve as phage receptors and also function as bacterial virulence determinants. For example, several virulence studies have showed that phage resistant *E. coli* mutants lost their capsules, which is a virulence factor protecting *E. coli* from phagocytosis, upon K-antigen targeting phage infections and showed significantly less virulence than their ancestral strains in mice (Smith & Huggins, 1982), as well as in domestic animals calves and piglets (Smith & Huggins, 1983). Similarly, phage-induced virulence mitigation may be attributed to alterations of OMPs. These proteins are known for their receptor roles in phage adsorption (Bertozzi Silva et al., 2016) and involved in various cellular functions including as a virulence determinant (Koebnik et al., 2000). A study published by Morona et al. (1984) showed that *E. coli* mutants conferred resistance to T-even-like

phages by altering receptor OmpA, which is a virulence contributor to bacterial adhesion, invasion, and evasion of the host immune system (Confer & Ayalew, 2013). However, the causative relationship between phage resistance-associated membrane alteration and reduction in virulence of pathogenic *E. coli* remains elusive.

Phage resistance conferred by modifying surface components in the outer membrane, which physically protects the bacteria and mediates the exchange of nutrients required for sustaining life, is frequently linked to changed membrane permeabilities of BIMs. For instance, studies showed that *E. coli* K-12 BIMs that lack glucose residues (*waaG* mutation) in LPS, exhibited super-sensitivity to novobiocin (Tamaki et al., 1971) and increased permeability to erythromycin (Wang et al., 2015). It was proposed later that mutations in *waaG* not only remove the glucose residues from the LPS, but also leads to complete absence of phosphate groups on Hep II (similar to *waaY* mutant) (Yethon et al., 1998) and 40% decreased phosphorylation on Hep I (Yethon et al., 2000). The lack of phosphate groups subsequently diminished the lateral interaction between LPS by releasing divalent cations, resulting in higher cell susceptibility to not only antibiotics but also the anionic surfactant sodium dodecyl sulfate (SDS) (Vaara, 1992; Yethon et al., 1998; Yethon et al., 2000). In addition, phage resistance-induced mutations in receptors that serves as proteinaceous channels through the OM can also compromise the uptake of certain substrates using these channels. Knowing that phage lambda binds to the maltose specific channel LamB during infection, Charbit et al. (Charbit et al., 1988) was able to isolate three classes of phage lambda *E. coli* BIMs with different maltose uptake levels. Class A was composed of BIMs with similar maltose transport as the parent strain; class B BIMs showed variable reduction in transportation; the single representative of class C abolished maltose transport completely. The diversified maltose uptake capability of these BIMs corresponds to the idea that while developing

phage resistance through spontaneously mutating phage receptors, the resulting alterations likely have variable impacts on the normal bacterial functionality, affecting bacterial survival in diverse environments.

Other fitness changes, such as reduced colony size (Mizoguchi et al., 2003; O'Flynn et al., 2004) and lowered mobility when exposed to flagella-targeting phages (Girgis et al., 2007), have also been reported sporadically in *E. coli* BIMs. Although the trade-off ensued by phage resistance is still elusive, studies have shown that the fitness changes of BIMs can be exploited in favor of phage antimicrobial effect (Alisky et al., 1998; Stephen T Abedon, 2019). A good example is the synergistic usage of phage and antibiotics. Essentially, the presence of phage selects for BIMs with modified membrane components because the membrane structure is disrupted and permeabilized. The synergy of phage and antibiotics ensures that the sensitized phage-resistant bacteria are subsequently challenged and most likely terminated, by the additional sub-lethal dosage of antibiotics (Comeau et al., 2007; Stephen T Abedon, 2019; C. G. Liu et al., 2020). This strategy not only maximizes the phage antimicrobial effect by suppressing phage resistance but also provides a potential solution to re-sensitize multidrug resistant bacteria to antibiotics. It is evident that understanding the mechanistic basis of the potential trade-off between phage resistance and bacterial fitness is of great importance, because phage-based biocontrol agents are increasingly popular as a food safety mitigation targeting foodborne pathogens like *E. coli* O157:H7.

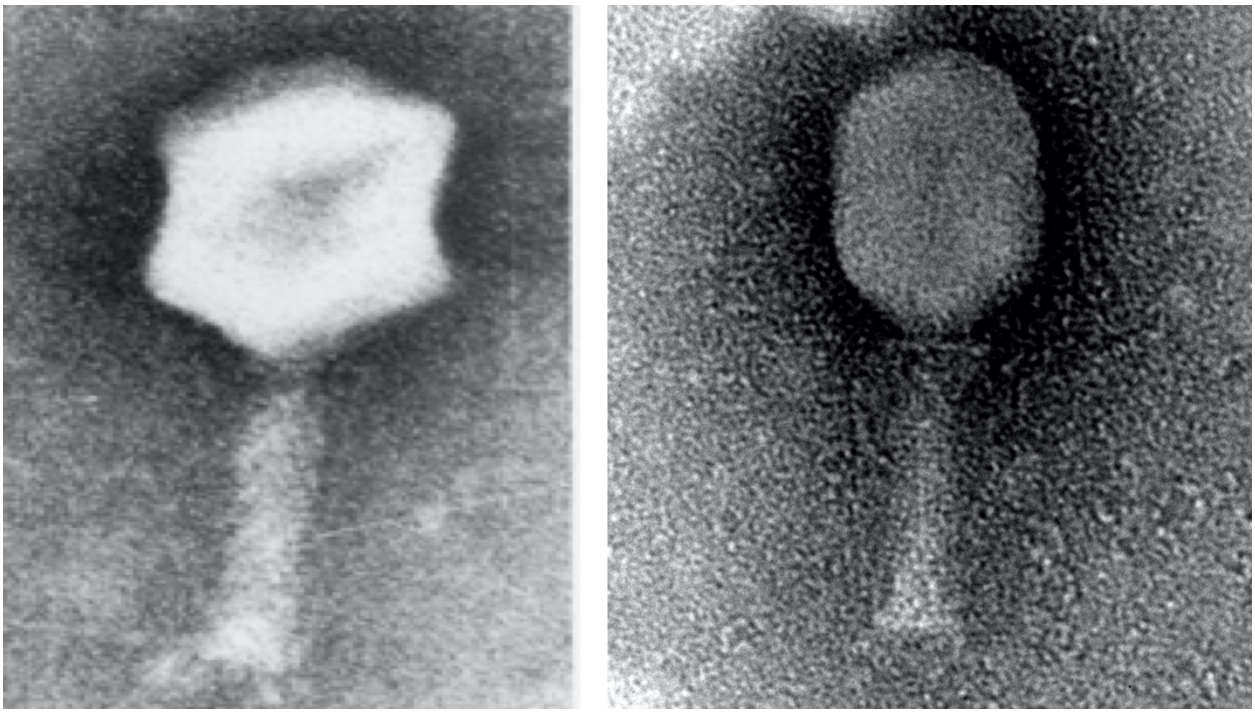

Figure 2.1. Transmission electron micrographs of phage AR1 (left), obtained from Goodridge et al. (2003), and phage T4 (right).

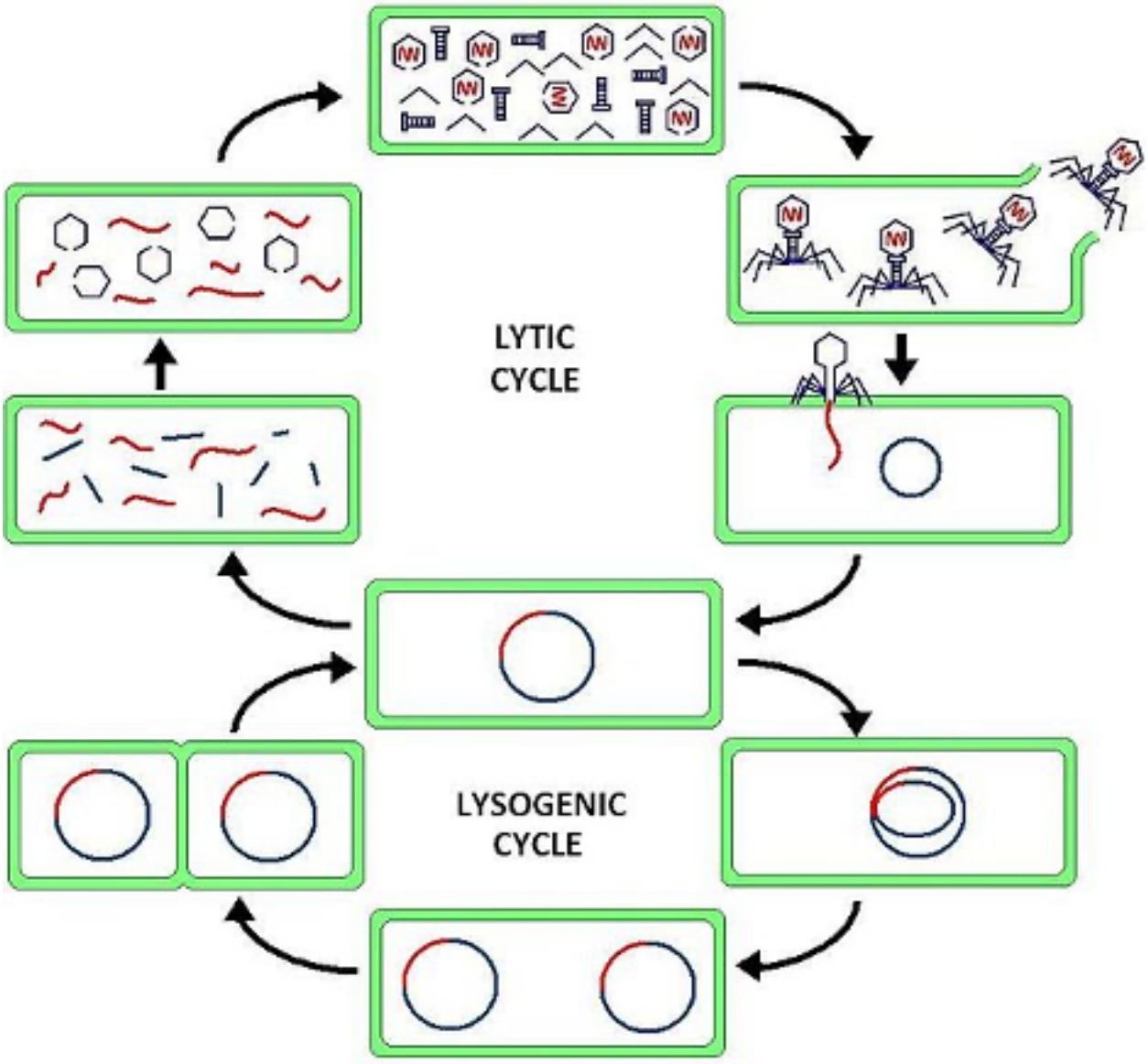

Figure 2.2. Lytic and lysogenic cycles of bacteriophages. Green boxes represent host cells. Red lines indicate phage nucleic acids; blue lines indicate host nucleic acids. This image is created by Suly12 and licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license (https://creativecommons.org/licenses/by-sa/3.0/deed.en).

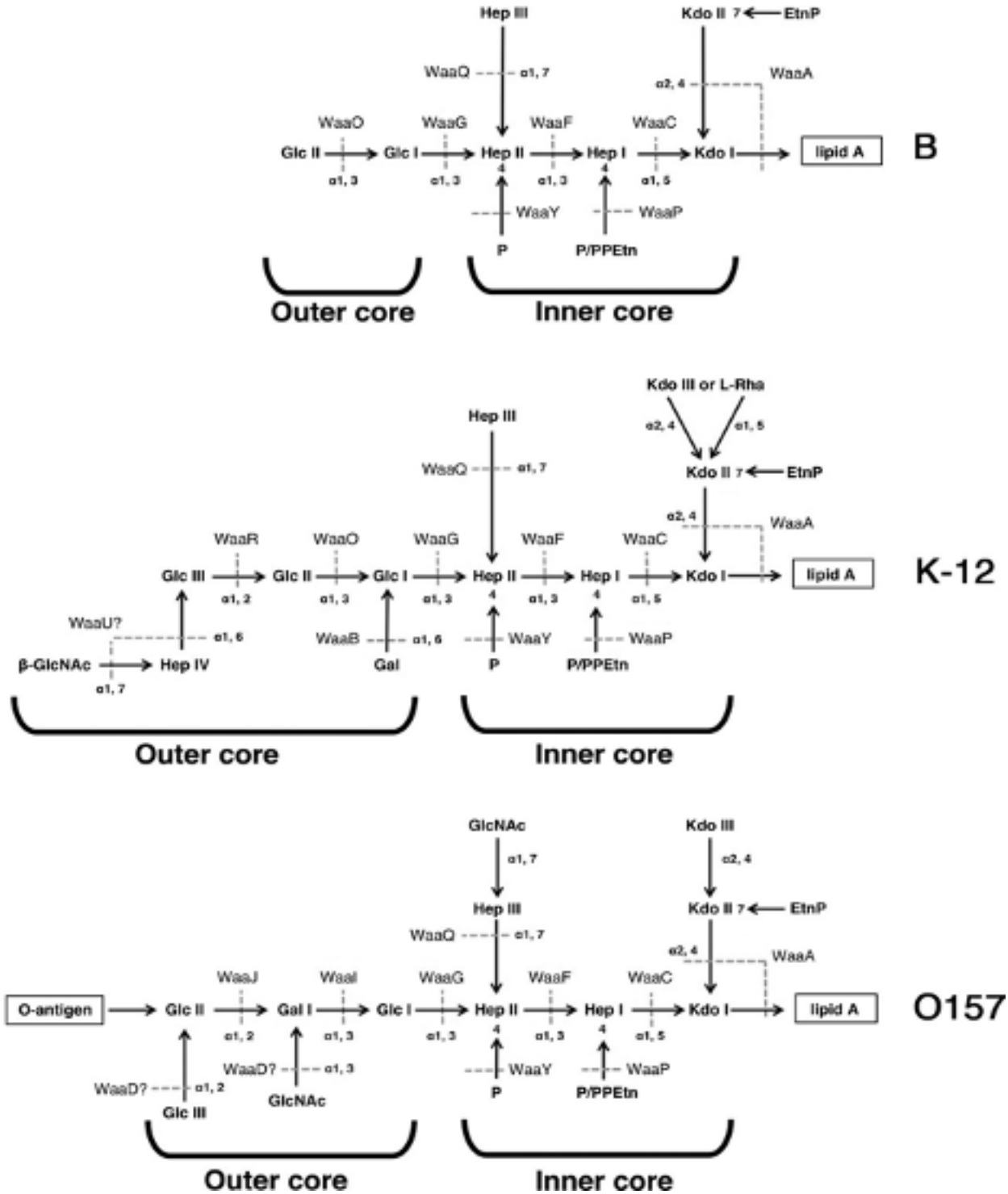

Figure 2.3. Lipopolysaccharide (LPS) structures of the *Escherichia coli* B, *Escherichia coli* K-12 and *Escherichia coli* O157 strains. Horizontal and vertical arrows respectively indicate the main backbone and branches. Dash lines represent the reactions catalyzed by glycosyltransferases encoded by waa operon. The abbreviations are as follows: Glc, glucose; Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; P, phosphate; P/PPEtn, phosphate or 2-aminoethyl diphosphate; and EtnP, ethanolamine phosphate. This figure was adapted from the publication by Washizaki et al. (2016)

2.4 References

Abedon, S. T. (2019). Phage-antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? *Antibiotics, 8*(4), 182.

Ackermann, H. W. (2003). Bacteriophage observations and evolution. *Res Microbiol, 154*(4), 245-251. doi:10.1016/S0923-2508(03)00067-6

Aldick, T., Bielaszewska, M., Zhang, W., Brockmeyer, J., Schmidt, H., Friedrich, A. W., Kim, K. S., Schmidt, M. A., & Karch, H. (2007). Hemolysin from Shiga toxin-negative *Escherichia coli* O26 strains injures microvascular endothelium. *Microbes and infection, 9*(3), 282-290.

Aldick, T., Bielaszewska, M., Uhlin, B. E., Humpf, H. U., Wai, S. N., & Karch, H. (2009). Vesicular stabilization and activity augmentation of enterohaemorrhagic *Escherichia coli* haemolysin. *Molecular microbiology, 71*(6), 1496-1508.

Alisky, J., Iczkowski, K., Rapoport, A., & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *Journal of Infection, 36*(1), 5-15.

Allos, B. M., Moore, M. R., Griffin, P. M., & Tauxe, R. V. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. In: The University of Chicago Press.

Amor, K., Heinrichs, D. E., Frirdich, E., Ziebell, K., Johnson, R. P., & Whitfield, C. (2000). Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*. *Infect Immun, 68*(3), 1116-1124. doi:10.1128/iai.68.3.1116-1124.2000

Bach, S., McAllister, T., Veira, D., Gannon, V., & Holley, R. (2002). Transmission and control of *Escherichia coli* O157:H7—a review. *Canadian journal of animal science, 82*(4), 475-490.

Bai, X., Hu, B., Xu, Y., Sun, H., Zhao, A., Ba, P., Fu, S., Fan, R., Jin, Y., & Wang, H. (2016). Molecular and phylogenetic characterization of non-O157 Shiga toxin-producing *Escherichia coli* strains in China. *Frontiers in cellular and infection microbiology, 6*, 143.

Bartual, S. G., Otero, J. M., Garcia-Doval, C., Llamas-Saiz, A. L., Kahn, R., Fox, G. C., & van Raaij, M. J. (2010). Structure of the bacteriophage T4 long tail fiber receptor-binding tip. *Proc Natl Acad Sci U S A, 107*(47), 20287-20292. doi:10.1073/pnas.1011218107

Beef Cattle Research Council. (2014). In-plant Mitigation of Pathogens. Retrieved from http://www.beefresearch.ca/research-topic.cfm/in-plant-mitigation-of-pathogens-12?language=. Accesed date:[Septermber 4th, 2020]

Bergh, O., BØrsheim, K. Y., Bratbak, G., & Heldal, M. (1989). High Abundance of Viruses Found in Aquatic Environments. *Nature, 340*(6233), 467.

Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host Receptors for Bacteriophage Adsorption. *FEMS Microbiol Lett, 363*(4). doi:10.1093/femsle/fnw002

Bielaszewska, M., Fell, M., Greune, L., Prager, R., Fruth, A., Tschäpe, H., Schmidt, M. A., & Karch, H. (2004). Characterization of cytolethal distending toxin genes and expression in Shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups. *Infection and immunity, 72*(3), 1812-1816.

Bielaszewska, M., Köck, R., Friedrich, A. W., Von Eiff, C., Zimmerhackl, L. B., Karch, H., & Mellmann, A. (2007). Shiga Toxin-mediated Hemolytic Uremic Syndrome: Time to Change the Diagnostic Paradigm? *PLoS One, 2*(10), e1024.

Bielaszewska, M., Rüter, C., Kunsmann, L., Greune, L., Bauwens, A., Zhang, W., Kuczius, T., Kim, K. S., Mellmann, A., & Schmidt, M. A. (2013). Enterohemorrhagic *Escherichia coli* Hemolysin Employs Outer Membrane Vesicles to Target Mitochondria and Cause Endothelial and Epithelial Apoptosis. *PLoS Pathog, 9*(12), e1003797.

Blanco, M., Blanco, J., Mora, A., Dahbi, G., Alonso, M., González, E., Bernárdez, M., & Blanco, J. (2004). Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). *Journal of Clinical Microbiology, 42*(2), 645-651.

Boerlin, P., McEwen, S. A., Boerlin-Petzold, F., Wilson, J. B., Johnson, R. P., & Gyles, C. L. (1999). Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology, 37*(3), 497-503.

Bondy-Denomy, J., & Davidson, A. R. (2014). When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. *Journal of Microbiology, 52*(3), 235-242.

Brenner, D. J., Fanning, G., Steigerwalt, A., Ørskov, I., & Ørskov, F. (1972). Polynucleotide sequence relatedness among three groups of pathogenic *Escherichia coli* strains. *Infection and immunity, 6*(3), 308-315.

Brockmeyer, J., Aldick, T., Soltwisch, J., Zhang, W., Tarr, P. I., Weiss, A., Dreisewerd, K., Müthing, J., Bielaszewska, M., & Karch, H. (2011). Enterohaemorrhagic *Escherichia coli* haemolysin is cleaved and inactivated by serine protease EspPα. *Environmental Microbiology, 13*(5), 1327-1341.

Brunder, W., Schmidt, H., & Karch, H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology, 142*(11), 3305-3315.

Brunder, W., Schmidt, H., & Karch, H. (1997). EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Molecular microbiology, 24*(4), 767-778.

Burland, V., Shao, Y., Perna, N. T., Plunkett, G., Blattner, F. R., & Sofia, H. J. (1998). The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic acids research, 26*(18), 4196-4204.

Caliezi, C., Wuillemin, W., Zeerleder, S., Redondo, M., Eisele, B., & Hack, C. (2000). C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacological reviews, 52*(1), 91-112.

Capparelli, R., Nocerino, N., Iannaccone, M., Ercolini, D., Parlato, M., Chiara, M., & Iannelli, D. (2010). Bacteriophage therapy of *Salmonella* enterica: a fresh appraisal of bacteriophage therapy. *J Infect Dis, 201*(1), 52-61. doi:10.1086/648478

Carlton, R. M. (1999). Phage therapy: past history and future prospects. *Arch Immunol Ther Exp (Warsz), 47*(5), 267-274.

Casjens, S. (2003). Prophages and bacterial genomics: what have we learned so far? *Molecular microbiology, 49*(2), 277-300.

CDC. (2020a). *Salmonella* Hompage. Retrieved from https://www.cdc.gov/*Salmonella*/index.html#:~:text=with%20backyard%20poultry-,Get%20tips%20to%20help%20protect%20you,family%20from%20a%20*Salmonella*%20infection.&text=CDC%20estimates%20*Salmonella*%20bacteria%20cause,the%20United%20States%20every%20year. Accesed date:[September 4th, 2020]

CDC. (2020b). Shigell-Shigellosis Fast Facts. Retrieved from https://www.cdc.gov/shigella/fast-facts.html. Accesed date:[September 4th, 2020]

CDC. (2020c). *E. coli* homepage. Retrieved from https://www.cdc.gov/ecoli/index.html. Accesed date:[September 4th, 2020]
Charbit, A., Gehring, K., Nikaido, H., Ferenci, T., & Hofnung, M. (1988). Maltose transport and starch binding in phage-resistant point mutants of maltoporin. Functional and topological implications. *J Mol Biol, 201*(3), 487-496. doi:10.1016/0022-2836(88)90630-4
Chart, H., Row, B., Threlfall, E. J., & Ward, L. R. (1989). Conversion of *Salmonella* enteritidis phage type 4 to phage type 7 involves loss of lipopolysaccharide with concomitant loss of virulence. *FEMS Microbiol Lett, 51*(1), 37-40. doi:10.1016/0378-1097(89)90073-6
Chatterjee, S., & Rothenberg, E. (2012). Interaction of bacteriophage l with its *E. coli* receptor, LamB. *Viruses, 4*(11), 3162-3178. doi:10.3390/v4113162
Clarke, S. C. (2001). Diarrhoeagenic *Escherichia coli*—an emerging problem? *Diagnostic microbiology and infectious disease, 41*(3), 93-98.
Comeau, A. M., Tetart, F., Trojet, S. N., Prere, M. F., & Krisch, H. M. (2007). Phage-Antibiotic Synergy (PAS): Beta-lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. *PLoS One, 2*(8), e799. doi:10.1371/journal.pone.0000799
Confer, A. W., & Ayalew, S. (2013). The OmpA family of proteins: roles in bacterial pathogenesis and immunity. *Vet Microbiol, 163*(3-4), 207-222. doi:10.1016/j.vetmic.2012.08.019
Coombes, B. K., Wickham, M. E., Mascarenhas, M., Gruenheid, S., Finlay, B. B., & Karmali, M. A. (2008). Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. *Applied and environmental microbiology, 74*(7), 2153-2160.
Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin Microbiol Rev, 26*(4), 822-880. doi:10.1128/CMR.00022-13
Currie, C. G., & Poxton, I. R. (1999). The lipopolysaccharide core type of *Escherichia coli* O157:H7 and other non-O157 verotoxin-producing *E. coli*. *FEMS Immunol Med Microbiol, 24*(1), 57-62. doi:10.1111/j.1574-695X.1999.tb01265.x
Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta, 1794*(5), 808-816. doi:10.1016/j.bbapap.2008.11.005
Deresinski, S. (2009). Bacteriophage therapy: exploiting smaller fleas. *Clinical Infectious Diseases, 48*(8), 1096-1101.
Destoumieux-Garzón, D., Duquesne, S., Peduzzi, J., Goulard, C., Desmadril, M., Letellier, L., Rebuffat, S., & Boulanger, P. (2005). The iron–siderophore transporter FhuA is the receptor for the antimicrobial peptide microcin J25: role of the microcin Val11–Pro16 β-hairpin region in the recognition mechanism. *Biochemical Journal, 389*(3), 869-876.
Doughty, S., Sloan, J., Bennett-Wood, V., Robertson, M., Robins-Browne, R. M., & Hartland, E. L. (2002). Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. *Infection and immunity, 70*(12), 6761-6769.
Duckworth, D. H. (1976). " Who discovered bacteriophage?". *Bacteriological reviews, 40*(4), 793.
Dunne, M., Hupfeld, M., Klumpp, J., & Loessner, M. J. (2018). Molecular Basis of Bacterial Host Interactions by Gram-Positive Targeting Bacteriophages. *Viruses, 10*(8), 397. doi:10.3390/v10080397

EFSA, & ECDC. (2019). The European Union one health 2018 zoonoses report. *EFSA Journal, 17*(12), e05926.

Endriss, F., & Braun, V. (2004). Loop deletions indicate regions important for FhuA transport and receptor functions in *Escherichia coli*. *J Bacteriol, 186*(14), 4818-4823. doi:10.1128/JB.186.14.4818-4823.2004

Farfan, M. J., Cantero, L., Vidal, R., Botkin, D. J., & Torres, A. G. (2011). Long polar fimbriae of enterohemorrhagic *Escherichia coli* O157:H7 bind to extracellular matrix proteins. *Infection and immunity, 79*(9), 3744-3750.

Faruque, S. M., & Mekalanos, J. J. (2012). Phage-bacterial interactions in the evolution of toxigenic *Vibrio cholerae*. *Virulence, 3*(7), 556-565.

FDA. (2017). Retail food protection: Employee health and personal hygiene handbook. Retrieved from https://www.fda.gov/media/77065/download. Accesed date:[Septerber 3rd, 2020]

Feige, U., & Stirm, S. (1976). On the structure of the *Escherichia coli* C cell wall lipopolysaccharide core and on its øX174 receptor region. *Biochemical and biophysical research communications, 71*(2), 566-573.

Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., & Spode, A. (2011). Epidemic profile of Shiga-toxin–producing *Escherichia coli* O104:H4 outbreak in Germany. *New England Journal of Medicine, 365*(19), 1771-1780.

Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B., & Knutton, S. (1998). Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Molecular microbiology, 30*(5), 911-921.

Fuller, C. A., Pellino, C. A., Flagler, M. J., Strasser, J. E., & Weiss, A. A. (2011). Shiga toxin subtypes display dramatic differences in potency. *Infection and immunity, 79*(3), 1329-1337.

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int J Food Microbiol, 134*(1-2), 37-45. doi:10.1016/j.ijfoodmicro.2009.05.021

Gill, J., & Abedon, S. T. (2003). Bacteriophage ecology and plants. *APSnet Feature*, 1-17.

Gilmore, B. F. (2012). Bacteriophages as anti-infective agents: recent developments and regulatory challenges. *Expert review of anti-infective therapy, 10*(5), 533-535.

Girgis, H. S., Liu, Y., Ryu, W. S., & Tavazoie, S. (2007). A comprehensive genetic characterization of bacterial motility. *PLoS Genet, 3*(9), 1644-1660. doi:10.1371/journal.pgen.0030154

Goerke, C., Koller, J., & Wolz, C. (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. *Antimicrob Agents Chemother, 50*(1), 171-177. doi:10.1128/AAC.50.1.171-177.2006

Goodridge, L., Gallaccio, A., & Griffiths, M. W. (2003). Morphological, host range, and genetic characterization of two coliphages. *Appl Environ Microbiol, 69*(9), 5364-5371. doi:10.1128/aem.69.9.5364-5371.2003

Grys, T. E., Siegel, M. B., Lathem, W. W., & Welch, R. A. (2005). The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. *Infection and immunity, 73*(3), 1295-1303.

Hammad, A. (1998). Evaluation of alginate-encapsulated Azotobacter chroococcum as a phage-resistant and an effective inoculum. *Journal of Basic Microbiology: An International*

Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 38(1), 9-16.
Hanlon, G. W., Denyer, S. P., Olliff, C. J., & Ibrahim, L. J. (2001). Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol., 67*(6), 2746-2753.
Hatfull, G. F., & Hendrix, R. W. (2011). Bacteriophages and their genomes. *Current opinion in virology, 1*(4), 298-303.
Heiman, K. E., Mody, R. K., Johnson, S. D., Griffin, P. M., & Gould, L. H. (2015). *Escherichia coli* O157 outbreaks in the United States, 2003–2012. *Emerging infectious diseases, 21*(8), 1293.
Heller, K., & Braun, V. (1982). Polymannose O-antigens of *Escherichia coli*, the binding sites for the reversible adsorption of bacteriophage T5+ via the L-shaped tail fibers. *J Virol, 41*(1), 222-227.
Ho, T. D., Davis, B. M., Ritchie, J. M., & Waldor, M. K. (2008). Type 2 secretion promotes enterohemorrhagic *Escherichia coli* adherence and intestinal colonization. *Infection and immunity, 76*(5), 1858-1865.
Hobbs, Z., & Abedon, S. T. (2016). Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. *FEMS microbiology letters, 363*(7).
Hu, B., Margolin, W., Molineux, I. J., & Liu, J. (2015). Structural remodeling of bacteriophage T4 and host membranes during infection initiation. *Proceedings of the National Academy of Sciences, 112*(35), E4919-E4928.
Hua, Y., An, X., Pei, G., Li, S., Wang, W., Xu, X., Fan, H., Huang, Y., Zhang, Z., & Mi, Z. (2014). Characterization of the morphology and genome of an *Escherichia coli* podovirus. *Archives of virology, 159*(12), 3249-3256.
Hussein, H. (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *Journal of animal science, 85*(suppl_13), E63-E72.
International Agency for Research on Cancer. (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. In *Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances*.
Janka, A., Bielaszewska, M., Dobrindt, U., & Karch, H. (2002). Identification and distribution of the enterohemorrhagic *Escherichia coli* factor for adherence (efa1) gene in sorbitol-fermenting *Escherichia coli* O157:H−. *International Journal of Medical Microbiology, 292*(3-4), 207-214.
Jeong, H., Barbe, V., Lee, C. H., Vallenet, D., Yu, D. S., Choi, S. H., Couloux, A., Lee, S. W., Yoon, S. H., Cattolico, L., Hur, C. G., Park, H. S., Segurens, B., Kim, S. C., Oh, T. K., Lenski, R. E., Studier, F. W., Daegelen, P., & Kim, J. F. (2009). Genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3). *J Mol Biol, 394*(4), 644-652. doi:10.1016/j.jmb.2009.09.052
Karam, J. D., & Drake, J. W. (1994). *Molecular biology of bacteriophage* (Vol. 4): Amer Society for Microbiology.
Karmali, M. A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., Isaac-Renton, J., Clark, C., Rahn, K., & Kaper, J. B. (2003). Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *Journal of Clinical Microbiology, 41*(11), 4930-4940.

Karmali, M. A., Gannon, V., & Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet Microbiol, 140*(3-4), 360-370. doi:10.1016/j.vetmic.2009.04.011

Kaufmann, A., Stierhof, Y. D., & Henning, U. (1994). New outer membrane-associated protease of *Escherichia coli* K-12. *J Bacteriol, 176*(2), 359-367. doi:10.1128/jb.176.2.359-367.1994

Killmann, H., Videnov, G., Jung, G., Schwarz, H., & Braun, V. (1995). Identification of receptor binding sites by competitive peptide mapping: phages T1, T5, and phi 80 and colicin M bind to the gating loop of FhuA. *J Bacteriol, 177*(3), 694-698. doi:10.1128/jb.177.3.694-698.1995

Kim, M., & Ryu, S. (2012). Spontaneous and transient defence against bacteriophage by phase-variable glucosylation of O-antigen in S almonella enterica serovar T yphimurium. *Molecular microbiology, 86*(2), 411-425.

Kirk, M. D., Angulo, F. J., Havelaar, A. H., & Black, R. E. (2017). Diarrhoeal disease in children due to contaminated food. *Bulletin of the World Health Organization, 95*(3), 233.

Kita, E., Yunou, Y., Kurioka, T., Harada, H., Yoshikawa, S., Mikasa, K., & Higashi, N. (2000). Pathogenic Mechanism of Mouse Brain Damage Caused by Oral Infection with Shiga Toxin-Producing *Escherichia coli* O157:H7. *Infection and immunity, 68*(3), 1207-1214.

Koebnik, R., Locher, K. P., & Van Gelder, P. (2000). Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol Microbiol, 37*(2), 239-253. doi:10.1046/j.1365-2958.2000.01983.x

Kondou, Y., Kitazawa, D., Takeda, S., Tsuchiya, Y., Yamashita, E., Mizuguchi, M., Kawano, K., & Tsukihara, T. (2005). Structure of the central hub of bacteriophage Mu baseplate determined by X-ray crystallography of gp44. *Journal of molecular biology, 352*(4), 976-985.

Konowalchuk, J., Speirs, J., & Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. *Infection and immunity, 18*(3), 775-779.

Koo, H. L., Jiang, Z.-D., Brown, E., Garcia, C., Qi, H., & DuPont, H. L. (2008). Coliform contamination of vegetables obtained from popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Clinical Infectious Diseases, 47*(2), 221-221.

Koskella, B., & Brockhurst, M. A. (2014). Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS microbiology reviews, 38*(5), 916-931.

Kumar, A., Taneja, N., Kumar, Y., & Sharma, M. (2012). Detection of S higa toxin variants among S higa toxin–forming *E scherichia coli* isolates from animal stool, meat and human stool samples in I ndia. *Journal of applied microbiology, 113*(5), 1208-1216.

Kutateladze, M. (2015). Experience of the Eliava Institute in bacteriophage therapy. *Virol Sin, 30*(1), 80-81. doi:10.1007/s12250-014-3557-0

Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat Rev Microbiol, 8*(5), 317-327. doi:10.1038/nrmicro2315

Lander, G. C., Khayat, R., Li, R., Prevelige, P. E., Potter, C. S., Carragher, B., & Johnson, J. E. (2009). The P22 tail machine at subnanometer resolution reveals the architecture of an infection conduit. *Structure, 17*(6), 789-799.

Lathem, W. W., Grys, T. E., Witowski, S. E., Torres, A. G., Kaper, J. B., Tarr, P. I., & Welch, R. A. (2002). StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Molecular microbiology, 45*(2), 277-288.

Leiman, P. G., Arisaka, F., Van Raaij, M. J., Kostyuchenko, V. A., Aksyuk, A. A., Kanamaru, S., & Rossmann, M. G. (2010). Morphogenesis of the T4 tail and tail fibers. *Virology journal, 7*(1), 1-28.

Leon, M., & Bastias, R. (2015). Virulence reduction in bacteriophage resistant bacteria. *Front Microbiol, 6*, 343. doi:10.3389/fmicb.2015.00343

Letarov, A. V., & Kulikov, E. E. (2017). Adsorption of Bacteriophages on Bacterial Cells. *Biochemistry (Mosc), 82*(13), 1632-1658. doi:10.1134/S0006297917130053

Levine, M. M., Xu, J.-g., Kaper, J. B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H., & Wachsmuth, K. (1987). A DNA probe to identify enterohemorrhagic *Escherichia coli* of 0157: H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *Journal of Infectious Diseases, 156*(1), 175-182.

Lewis, R., & Hill, C. (2020). Overcoming barriers to phage application in food and feed. *Current Opinion in Biotechnology, 61*, 38-44.

Lhuillier, S., Gallopin, M., Gilquin, B., Brasilès, S., Lancelot, N., Letellier, G., Gilles, M., Dethan, G., Orlova, E. V., & Couprie, J. (2009). Structure of bacteriophage SPP1 head-to-tail connection reveals mechanism for viral DNA gating. *Proceedings of the National Academy of Sciences, 106*(21), 8507-8512.

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of microbiology and biotechnology, 20*(1), 5.

Liu, C. G., Green, S. I., Min, L., Clark, J. R., Salazar, K. C., Terwilliger, A. L., Kaplan, H., Trautner, B., Ramig, R. F., & Maresso, A. W. (2020). Phage-Antibiotic Synergy Is Driven By A Unique Combination Of Antibacterial Mechanism Of Action And Stoichiometry. *bioRxiv*.

Lu, Z., & Breidt, F. (2015). *Escherichia coli* O157: H7 bacteriophage Φ241 isolated from an industrial cucumber fermentation at high acidity and salinity. *Frontiers in microbiology, 6*, 67.

Luck, S. N., Bennett-Wood, V., Poon, R., Robins-Browne, R. M., & Hartland, E. L. (2005). Invasion of epithelial cells by locus of enterocyte effacement-negative enterohemorrhagic *Escherichia coli*. *Infection and immunity, 73*(5), 3063-3071.

Luck, S. N., Badea, L., Bennett-Wood, V., Robins-Browne, R., & Hartland, E. L. (2006). Contribution of FliC to epithelial cell invasion by enterohemorrhagic *Escherichia coli* O113:H21. *Infection and immunity, 74*(12), 6999-7004.

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., Yeung, D. H., & Kirk, M. D. (2014). Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog Dis, 11*(6), 447-455. doi:10.1089/fpd.2013.1704

Mathews, C. K. (1983). *Bacteriophage T4*: Wiley Online Library.

Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., Wakiguchi, H., & Imai, S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *J Infect Chemother, 11*(5), 211-219. doi:10.1007/s10156-005-0408-9

Melton-Celsa, A. R. (2015). Shiga toxin (Stx) classification, structure, and function. *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli*, 37-53.

Meza-Segura, M., Zaidi, M. B., Maldonado-Puga, S., Huerta-Cantillo, J., Chavez-Dueñas, L., Navarro-Garcia, F., & Estrada-Garcia, T. (2017). Cytolethal Distending Toxin-producing

Escherichia coli Strains Causing Severe Diarrhoea in Young Mexican Children. *JMM Case Reports, 4*(2).
Miller, E. S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T., & Rüger, W. (2003). Bacteriophage T4 genome. *Microbiology and Molecular Biology Reviews, 67*(1), 86-156.
Miyamoto, Y., Iimura, M., Kaper, J. B., Torres, A. G., & Kagnoff, M. F. (2006). Role of Shiga toxin versus H7 flagellin in enterohaemorrhagic *Escherichia coli* signalling of human colon epithelium in vivo. *Cellular microbiology, 8*(5), 869-879.
Mizoguchi, K., Morita, M., Fischer, C. R., Yoichi, M., Tanji, Y., & Unno, H. (2003). Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. *Appl Environ Microbiol, 69*(1), 170-176. doi:10.1128/aem.69.1.170-176.2003
Morona, R., Klose, M., & Henning, U. (1984). *Escherichia coli* K-12 outer membrane protein (OmpA) as a bacteriophage receptor: analysis of mutant genes expressing altered proteins. *Journal of bacteriology, 159*(2), 570-578.
Motto, D. G., Chauhan, A. K., Zhu, G., Homeister, J., Lamb, C. B., Desch, K. C., Zhang, W., Tsai, H.-M., Wagner, D. D., & Ginsburg, D. (2005). Shiga toxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *The Journal of clinical investigation, 115*(10), 2752-2761.
Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. *Viruses, 10*(4), 205. doi:10.3390/v10040205
Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol Mol Biol Rev, 67*(4), 593-656. doi:10.1128/mmbr.67.4.593-656.2003
Nordstrom, K., & Forsgren, A. (1974). Effect of protein A on adsorption of bacteriophages to Staphylococcus aureus. *J Virol, 14*(2), 198-202.
O'Brien, A. D., Newland, J. W., Miller, S. F., Holmes, R. K., Smith, H. W., & Formal, S. B. (1984). Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science, 226*(4675), 694-696.
O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Appl Environ Microbiol, 70*(6), 3417-3424. doi:10.1128/AEM.70.6.3417-3424.2004
Oechslin, F. (2018). Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. *Viruses, 10*(7), 351. doi:10.3390/v10070351
Ogura, Y., Ooka, T., Iguchi, A., Toh, H., Asadulghani, M., Oshima, K., Kodama, T., Abe, H., Nakayama, K., & Kurokawa, K. (2009). Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Proceedings of the National Academy of Sciences, 106*(42), 17939-17944.
Orth, D., Ehrlenbach, S., Brockmeyer, J., Khan, A. B., Huber, G., Karch, H., Sarg, B., Lindner, H., & Würzner, R. (2010). EspP, a serine protease of enterohemorrhagic *Escherichia coli*, impairs complement activation by cleaving complement factors C3/C3b and C5. *Infection and immunity, 78*(10), 4294-4301.
Otsuji, N., Sekiguchi, M., Iijima, T., & Takagi, Y. (1959). Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin C. *Nature, 184(Suppl 14)*(4692), 1079-1080. doi:10.1038/1841079b0
Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., Morabito, S., & Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. *Frontiers in microbiology, 8*, 2390.

Paton, A. W., Ratcliff, R. M., Doyle, R. M., Seymour-Murray, J., Davos, D., Lanser, J. A., & Paton, J. C. (1996). Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *Journal of Clinical Microbiology, 34*(7), 1622-1627.

Paton, A. W., Srimanote, P., Woodrow, M. C., & Paton, J. C. (2001). Characterization of Saa, a Novel Autoagglutinating Adhesin Produced by Locus of Enterocyte Effacement-Negative Shiga-Toxigenic *Escherichia coli* Strains That Are Virulent for Humans. *Infection and immunity, 69*(11), 6999-7009.

Pedruzzi, I., Rosenbusch, J. P., & Locher, K. P. (1998). Inactivation in vitro of the *Escherichia coli* outer membrane protein FhuA by a phage T5-encoded lipoprotein. *FEMS Microbiol Lett, 168*(1), 119-125. doi:10.1111/j.1574-6968.1998.tb13264.x

Perera, M. N., Abuladze, T., Li, M., Woolston, J., & Sulakvelidze, A. (2015). Bacteriophage cocktail significantly reduces or eliminates Listeria monocytogenes contamination on lettuce, apples, cheese, smoked salmon and frozen foods. *Food microbiology, 52*, 42-48.

PHAC. (2017). Causes of *E. coli* (*Escherichia coli*) infection. Retrieved from https://www.canada.ca/en/public-health/services/diseases/e-coli/causes-e-coli.html. Accesed date:[September 4th, 2020]

Picken, R., & Beacham, I. (1977). Bacteriophage-resistant mutants of *Escherichia coli*K12. Location of receptors within the lipopolysaccharide. *Microbiology, 102*(2), 305-318.

Pollari, F., Christidis, T., Pintar, K. D., Nesbitt, A., Farber, J., Lavoie, M.-C., Gill, A., Kirsch, P., & Johnson, R. P. (2017). Evidence for the benefits of food chain interventions on *E. coli* O157:H7/NM prevalence in retail ground beef and human disease incidence: A success story. *Canadian Journal of Public Health, 108*(1), e71-e78.

Prehm, P., Jann, B., Jann, K., Schmidt, G., & Stirm, S. (1976). On a Bacteriophage T3 and T4 Receptor Region within the Cell Wall Lipopolysaccharide of *Escherichia coli* B. *Journal of molecular biology, 101*(2), 277-281.

Public Health Agency of Canada. (2012). National Enteric Surveillance Program (NESP). In: Winnipeg Manitoba, Canada.

Qadri, F., Svennerholm, A.-M., Faruque, A., & Sack, R. B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical microbiology reviews, 18*(3), 465-483.

Qin, J., Cui, Y., Zhao, X., Rohde, H., Liang, T., Wolters, M., Li, D., Campos, C. B., Christner, M., & Song, Y. (2011). Identification of the Shiga toxin-producing *Escherichia coli* O104: H4 strain responsible for a food poisoning outbreak in Germany by PCR. *Journal of Clinical Microbiology, 49*(9), 3439-3440.

Rakonjac, J., Bennett, N. J., Spagnuolo, J., Gagic, D., & Russel, M. (2011). Filamentous bacteriophage: biology, phage display and nanotechnology applications. *Current issues in molecular biology, 13*(2), 51.

Randall-Hazelbauer, L., & Schwartz, M. (1973). Isolation of the bacteriophage lambda receptor from *Escherichia coli*. *J Bacteriol, 116*(3), 1436-1446.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerging infectious diseases, 11*(4), 603.

Ratnam, S., March, S., Ahmed, R., Bezanson, G., & Kasatiya, S. (1988). Characterization of *Escherichia coli* serotype O157:H7. *Journal of Clinical Microbiology, 26*(10), 2006-2012.

Riede, I. (1987). Receptor specificity of the short tail fibres (gp12) of T-even type *Escherichia coli* phages. *Mol Gen Genet, 206*(1), 110-115. doi:10.1007/BF00326544
Ripp, S., & Miller, R. V. (1997). The role of pseudolysogeny in bacteriophage-host interactions in a natural freshwater environment. *Microbiology, 143*(6), 2065-2070.
Rohwer, F., & Edwards, R. (2002). The Phage Proteomic Tree: a genome-based taxonomy for phage. *J Bacteriol, 184*(16), 4529-4535. doi:10.1128/jb.184.16.4529-4535.2002
Salmond, G. P., & Fineran, P. C. (2015). A century of the phage: past, present and future. *Nat Rev Microbiol, 13*(12), 777-786. doi:10.1038/nrmicro3564
Samson, J. E., Magadán, A. H., Sabri, M., & Moineau, S. (2013). Revenge of the phages: defeating bacterial defences. *Nature Reviews Microbiology, 11*(10), 675-687.
Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging infectious diseases, 17*(1), 7.
Scaletsky, I., Silva, M., & Trabulsi, L. R. (1984). Distinctive patterns of adherence of enteropathogenic Escherichia coli to HeLa cells. *Infection and immunity, 45*(2), 534-536.
Schmidt, H., Beutin, L., & Karch, H. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. *Infection and immunity, 63*(3), 1055-1061.
Schmidt, H., Henkel, B., & Karch, H. (1997). A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. *FEMS microbiology letters, 148*(2), 265-272.
Scholl, D., Adhya, S., & Merril, C. (2005). *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. *Appl. Environ. Microbiol., 71*(8), 4872-4874.
Sciara, G., Bebeacua, C., Bron, P., Tremblay, D., Ortiz-Lombardia, M., Lichière, J., van Heel, M., Campanacci, V., Moineau, S., & Cambillau, C. (2010). Structure of lactococcal phage p2 baseplate and its mechanism of activation. *Proceedings of the National Academy of Sciences, 107*(15), 6852-6857.
Scuron, M. D., Boesze-Battaglia, K., Dlakić, M., & Shenker, B. J. (2016). The Cytolethal Distending Toxin Contributes to Microbial Virulence and Disease Pathogenesis by Acting as A Tri-perditious Toxin. *Frontiers in cellular and infection microbiology, 6*, 168.
Siringan, P., Connerton, P. L., Cummings, N. J., & Connerton, I. F. (2014). Alternative bacteriophage life cycles: the carrier state of *Campylobacter jejuni*. *Open biology, 4*(3), 130200.
Smith, H. W., & Huggins, M. B. (1982). Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. *Microbiology, 128*(2), 307-318.
Smith, H. W., & Huggins, M. B. (1983). Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J Gen Microbiol, 129*(8), 2659-2675. doi:10.1099/00221287-129-8-2659
Stevens, M. P., Roe, A. J., Vlisidou, I., Van Diemen, P. M., La Ragione, R. M., Best, A., Woodward, M. J., Gally, D. L., & Wallis, T. S. (2004). Mutation of toxB and a truncated version of the efa-1 gene in *Escherichia coli* O157:H7 influences the expression and secretion of locus of enterocyte effacement-encoded proteins but not intestinal colonization in calves or sheep. *Infection and immunity, 72*(9), 5402-5411.

Stirm, S. (1968). *Escherichia coli* K bacteriophages. I. Isolation and introductory characterization of five *Escherichia coli* K bacteriophages. *J Virol, 2*(10), 1107-1114.
Strockbine, N. A., Marques, L. R., Newland, J. W., Smith, H. W., Holmes, R. K., & O'Brien, A. D. (1986). Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect Immun, 53*(1), 135-140.
Sulakvelidze, A., Alavidze, Z., & Morris, J. G., Jr. (2001). Bacteriophage therapy. *Antimicrob Agents Chemother, 45*(3), 649-659. doi:10.1128/AAC.45.3.649-659.2001
Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V., & Force, C. P. T. (2001). PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging infectious diseases, 7*(3), 382.
Tamaki, S., Sato, T., & Matsuhashi, M. (1971). Role of Lipopolysaccharides in Antibiotic Resistance and Bacteriophage Adsorption of *Escherichia coli* K-12. *J Bacteriol, 105*(3), 968-975.
Tan, L., & Darby, C. (2005). *Yersinia pestis* is viable with endotoxin composed of only lipid A. *Journal of bacteriology, 187*(18), 6599-6600.
Tatsuno, I., Horie, M., Abe, H., Miki, T., Makino, K., Shinagawa, H., Taguchi, H., Kamiya, S., Hayashi, T., & Sasakawa, C. (2001). toxB gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. *Infection and immunity, 69*(11), 6660-6669.
Tilden Jr, J., Young, W., McNamara, A.-M., Custer, C., Boesel, B., Lambert-Fair, M. A., Majkowski, J., Vugia, D., Werner, S., & Hollingsworth, J. (1996). A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American journal of public health, 86*(8_Pt_1), 1142-1145.
Todd, E. (2014). Foodborne diseases: Overview of biological hazards and foodborne diseases. *Encyclopedia of Food Safety*, 221.
Toivonen, P. M., & Lu, C. (2013). Differential quenching of free chlorine by organic compounds potentially exuded from injured plant tissues. *Postharvest biology and technology, 86*, 192-194.
Totten, S., Medaglia, A., & McDermott, S. (2019). Updates to Canadian Notifiable Disease Surveillance System.
Trofa, A. F., Ueno-Olsen, H., Oiwa, R., & Yoshikawa, M. (1999). Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. *Clinical Infectious Diseases, 29*(5), 1303-1306.
Tzipori, S., Chow, C., & Powell, H. (1988). Cerebral infection with *Escherichia coli* O157:H7 in humans and gnotobiotic piglets. *Journal of clinical pathology, 41*(10), 1099-1103.
Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R. P., & Penades, J. R. (2005). Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol Microbiol, 56*(3), 836-844. doi:10.1111/j.1365-2958.2005.04584.x
Uhlich, G. A. (2009). KatP contributes to OxyR-regulated hydrogen peroxide resistance in *Escherichia coli* serotype O157:H7. *Microbiology, 155*(11), 3589-3598.
USDA. USDA Targeting Six Additional Strains of E.coli in Raw Beef Trim Starting Monday [Press release]. Retrieved from https://www.usda.gov/media/press-releases/2012/05/31/usda-targeting-six-additional-strains-ecoli-raw-beef-trim-starting. Accesed date:[September 4th, 2020]

Vaara, M. (1992). Agents that increase the permeability of the outer membrane. *Microbiological reviews, 56*(3), 395-411.
Van der Ley, P., De Graaff, P., & Tommassen, J. (1986). Shielding of *Escherichia coli* outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. *Journal of bacteriology, 168*(1), 449-451.
van Deurs, B., & Sandvig, K. (1995). Furin-induced cleavage and activation of Shiga toxin. *Journal of Biological Chemistry, 270*(18), 10817-10821.
Viator, C., Muth, M., & Brophy, J. (2015). Costs of Food Safety Investments. *RTI International. Prepared for USDA, Food Safety and Inspection Service, Office of Policy and Program Development*.
Vigil, K. J., Jiang, Z.-D., Chen, J. J., Palumbo, K. L., Galbadage, T., Brown, E. L., Yiang, J., Koo, H., DuPont, M. W., & Ericsson, C. (2009). Coliform and *Escherichia coli* contamination of desserts served in public restaurants from Guadalajara, Mexico, and Houston, Texas. *The American journal of tropical medicine and hygiene, 80*(4), 606-608.
Wang, Z., Wang, J., Ren, G., Li, Y., & Wang, X. (2015). Influence of Core Oligosaccharide of Lipopolysaccharide to Outer Membrane Behavior of *Escherichia coli*. *Mar Drugs, 13*(6), 3325-3339. doi:10.3390/md13063325
Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the Interactions between *Escherichia coli* Receptors, LPS and OmpC, and Bacteriophage T4 Long Tail Fibers. *Microbiologyopen, 5*(6), 1003-1015. doi:10.1002/mbo3.384
Weber-Dąbrowska, B., Mulczyk, M., & Górski, A. (2001). Bacteriophage therapy of bacterial infections: an update of our institute's experience. In *Inflammation* (pp. 201-209): Springer.
WHO. (2015). *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015*. Retrieved from
WHO. (2019). *Shiga Toxin-producing Escherichia Coli (STEC) and Food: Attribution Characterization and Monitoring* (Vol. 19): World Health Organization.
Wolfe, M. M., & Soll, A. H. (1988). The physiology of gastric acid secretion. *New England Journal of Medicine, 319*(26), 1707-1715.
Xicohtencatl-Cortes, J., Monteiro-Neto, V., Ledesma, M. A., Jordan, D. M., Francetic, O., Kaper, J. B., Puente, J. L., & Girón, J. A. (2007). Intestinal adherence associated with type IV pili of enterohemorrhagic *Escherichia coli* O157:H7. *The Journal of clinical investigation, 117*(11), 3519-3529.
Yethon, J. A., Heinrichs, D. E., Monteiro, M. A., Perry, M. B., & Whitfield, C. (1998). Involvement of waaY, waaQ, andwaaP in the Modification of *Escherichia coli*Lipopolysaccharide and Their Role in the Formation of a Stable Outer Membrane. *Journal of Biological Chemistry, 273*(41), 26310-26316.
Yethon, J. A., Vinogradov, E., Perry, M. B., & Whitfield, C. (2000). Mutation of the Lipopolysaccharide Core Glycosyltransferase Encoded by waaG Destabilizes the Outer Membrane of *Escherichia coli* by Interfering with Core Phosphorylation. *J Bacteriol, 182*(19), 5620-5623. doi:10.1128/jb.182.19.5620-5623.2000
Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. *J Bacteriol, 151*(2), 718-722.

Yu, S., Ko, K., Chen, C., Chang, Y., & Syu, W. (2000). Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1, Which Specifically Infects*Escherichia coli* O157: H7. *Journal of bacteriology, 182*(21), 5962-5968.

Zhong, Z., Emond-Rheault, J.-G., Bhandare, S., Lévesque, R., & Goodridge, L. (2020). Bacteriophage-Induced Lipopolysaccharide Mutations in *Escherichia coli* Lead to Hypersensitivity to Food Grade Surfactant Sodium Dodecyl Sulfate. *Antibiotics, 9*(9), 552.

Zumbrun, S. D., Hanson, L., Sinclair, J. F., Freedy, J., Melton-Celsa, A. R., Rodriguez-Canales, J., Hanson, J. C., & O'Brien, A. D. (2010). Human intestinal tissue and cultured colonic cells contain globotriaosylceramide synthase mRNA and the alternate Shiga toxin receptor globotetraosylceramide. *Infection and immunity, 78*(11), 4488-4499.

Connecting Statement

In the previous chapter, a comprehensive review of related literature was conducted, introducing different foodborne diarrheal pathogens with an emphasis on *E. coli* O157:H7. On the other hand, this review provides an overview of T4-like bacteriophages, showcasing their histories, biological features, and the typical infection process. Most importantly, we reviewed different anti-phage mechanisms as well as the resulting fitness changes identified in BIMs. In this study, BIMs were isolated from a wildtype *E. coli* B strain and analyzed by genomic and phenotypic methods, attempting to delineate the fitness costs of acquiring phage resistance to T4.

Chapter III

Bacteriophage-induced Lipopolysaccharide Mutations in *Escherichia coli* Lead to Hypersensitivity to Food Grade Surfactant Sodium Dodecyl Sulfate

Abstract

Bacteriophages are considered as one of the most promising antibiotic alternatives in combatting bacterial infectious diseases. However, one concern of employing phage application is the emergence of bacteriophage-insensitive mutants. Here, we isolated six BIMs from *E. coli* B in the presence of phage T4 and characterized them using genomic and phenotypic methods. Of all six BIMs, a six-amino acid deletion in glucosyltransferase WaaG likely conferred phage resistance by deactivating the addition of T4 receptor glucose to the LPS. This finding was further supported by the impaired phage adsorption to BIMs and glycosyl composition analysis which quantitatively confirmed the absence of glucose in the LPS of BIMs. Since LPSs actively maintain outer membrane (OM) permeability, phage-induced truncations of LPSs destabilized the OM and sensitized BIMs to various substrates, especially to the food-grade surfactant sodium dodecyl sulfate (SDS). This hypersensitivity to SDS was exploited to design a T4–SDS combination which successfully prevented the generation of BIMs and eliminated the inoculated bacteria. Collectively, phage-driven modifications of LPSs immunized BIMs from T4 predation but increased their susceptibilities as a fitness cost. The findings of this study suggest a novel strategy to enhance the effectiveness of phage-based food safety interventions.

3.1 Introduction

Antimicrobial resistance (AMR) is now recognized as a global health crisis that demands immediate intervention. A high-profile report published by British economist Jim O'Neil stressed that AMR would be more life-threatening than cancerous diseases by 2050, causing 10 million deaths a year globally. Almost 90% of this fatality is expected to come from developing countries in Asia and Africa (O'Neill, 2016). A panel of experts from the Council of Canadian Academies also forecasts that the resistance rates to first-line antimicrobials would likely rise from 26% in 2018 to 40% by 2050, leading to a cumulative loss of 396,000 lives in Canada (Council of Canadian Academies, 2019). To slow down the wheel, the World Health Organization (WHO) has implemented a global action plan on AMR, aiming to improve public awareness, strengthen knowledge through surveillance and research, reduce the incidence of infection, optimize the use of antimicrobials and increase investment in new alternative medicine development (WHO, 2015a).

Based on the gathered data from AMR surveillance systems like GLASS (WHO, 2016), WHO identified seven bacteria that confer high resistance rates to drugs commonly used for bacterial infectious diseases; among these species, the AMR situation of *Escherichia coli* (*E. coli*) is considered as the most critical (WHO, 2014). Originally, this bacterium was merely a benign member of the commensal community in human and animal gastrointestinal tracts and intrinsically susceptible to many antimicrobial agents (Poirel et al., 2018); however, due to the increasing demand of animal protein, misuse and abuse of antibiotics in agricultural practices facilitate the acquisition of antibiotic genes in bacterial communities through horizontal gene transfer, consequently leading to the emergence of multiantibiotic-resistant *E. coli* strains (Manyi-Loh et al., 2018). It is estimated that infection caused by various multidrug-resistant *E. coli* strains will lead to more than 3 million deaths by 2050 (O'Neill, 2016). Recently, Poirel et al. summarized the

AMR found in *E. coli* isolates from human and animal sources to different antibiotic classes (Poirel et al., 2018). Of note, this widespread AMR is undermining the effectiveness of critically important antimicrobials used in common infection treatments. For example, while cephalosporins and fluoroquinolones are considered as the first-line agents to treat lower urine tract infection (UTI) caused by the uropathogenic *E. coli* (Pitout, 2012; Shepherd & Pottinger, 2013), resistance to these compounds has been reported repeatedly (Rasheed et al., 2014; Gao et al., 2015; Bonyadian et al., 2017; Manyi-Loh et al., 2018) and predicted as a continuous growing trend in the next decade (McDanel et al., 2017; Alvarez-Uria et al., 2018). Since the high AMR prevalence is accelerating the extinction of effective antibiotics and limiting options for treatments, novel solutions are in great demand.

Phages, the viruses that can infect and kill bacteria, are considered as a promising alternative for antibiotics (Lin et al., 2017; Ghosh et al., 2019). These microorganisms have gained popularity because of their unique characteristics as an antimicrobial agent. Approximately 10^{31} phage particles distribute ubiquitously in aquatic environments, gastrointestinal tracts in animals and other natural habitats on Earth (Bergh et al., 1989), offering an immense reservoir for screening the proper bactericidal agents. Unlike the static effect of antibiotics, phages are continuously evolving with their bacterial hosts in an antagonistic manner. In other words, the dynamic interaction between phages and hosts allows the constant generation of new variants that can overcome resistance and infect bacteria. All these features advocate phages as a valuable candidate for developing antibacterial agents. In fact, a number of phage-based antimicrobial products now have been incorporated in food processing as a safety intervention, targeting foodborne pathogens such as *Listeria monocytogenes*, *Salmonella* and *E. coli* O157:H7 (Greer, 2005; Moye et al., 2018).

Remarkably, phage resistance has also been linked to the restoration of bacterial sensitivity to antimicrobial agents such as antibiotics. Chan et al. evaluated the antibiotic susceptibilities of *Pseudomonas aeruginosa* mutants that are resistant to lytic phage OMKO1, which absorbs to the outer membrane protein M (OprM) of the multidrug efflux systems' MEX as a receptor site (Chan et al., 2016). The results showed that these OMKO1-insensitive mutants were more susceptible to ciprofloxacin, tetracycline, ceftazidime and erythromycin than the parental strains, possibly due to the phage resistance-associated alterations in receptor OprM (Chan et al., 2016). This trade-off between phage resistance and increased antibiotic sensitivity was also further explored using phage–antibiotic synergy to control uropathogenic *E. coli* isolates. The study conducted by Valério et al. (Valério et al., 2017) demonstrated that while using either phage ECA2 or ciprofloxacin (0.05 mg/L) alone showed a limited suppression effect on cell growth after 8 h incubation in urine samples, the combination of phage ECA2 and same amount of ciprofloxacin was able to eliminate the bacteria after 4 h incubation in the same environment. However, in an attempt of using phages to control *E. coli* in a food matrix, further studies are needed to characterize the genetic and phenotypic changes of these bacteriophage-insensitive mutants (BIMs) and evaluate whether the combination of phage and other food-grade agents can suppress phage resistance and confer a synergic antimicrobial effect.

In this work, by using the classic model of *E. coli* B and phage T4, we genomically and phenotypically characterized the BIMs isolated from *E. coli* B in the presence of phage T4 and evaluated the antibacterial efficacy of the combination of phage T4 and the food-grade surfactant sodium dodecyl sulfate (SDS). Given that previous studies had shown that phage T4 recognizes and binds to the exposed glucose terminus in the outer core of *E. coli* B's LPS (Figure 3.1) as the only receptor site (Prehm et al., 1976; Washizaki et al., 2016), six isolated BIMs (ZZa0, ZZa1,

ZZa2, ZZa3, ZZa4 and ZZa5) were whole-genome sequenced and examined for their structural modifications in the receptor LPS. Loss of terminal glucose residues in this LPS in the presence of T4 was found to confer phage resistance and also sensitize bacteria to different substrates, especially SDS, suggesting a novel approach of combining phages with a food-grade surfactant to prevent the emergence of phage resistance, one of the major challenges of phage application.

3.2 Materials and Methods

3.2.1 Bacterial strain and growth conditions

Escherichia coli B ATCC 11303 was cultured at 37 °C for 18 h in Luria-Bertani broth (LB; Sigma-Aldrich, St-Louis, Missouri, USA) in an orbital shaker at a speed of 225 rpm.

3.2.2 Bacteriophage T4 preparation

Bacteriophage T4 was purchased from American Type Culture Collection (Manassas, VA, USA). High-titre phage stocks were prepared as previously described (Sambrook et al., 1989). Briefly, approximately 104 PFU T4 particles were added to 4 mL of molten agar (LB with 0.8% agar) containing 100 µL of E. coli B overnight culture and overlaid onto a pre-warmed LB agar plate (LB with 1.5% agar). After incubation at 37 °C for 18 h, the top agar containing phages was scraped off and submerged in 20 mL lambda buffer to allow phage elution, followed by centrifugation at 6,000 g for 10 min at 4 °C. This crude phage lysate was further purified with DNase (Roche Diagnostics, Mannheim, Germany), RNase (Roche Diagnostics, Germany) and Proteinase K (Roche Diagnostics, Germany), then precipitated by polyethylene glycol 8000 (Fisher Scientific, Fair Lawn, New Jersey, USA) before centrifugation at 11,000 × g for 15 min at 4°C. Phage pellet was collected from the centrifuged lysate and re-suspended in SM buffer (v/v = 62.5/1). Residual bacterial debris and precipitator were then removed by an equal volume of chloroform and centrifugation at 3,000 × g for 15 min at 4°C.

3.2.3 BIMs isolation and confirmation

Bacteriophage-insensitive mutants were generated using the double agar overlay method by Kropinski et al. (2009). Briefly, a uniform bacterial lawn was formed by overlaying 4 mL molten agar (LB with 0.8% agar; Sigma-Aldrich, USA) inoculated with 100ul of the wildtype *E. coli* B overnight culture onto an LB agar plate (Sigma-Aldrich, USA). After the molten agar was solidified, 100 µL of each 10-fold serial diluted phage T4 lysate (from 10^{10} to 10^{0} PFU/mL) was dispensed onto individual bacterial lawns and incubated at 37 °C for 18 h. Colonies that appeared within the phage clearing after incubation were considered as potential BIMs. Phage resistance of these candidates was further confirmed by a spot test as described by Clokie et al. (2009). Ten microliters of high-titre phage T4 lysate (10^{10} PFU/mL) was spot-inoculated on the bacterial lawn populated with each putative BIM. The resistance to phage T4 can be finally confirmed by the lack of plaques on the bacterial lawn.

3.2.4 Bacteriophage adsorption measurements

The adsorption curves of phage T4 to the wildtype and each BIM were determined as described by Kropinski (2009). Briefly, a mid-logarithm phase culture of the target bacterium was diluted into LB broth supplemented with 10 mM calcium chloride to give OD_{600} of 0.3, followed by addition of phage T4 at a multiplicity of infection (MOI) of 0.01. At several timepoints (1, 2, 4, 7 and 10 min), an aliquot was taken from the mixture and further diluted into a prechilled tube with the same media and three drops of chloroform. The titre of unadsorbed phages in each collected sample was then enumerated using the double agar overlay method, proliferating with the wildtype *E. coli* B overnight. A nonadsorbing control in the absence of bacteria was used to determine the initial phage titre which was then normalized to 100%. Each assay was performed

in duplicate and repeated at least three times. Phage adsorption rate constants were calculated using the following equation (1) (Krueger, 1931):

$$k = \frac{2.3}{\mathrm{B}t}\log\frac{P_0}{P}, (1)$$

where k represents the adsorption rate constant (mL/min); B stands for the inoculated bacterial load; and t is the time (min) required for a phage titre change from initial P_0 to final P.

3.2.5 DNA extraction and whole genome sequencing

Genomic DNA of the wildtype *E. coli* B ATCC 11303 and BIMs were extracted from LB broth cultures after 37 °C overnight incubation using the E-Z 96 Tissue DNA Kit (Omega Bio-tek, Norcross GA, USA) following the manufacturer's instructions. Approximately 500 ng of genomic DNA was extracted and mechanically fragmented for 40 s by ultra-sonicator Covaris M220 (Covaris, Woburn MA, USA) using the default settings. Libraries were synthesized using the NEBNext Ultra II DNA library prep kit for Illumina (New England Biolabs, Ipswich MA, USA) according to the manufacturer's instructions and were sequenced using the EcoGenomics Analysis Platform on an Illumina® MiSeq sequencer with TruSeq 300 bps paired-end libraries and 30 × coverage giving approximately 75 contigs per genome.

3.2.6 Bioinformatic analysis

The raw reads of sequenced strains were *de novo* assembled using the A5-miseq pipeline (Tritt et al., 2012). The sequence of the wildtype *E. coli* B ATCC 11303 was used as a reference for alignment and comparison to the BIMs. Retrieved genomes were then annotated by the Rapid Annotation using Subsystem Technology (RAST) service (Aziz et al., 2008). Single nucleotide polymorphism (SNP) detection was performed using the Geneious software. Genome comparison was performed using the MAUVE software v2.3.1. Gene sequences were aligned using the online platform Clustal Omega Multiple Sequence Alignment and visualized by Jalview version 2.11.0

(Waterhouse et al., 2009; Madeira et al., 2019). Lastly, ResFinder was used to compare the acquired antibiotic resistance profiles between the wildtype and BIMs (Zankari et al., 2012).

3.2.7 Isolation of LPS and glycosyl composition analysis

The LPS of wildtype E. coli B ATCC 11303 (WT-B) and BIM ZZa3 were prepared using the hot phenol-water extraction procedure (Westphal & Jann, 1965). Essentially, bacterial cells were collected via centrifugation and resuspended into molecular-grade water. The same volume of preheated 90% phenol was added to extract the LPSs at 70 °C for 20 min. The mixture was then cooled down in ice and centrifuged at 5,000 × g at 4 °C to promote phase separation. The upper (aqueous) phase of the mixture was collected. Extraction was repeated by adding the same volume of water into the lower (organic) phase of the mixture three times. Next, crude LPSs in the water phase were dialyzed (6,000 MWCO) at 4 °C against water until no detectable phenol remained, followed by treatments with RNase, DNase and proteinase K. The purified LPSs were dialyzed again and finally ultra-centrifuged at 100,000 × g at 4 °C for 18 h. Both LPS pellets and supernatants were collected for the following analysis.

The sugar components of the LPS from the wildtype and one of the BIMs, ZZa3, were determined. Extracted LPSs were converted to trimethylsilyl (TMS) methylglycosides through methanolysis where 1M HCl in methanol was added to the sample in the presence of an internal standard inositol and held at 80 °C for 18 h. The TMS derivatives were analyzed by GC-MS as previously described (York et al., 1986; Bhat et al., 1994) on a HP5890 gas chromatograph (Hewlett-Packard, Wilmington, Delaware, USA) equipped with a mass selective detector 5970 (Hewlett-Packard, USA) using an EC-1 fused silica capillary column (30m × 0.25 mm I.D.). Temperature cycle started at 80 °C for 2 min, then ramped to 160 °C at a rate of 20 °C/min, and to 200 °C at 2 °C/min, followed by an increase to 250 °C at 10 °C/min with an 11-min hold.

3.2.8 Minimum inhibitory concentration (MIC) assays

Minimum inhibitory concentration assays were conducted to compare the susceptibilities of the wildtype strain and six BIMs to various compounds, i.e., kanamycin sulfate (Sigma-Aldrich, USA), ampicillin (Sigma-Aldrich, USA), ethylenediaminetetraacetic acid (EDTA, ThermoFisher, Waltham, MA, USA), novobiocin (Sigma-Aldrich, USA), polymyxin B sulfate (ThermoFisher, USA), sodium cholate sulfate (ThermoFisher, USA) and SDS (ThermoFisher, USA), as previously described (Yethon et al., 2000).

Briefly, minimum inhibitory concentration assays were carried out in culture tubes where each contained 5 mL of LB broth. Two-fold serial dilutions of kanamycin sulfate (from 64 to 1 μg/mL), ampicillin (from 64 to 1 μg/mL), novobiocin (from 200 to 1 μg/mL), polymyxin B sulfate (from 200 to 1 μg/mL), EDTA (from 200 to 1 mg/mL), sodium cholate sulfate (from 128 to 1 mg/mL) and SDS (from 200 to 1 mg/mL) were made in these tubes. Each series of tubes was inoculated with 100 μL of overnight *E. coli* B cultures and incubated with orbital shaking at 225 rpm at 37 °C for 18 h. A positive score was recorded if the culture was visibly turbid. The MIC of each isolate to a certain compound was determined based on the tube with the highest concentration and visibly clear appearance. Each trial was performed in duplicate and repeated in three individual experiments.

3.2.9 Bacteriophage resistance inhibition assay

Since BIMs conferred hypersensitivity to the anion detergent SDS, a phage resistance inhibition test consisting of four settings (A, B, C and D) was conducted as follows (Table 3.3). This experiment was performed simultaneously in culture tubes and a 96-well microplate. Each setting was inoculated with an equal amount of *E. coli* B. While setting A was used as a negative control, setting B and C described the solo impact of phage T4 and low level of SDS on bacterial

growth, respectively. Lastly, in setting D, both phage T4 and a sublethal amount of SDS were used to assess the synergic effect of this combination on suppressing the emergence of phage resistance. After overnight incubation with shaking at 225 rpm at 37 °C, 100 μL of culture from each setting was enumerated using spread plating to determine the bacterial loads (detection limit = 10 CFU/mL) and the visual appearance of these four tubes was also captured. In the parallel study, the microbial growth in the designed settings was recorded at OD_{600} using a Synergy HTX Multi-Mode Reader (Winooski, VT, USA). Each trial was performed in duplicate and repeated on three individual experiments.

3.2.10 Statistical analysis

Single factor ANOVA was used to detect differences in bacterial growth in the four settings of the phage resistance inhibition test. Data were analyzed using Microsoft Excel and a difference was considered significant at $p < 0.05$.

3.3 Results

3.3.1 Bacteriophage T4 insensitive mutant isolation and phage adsorption assays

Wildtype (WT) *E. coli* B ATCC 11303 was challenged by phage T4 at different titre levels. Six BIMs, i.e., ZZa0 (accession no. PQWJ00000000), ZZa1 (accession no. PQWI00000000), ZZa2 (accession no. PQWH00000000), ZZa3 (accession no. PQWG00000000), ZZa4 (accession no. PQWF00000000) and ZZa5 (accession no. PQWE00000000) were derived from infection by 10^9, 10^8, 10^7, 10^6, 10^5 and 10^0 PFU of phage T4, respectively. Phage resistance were further evidenced by phage adsorption assays (Figure 3.2). While adsorption to WT *E. coli* B occurred immediately and only 20% of phages left at the end, the titre of the free phage remained similar to the initial level when T4 was proliferated with six BIMs. Based on formula (1), the calculated adsorption rate constant k of WT *E. coli* B was 1.64×10^{-9} mL/min, while T4 adsorbed to BIM

ZZa0, ZZa1, ZZa2, ZZa3, ZZa4 and ZZa5 at a rate of 1.09×10^{-11}, 1.25×10^{-11}, 2.26×10^{-10}, 1.51×10^{-10}, 5.79×10^{-11} and 9.51×10^{-11} mL/min, respectively, suggesting that these BIMs would be at least 7.26 times less likely than the wildtype to be infected by phage T4. Collectively, these findings suggest that phage T4 absorbed promptly to the WT strain but might not be able to attach to the BIMs.

3.3.2 Identification of genetic mutations associated with phage resistance

Mapping to the sequence of wildtype *E. coli* B, single nucleotide polymorphism (SNP) analysis identified a mutated gene, *waaG*, in operon waa of all BIMs consistently. Gene *waaG* is involved in the biosynthesis of LPSs, encoding an alpha-1,3-glucosyltransferase WaaG which catalyzes the transfer of a single glucose residue from an activated uridine diphosphate-D-glucose (UDP-Glc) to the nonreducing end of the inner core of the LPS (see Figure 3.1) (Coutinho et al., 2003; Lairson et al., 2008). A total of 374 and 368 amino acid residues were identified in the WaaG sequence of wildtype and BIMs, respectively (Figure 3.3). The six-amino acid (ADVCYA) difference was due to a deletion located at residues 99 to 104 found in the WaaG sequence of all BIMs. As a result, the mutated glucosyltransferase WaaG might be inactivated, subsequently disabling the addition of glucose, the phage receptor site, to the core area of the LPS.

3.3.3 LPS sugar composition analysis

Comparative gene alignments have shown that the gene encoding glucosyltransferase WaaG was mutated in all the BIMs, and the sugar composition of LPSs extracted from these mutants might have a different profile from the wildtype. Therefore, LPS sugar composition analysis was conducted using gas chromatograph mass spectrometry (GC-MS) to quantitatively compare the glycosyl profiles of the wildtype *E. coli* B and one of the BIMs, ZZa3, delineating the impact of WaaG mutation on LPS integrity.

Approximately 2 g of cells was harvested from 1 litre of overnight culture and used to extract LPSs. After the purification process, 400 μg dry weight of LPS was collected to perform comparative chemical analysis by GC-MS. The glycosyl composition profiles of WT and ZZa3 are shown in Figure 3.4 and quantified in Table 3.1. As shown in Figure 3.1, the main sugar moieties present in both the wildtype and ZZa3 LPS were Glc, Hep, Kdo and GlcN (Figure 3.4). The presence of Rib, however, might be due to the residual ribose nucleic acid that was not fully removed during ultracentrifugation. A striking difference was observed in the Glc content where the relative mole percentage was 17.8% in the WT LPS, while only 1% was detected in the ZZa3 LPS (Table 3.1). Due to the significant drop in glucose content, the relative percentages of heptose and glucosamine were increased proportionally. Together, these findings implied that BIM ZZa3 was likely producing a truncated LPS where the glucose residues are absent from the core area due to the gene *waaG* mutation triggered by phage T4 infection. In addition to sugar residues, the fatty acid profiles were also shown in the chromatograms. Based on the relative peak area, the LPS of both samples consisted of mainly hydroxylated fatty acid chain (3-OH)14:0 and small amounts of (3-OH)15:0, (3-OH)12:0 and (3-OH)13:0, 12:0, 14:0 and 16:0, demonstrating acylation heterogeneity in lipid A. However, the mole percentage of each fatty acid was not measured in this study.

3.3.4 Evaluation of bacterial membrane permeability

It is noteworthy that LPSs play a crucial role in maintaining the integrity of the OM structure and phage-induced mutations in WaaG subsequently resulted in LPS truncation and membrane destabilization, as a cost of phage resistance. Therefore, the structural integrity of the WT and BIMs were evaluated by measuring the cross-membrane permeability of different substrates, including ampicillin (penicillin), kanamycin (aminoglycoside), novobiocin

(aminocoumarin), polymyxin B (polypeptide), EDTA (membrane permeabilizer), sodium cholate (bile salt) and SDS (food-grade surfactant) (Table 3.2). Overall, mutants showed higher sensitivities to most of the substrates than the WT strain. An exception was made for the tolerance to novobiocin which was not affected by the *waaG* mutation. For ampicillin, kanamycin, polymyxin B and EDTA, BIMs showed at least 50% lower MIC values. Strikingly, the WT was relatively impermeable to SDS (MIC > 200 mg/mL), but the phage-resistant mutants conferred hypersensitivity (MIC ≤ 3 mg/mL) to this food-grade surfactant. The overall increased sensitivity to the tested compound demonstrated that the OM structure of BIMs was destabilized due to the phage-induced LPS truncation which permeabilized the cell to the surrounding environment.

3.3.5 Bacteriophage resistance inhibition assay

Given that all BIMs showed hypersensitivity to SDS (Valério et al., 2017), we evaluated the synergic effect of phage T4 and the sublethal amount of SDS (10 mg/mL) in suppressing the emergence of resistance. As shown in Figure 3.5A, after overnight incubation, settings containing either phage (setting B) or SDS (setting C) alone showed a similar recovered bacterial load as the control (about 9 $\log_{10}$ CFU/mL), while the bacterial rate in setting D was significantly lower than the other settings ($p < 0.05$), showing a 5-log reduction compared to the initial inoculum. This finding was supported by the result that the visual appearances of culture tubes A, B and C appeared to be cloudy, but tube D remained clear after incubation (Appendix Figure S3.1). Based on the recovery rates and visual confirmation, it was obvious that the inoculated bacteria populated readily in settings A, B and C but were completely inhibited in setting D. The growth curve study (Figure 3.5B) further supported this argument by characterizing bacterial growth over time. Bacterial proliferation in settings A, B and C had a lag phase of 3 h and showed similar OD_{600} values throughout the trial ($p > 0.05$). The similar bacterial growths in settings A and B suggested

that using phage T4 alone had a limited effect on suppressing bacterial growth, possibly owing to the emergence of BIMs in setting B. However, in the presence of the T4–SDS combination, the concentration of bacterial cells in setting D was not significantly different from the bacteria-free negative control ($p > 0.05$). These findings demonstrate that the combination of phage and surfactant exerted a synergic antimicrobial effect not only suppressing the emergence of phage resistance but also eliminating bacterial growth which neither phage nor SDS alone could achieve.

3.4 Discussion

The rise of phage-resistant mutants is one of the major obstacles limiting the efficacy of phage application. It is believed that using phages simultaneously with antibiotics can suppress the emergence of phage resistance and thus improve the antimicrobial effect of phages (Comeau et al., 2007; Valério et al., 2017). However, little is known about the physiological changes of mutants after acquiring phage resistance and if other food-grade substrates can be incorporated to enhance the efficacy of phage-based food safety interventions. In this study, while T4 attached to the WT *E. coli* B rapidly as previously described (Karam & Drake, 1994; Kasman et al., 2002), six BIMs resisted T4 adsorption and therefore prevented subsequent formation of a lysis zone. The identified mutation in glycosyltransferase WaaG and the comparative LPS glycosyl composition analysis collectively suggested that the receptor glucose in BIMs' LPS was truncated, consequently conferring resistance by preventing phage T4 from adsorbing to the bacterial surface. However, this truncation in the LPS increased the OM permeabilities to different compounds, especially to the food-grade surfactant SDS. By exploiting the hypersensitivity to SDS, the design of phage–SDS combination not only successfully eliminated the inoculated bacteria but also suppressed the emergence of phage resistance.

Escherichia coli B is a common laboratory strain which has been used for studying bacteriophages since the 1920s (d'Herelle, 1918). Its interaction with phage T4 has been characterized by extensive studies (Weidel, 1953; S. Brenner, 1955; Prehm et al., 1976; Karam & Drake, 1994; Washizaki et al., 2016). It is a common perception that T4 uses both the LPS and outer membrane protein C (ompC) as the receptor while infecting *E. coli* (Bertozzi Silva et al., 2016). However, in the case of *E. coli* B, phage T4 only absorbs to the receptor site (glucose) in the LPS because ompC expression is absent in this particular strain (Yoon et al., 2012; Washizaki et al., 2016). Therefore, this classic model system allows us to specifically study the impact of phage-induced LPS truncation on the OM structure by excluding the influence of general porin ompC.

It is known that phage T4 tail fiber protein gp 37 interacts with the glucose terminus in the outer core area of the LPS and then anchors the phage particle onto the *E. coli* B surface (Prehm et al., 1976). Mutations in the chromosomal operon waa (formerly known as rfa), which contains a cluster of structural genes that are responsible for the biosynthesis of LPSs, have been associated with the loss of phage receptors due to LPS truncation (Tamaki et al., 1971; Yethon et al., 2000; Chang et al., 2010). In this study, we identified six-amino acid deletion in glucosyltransferase WaaG which is responsible for transferring the first phage-binding glucose to the LPS. Recently, Liebau et al. constructed the 3D structure of protein WaaG isolated from *E. coli* K-12 W3110 (PDB accession number 2IW1) and characterized its membrane interaction (Appendix Figure S3.2) (Liebau et al., 2015). Most importantly, they identified a membrane-interacting region in the N-terminal domain of WaaG (MIR-waaG) which anchors this enzyme to the cytosolic side of the *E. coli* inner membrane (Liebau et al., 2015). This putative MIR-waaG was proposed as a 30-amino acid region (YAEKVAQEKGFLYRLTSRYRHYAAFERATF) situated at residues 103-132 in the

WaaG sequence. Comparative gene alignment (Figure 3.3) showed that the K-12 WaaG sequence showed a high degree of protein similarity to that of the WT *E. coli* B (identities=90%, coverage=100%, and E-value=0) used in this study; an almost identical sequence of MIR-waaG was found in *E. coli* B at the same position of residues 103-132. Thus, it is conceivable that the *E. coli* B WaaG possesses a highly homologous and functional membrane-interacting region as the K-12 WaaG. Interestingly, the putative membrane-interacting region in *E. coli* B WaaG overlapped two hydrophobic amino acids (tyrosine [Y] and alanine [A]) with the deletion region (ADVCYA) in BIMs' waaG sequences (Figure 3.3). Since hydrophobic interactions in the N-terminal domain are considered as the key contributors to glycosyltransferase membrane anchoring (Albesa-Jove et al., 2014; Liebau et al., 2015), the loss of the first two hydrophobic amino acid residues in BIM's MIR-waaG might obstruct the enzyme anchoring to the inner membrane, and thus inactivating the reaction of glucose transfer and resulting in the loss of phage T4 receptor.

In the LPS sugar composition analysis, although a trace amount of glucose was still detected, possibly due to the residual biofilm which also accounts for the presence of mannose (Beloin et al., 2008; Yi et al., 2009), the substantial difference in glucose content between the LPS from WT *E. coli* B and ZZa3 validated the glucose truncation of the LPS inner core, conferring a phenotype similar to the *waaG* mutant derived from *E. coli* F470 (Yethon et al., 2000). More importantly, Yethon et al. pointed out that the *waaG* mutation could not only trim the LPS but also destabilize the entire OM structure by discouraging the phosphorylation of heptose residues in the inner core (Yethon et al., 2000). As an effective permeability barrier, the negatively charged phosphate substituents in the LPS are essential for binding to divalent cations which laterally cross-link the neighboring LPS molecules. The fact that a total of 80% reduction in heptose phosphorylation was observed in the *waaG* mutant (Yethon et al., 2000) could substantially disrupt

the cross-linkage among neighboring LPS molecules and permeabilize the OM (Nikaido, 2003). This loss of lateral strength among LPS molecules likely permeabilized the membrane of BIMs and led to the decreases in MIC values of BIMs to most tested compounds (Table 3.2) since ResFinder discovered no difference in the antibiotic resistance gene profiles between the parental strain and six mutants.

To maximize the chance of survival, bacteria alter their OM structure on environmental cues. Upon phage T4 infection, *E. coli* B mutants survived by removing the phage receptor site glucose on the LPS chain. As a cost of this modulation, the OM of these BIMs became highly permeable to the surfactant SDS. In the phage resistance inhibition test, high fluctuation of OD_{600} values was observed in setting B, possibly due to the varied bacteria–phage interactions in each independent trial. Similar to the phage-antibiotic synergy (PAS) reported previously (Valério et al., 2017), our results collectively showed that the T4–SDS combination significantly reduced bacterial growth and suppressed phage resistance. This is also consistent with the observations by Scanlan et al. (Scanlan et al., 2017). However, the same study also argued that the combination of phage and surfactant did not necessarily achieve a synergic antibacterial effect (Scanlan et al., 2017). They tested the combination of SDS with three phages (T4, λcl6 and T7) from different families of Caudovirales and showed that except for phage T4, the presence of a small amount of SDS (0.3 mg/mL) would actually negatively affect the survivals of *Siphoviridae* phage λcl6 and *Podoviridae* phage T7 in liquid media, and thus buffer the antibacterial effects. Similar results were observed in the combination of bile salt with phages (Gabig et al., 2002; Scanlan et al., 2017). This evidence of varied synergic effects suggests that caution must be taken in future studies while selecting the specific combination of phage and surfactant, since the success of the synergic effect

depends on not only the bacterial target but also the potential interactions between selected phages and surfactants.

Not only a cleaning and hygienic detergent, SDS is also generally a food additive that can be safely used in egg white products, marshmallows and fruit juice (Canadian Food Inspection Agency; Food and Drug Administration). Although our phage resistance inhibition design was able to achieve a 5-log reduction and suppress phage resistance, other aspects need to be considered. For example, the maximum limit of SDS allowed in food (solid egg white) is ten times less than the dose used in this study (Food and Drug Administration). Moreover, further experiments are required to test this method on different food matrices in which the influences of food properties and environmental factors on the synergic effect remain elusive. Most importantly, the antibacterial impact on pathogenic *E. coli* strains might not be as straightforward as in *E. coli* B. Preliminary studies for *E. coli* O157:H7 BIMs found various mutations in genes related to receptors OmpC and LPS, suggesting inconsistent membrane permeabilities which would render the antimicrobial outcome of the combination of phages and a secondary substrate ineffective (Zhong & Goodridge, 2020).

Having been exposed to phage T4, *E. coli* BIMs harboring a mutation in *waaG* acquired phage resistance by detaching glucose residues from their LPS. However, this truncation of the LPS came with a costly change in OM permeability including the hypersensitivity to SDS. The T4–SDS combination designed in this study was shown to be successful in eliminating phage resistance and bacterial growth simultaneously. These findings thereby evidence that the combination of a phage and sublethal amount of a food-grade surfactant could be a potential novel phage-based food safety intervention.

Table 3.1: Design of the bacteriophage resistance inhibition assay

	Setting A	Setting B	Setting C	Setting D
E. coli B (10^6 CFU)	+	+	+	+
Phage T4 (10^7 PFU)	-	+	-	+
SDS (10 mg/mL)	-	-	+	+

Table 3.2: Glycosyl composition of LPS isolated from wildtype *Escherichia coli* B (WT-B) and bacteriophage insensitive mutant ZZa3

Strain	**LPS Glycosyl Composition [Mole %]**					
	Rib	**Man**	**Glc**	**Hep**	**GlcN**	**Kdo***
WT *E. coli* B	12.2	4.3	**17.8**	62.2	3.5	+
ZZa3	12.8	2.1	**1**	78.5	5.7	+

*Due to unavailability of Kdo standard, the relative mole % of Kdo was not included in the calculation.

Table 3.3: Minimum inhibitory concentration assays for wildtype *Escherichia coli* B (WT-B) and bacteriophage insensitive mutants

Substrates	**WT-B**	**ZZa0**	**ZZa1**	**ZZa2**	**ZZa3**	**ZZa4**	**ZZa5**
Ampicillin (µg/mL)	4	2	2	2	2	1	1
Kanamycin (µg/mL)	32	16	16	16	16	16	16
Novobiocin (µg/mL)	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Polymyxin B (µg/mL)	64	32	32	32	32	32	32
EDTA (mg/mL)	50	25	25	25	25	25	25
Sodium Cholate (mg/mL)	> 128	128	128	128	128	128	128
SDS (mg/mL)	> 200	3	1.5	3	3	3	1.5

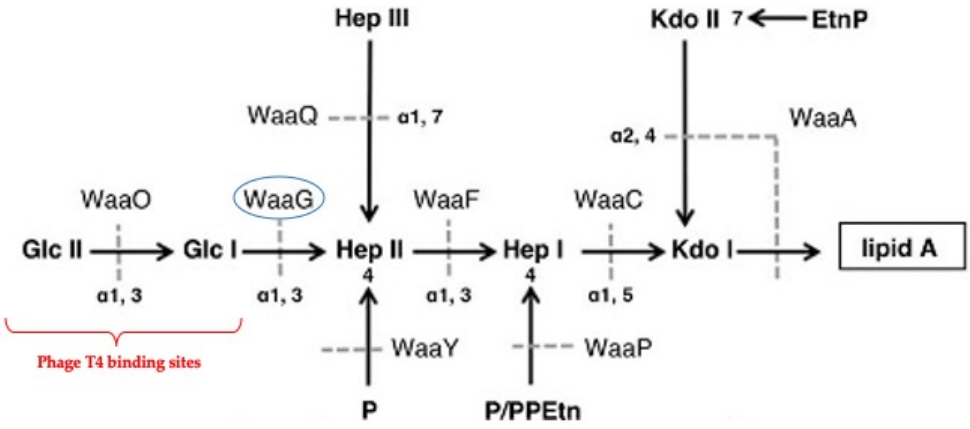

Figure 3.1. Glucosyltransferase WaaG addes glucose residues to the LPS of the wildtype *E. coli* B (WT-B). Horizontal and vertical arrows indicate the main backbone and branches, respectively. Dotted lines represent the reactions catalyzed by glycosyltransferases in waa operon. The abbreviations are as follows: Glc, glucose; Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; P, phosphate; P/PPEtn, phosphate or 2-aminoethyl diphosphate; and EtnP, ethanolamine phosphate. The specific receptor sites of phage T4 and glucosyltransferase waaG are highlighted in the red bracket and blue circle, respectively. This figure was adapted from the publication of Washizaki et al. (Washizaki et al., 2016).

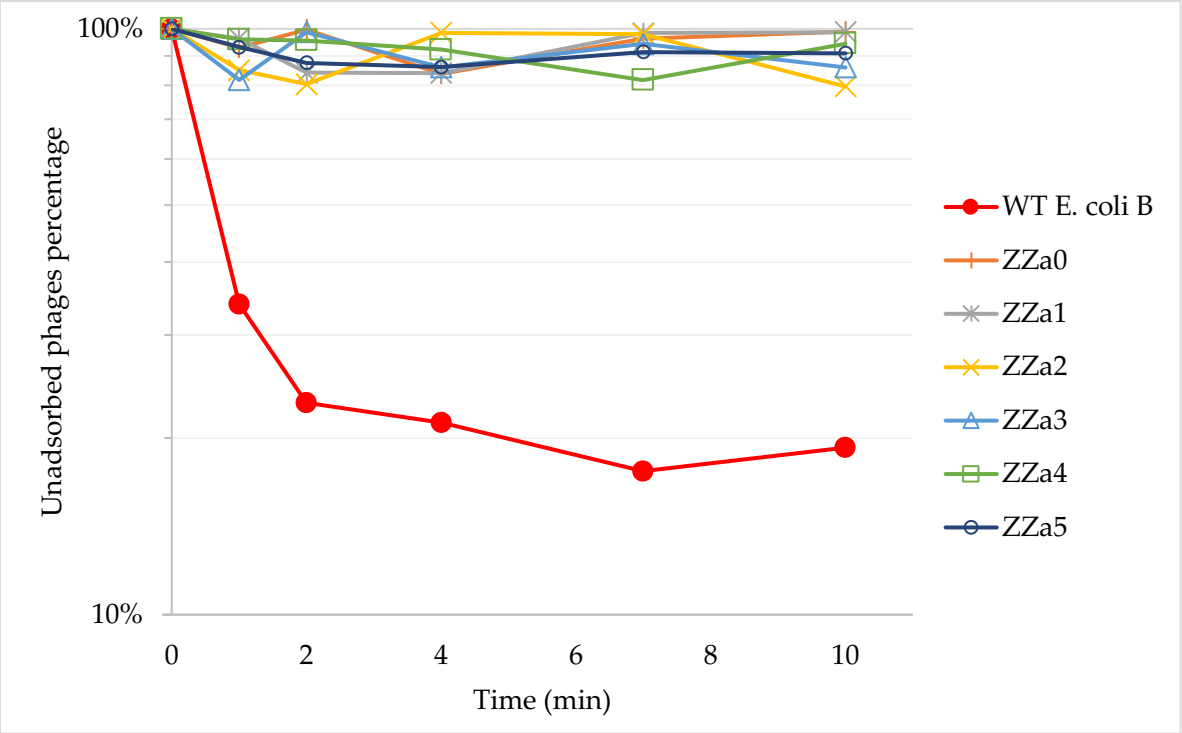

Figure 3.2. Adsorption kinetics of phage T4 to wildtype *Escherichia coli* B (WT-B) and bacteriophage insensitive mutants, shown as unadsorbed phage percentages. Each data point was generated using the average results from three measurements.

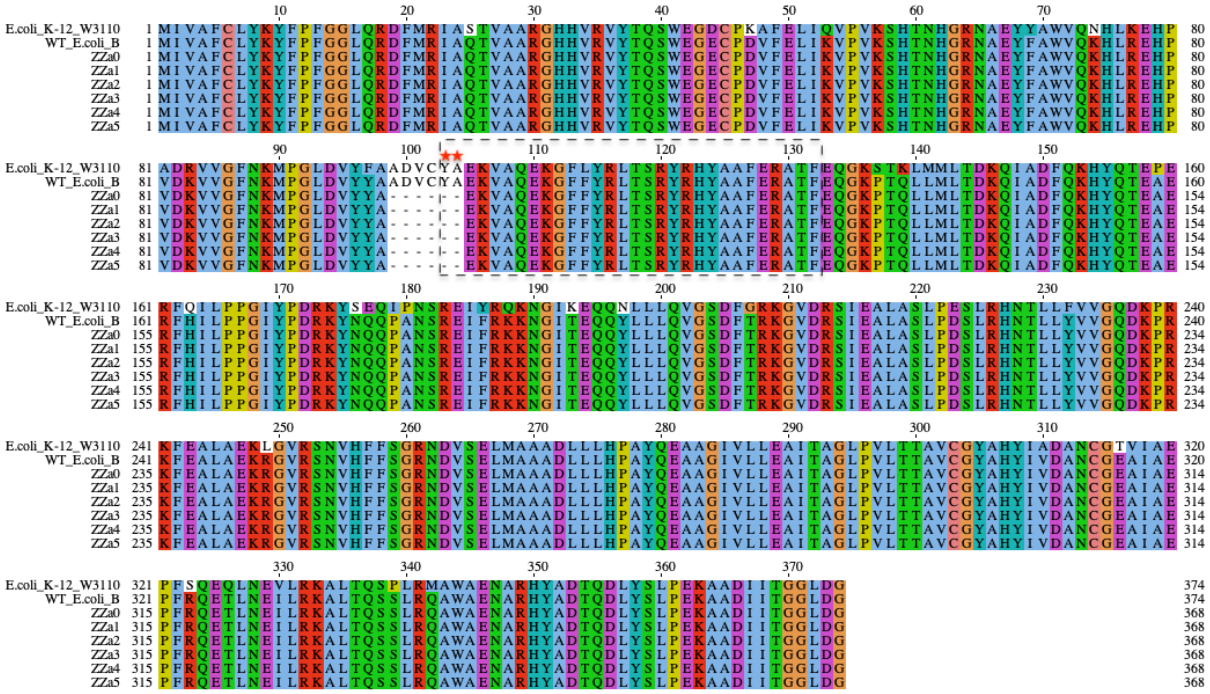

Figure 3.3. Amino acid alignments of WaaG of *Escherichia coli* K-12 W3110 (PDB accession number 2IW1), wildtype *Escherichia coli* B and six bacteriophage insensitive mutants. Sequences were aligned using Clustal Omega and visualized by Jalview. The black box highlights the putative membrane-interacting region (MIR). The red asterisks signify the two amino acids deleted in the putative MIR of BIMs' waaG. Amino acids were colored by the Clustal X scheme (orange: G; yellow: P; pink: C; red: K and R; magenta: E and D; cyan: H and Y; green: S, T, N and Q; blue: A, I, L, M, F, W and V). Gap is indicated by a dash.

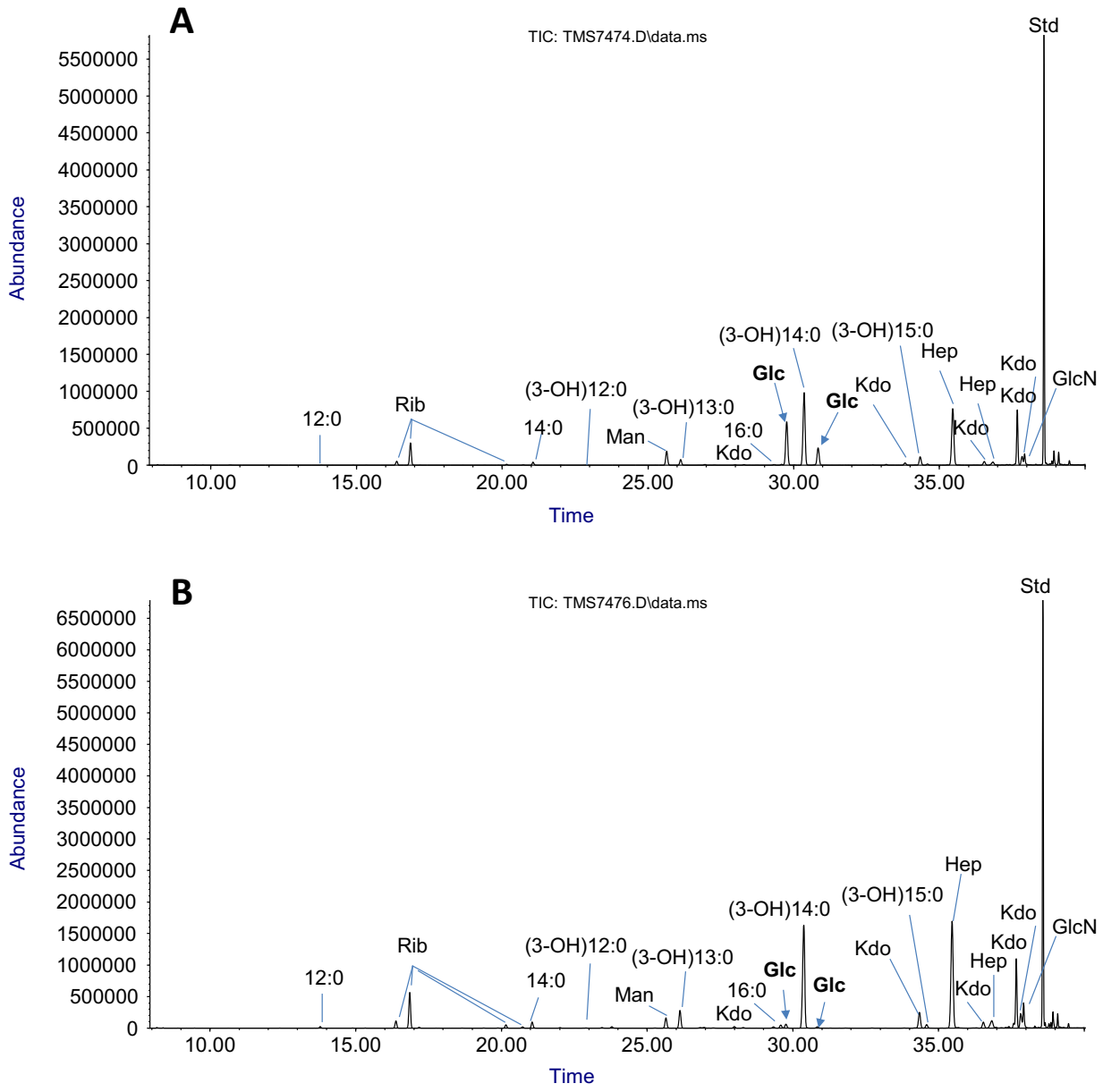

Figure 3.4. Sugar and fatty acid composition chromatograms of LPS isolated from wildtype *Escherichia coli* B (**A**) and bacteriophage insensitive mutant ZZa3 (**B**). A significant drop in the content of Glc in the ZZa3 mutant (Panel B) in comparison with the parental strain (Panel B) is highlighted (bold font and arrows).

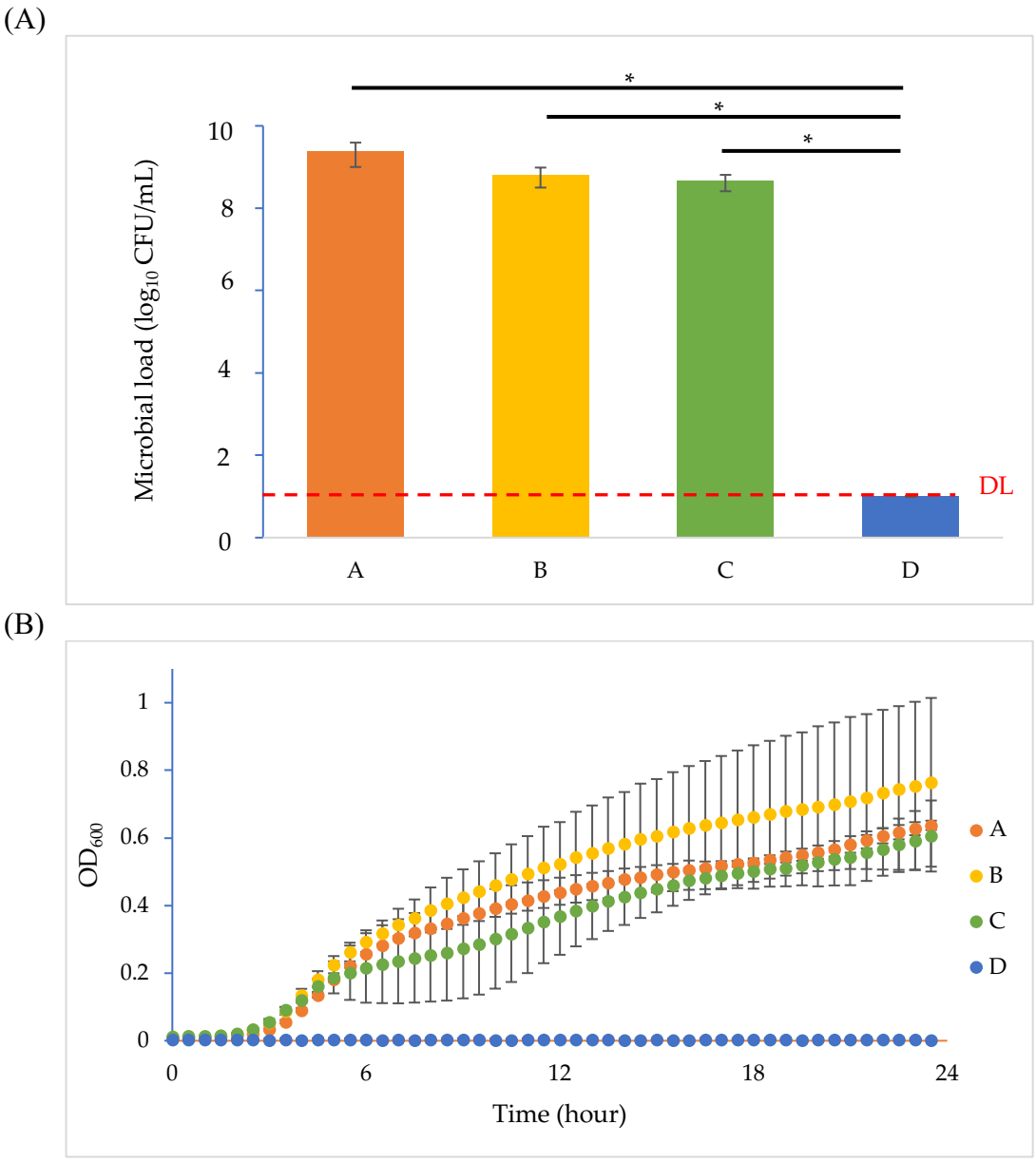

Figure 3.5. Bacteriophage resistance inhibition assay. (**A**) Recovered microbial loads in four designed settings (A, B, C and D) after overnight incubation. Asterisks indicate statistical significance using ANOVA ($p < 0.05$). Detection limit (DL) is indicated with red dash line. (**B**) Bacterial growth curves in the settings of phage inhibition test over time. Each trial was performed in triplicate.

3.5 References

Albesa-Jove, D., Giganti, D., Jackson, M., Alzari, P. M., & Guerin, M. E. (2014). Structure-Function Relationships of Membrane-associated GT-B Glycosyltransferases. *Glycobiology, 24*(2), 108-124. doi:10.1093/glycob/cwt101

Alvarez-Uria, G., Gandra, S., Mandal, S., & Laxminarayan, R. (2018). Global Forecast of Antimicrobial Resistance in Invasive Isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Int J Infect Dis, 68*, 50-53. doi:10.1016/j.ijid.2018.01.011

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., & Kubal, M. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC genomics, 9*(1), 75.

Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In *Bacterial Biofilms* (pp. 249-289): Springer-Verlag Berlin Heidelberg. Berlin, Germany.

Bergh, O., BØrsheim, K. Y., Bratbak, G., & Heldal, M. (1989). High Abundance of Viruses Found in Aquatic Environments. *Nature, 340*(6233), 467.

Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host Receptors for Bacteriophage Adsorption. *FEMS Microbiol Lett, 363*(4). doi:10.1093/femsle/fnw002

Bhat, U. R., Forsberg, L. S., & Carlson, R. W. (1994). Structure of Lipid A Component of *Rhizobium leguminosarum* bv. *phaseoli* Lipopolysaccharide. . *J Biol Chem, 269*(20), 14402-14410.

Bonyadian, M., Moshtaghi, H., & Mohamadtaghipour, L. (2017). Antibiotic Resistance of Verotoxigenic *Escherichia coli* Isolated from Vegetables. *Biological Journal of Microorganism, 5*(20), 9-16.

Brenner, S. (1955). The Adsorption of Bacteriophages by Sensitive and Resistant Cells of *Escherichia coli* strain B. *Proc R Soc Lond B Biol Sci, 144*(914), 93-99. doi:10.1098/rspb.1955.0036

Canadian Food Inspection Agency. Permitted Synonyms for Food Additives Table. Retrieved from https://www.inspection.gc.ca/food-label-requirements/labelling/industry/food-additives/permitted-synonyms/eng/1369857665232/1369857767799. Accesed date:[April 16, 2020]

Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas aeruginosa*. *Sci Rep, 6*, 26717. doi:10.1038/srep26717

Chang, V., Chen, L. Y., Wang, A., & Yuan, X. (2010). The Effect of Lipopolysaccharide Core Structure Defects on Transformation Efficiency in Isogenic *Escherichia coli* BW25113 rfaG, rfaP, and rfaC Mutants. *J ExpMicrobiol Immunol (JEMI), 14*, 101-107.

Clokie, M. R., Kropinski, A. M., & Lavigne, R. (2009). *Bacteriophages*: Springer. Berlin, Germany.

Comeau, A. M., Tetart, F., Trojet, S. N., Prere, M. F., & Krisch, H. M. (2007). Phage-Antibiotic Synergy (PAS): Beta-lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. *PLoS One, 2*(8), e799. doi:10.1371/journal.pone.0000799

Council of Canadian Academies. (**2019**). *When Antibiotics Fail. The Expert Panel on the Potential Socio-Economic Impacts of Antimicrobial Resistance in Canada*. Retrieved from

Coutinho, P. M., Deleury, E., Davies, G. J., & Henrissat, B. (2003). An Evolving Hierarchical Family Classification for Glycosyltransferases. *J Mol Biol, 328*(2), 307-317. doi:10.1016/s0022-2836(03)00307-3

d'Herelle, F. (1918). Sur le rôle du microbe filtrant bactériophage dans la dysentérie bacillaire. *CR Acad Sci, 167*, 970.

Food and Drug Administration. Code of Federal Regulations Title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.822. Accesed date:[Apr 16, 2020]

Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Los, M., Thomas, M. S., & Wegrzyn, G. (2002). The Cell Surface Protein Ag43 Facilitates Phage Infection of *Escherichia coli* in the Presence of Bile Salts and Carbohydrates. *Microbiology, 148*(Pt 5), 1533-1542. doi:10.1099/00221287-148-5-1533

Gao, L., Hu, J., Zhang, X., Wei, L., Li, S., Miao, Z., & Chai, T. (2015). Application of Swine Manure on Agricultural Ffields Contributes to Extended-spectrum Beta-lactamase-producing *Escherichia coli* Spread in Tai'an, China. *Front Microbiol, 6*, 313. doi:10.3389/fmicb.2015.00313

Ghosh, C., Sarkar, P., Issa, R., & Haldar, J. (2019). Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance. *Trends Microbiol, 27*(4), 323-338. doi:10.1016/j.tim.2018.12.010

Greer, G. G. (2005). Bacteriophage Control of Foodborne Bacteria. *Journal of Food Protection, 68*(5), 1102-1111.

Karam, J. D., & Drake, J. W. (1994). *Molecular Biology of Bacteriophage* (Vol. 4): American Society for Microbiology. Washington DC, USA.

Kasman, L. M., Kasman, A., Westwater, C., Dolan, J., Schmidt, M. G., & Norris, J. S. (2002). Overcoming the Phage Replication Threshold: A Mathematical Model with Implications for Phage Therapy. *J Virol, 76*(11), 5557-5564. doi:10.1128/jvi.76.11.5557-5564.2002

Kropinski, A. M. (2009). Measurement of the Rate of Attachment of Bacteriophage to Cells. In *Bacteriophages* (pp. 151-155): Springer. Berlin, Germany.

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. In *Bacteriophages* (pp. 69-76): Springer. Berlin, Germany.

Krueger, A. P. (1931). The Sorption of Bacteriophage by Living and Dead Susceptible Bacteria. *J Gen Physiol, 14*(4), 493-516. doi:10.1085/jgp.14.4.493

Lairson, L. L., Henrissat, B., Davies, G. J., & Withers, S. G. (2008). Glycosyltransferases: Structures, Functions, and Mechanisms. *Annu Rev Biochem, 77*, 521-555. doi:10.1146/annurev.biochem.76.061005.092322

Liebau, J., Pettersson, P., Szpryngiel, S., & Maler, L. (2015). Membrane Interaction of the Glycosyltransferase WaaG. *Biophys J, 109*(3), 552-563. doi:10.1016/j.bpj.2015.06.036

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage Therapy: An Alternative to Antibiotics in the Age of Multi-drug Resistance. *World J Gastrointest Pharmacol Ther, 8*(3), 162-173. doi:10.4292/wjgpt.v8.i3.162

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. *Nucleic Acids Res, 47*(W1), W636-W641. doi:10.1093/nar/gkz268

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules, 23*(4), 795.

McDanel, J., Schweizer, M., Crabb, V., Nelson, R., Samore, M., Khader, K., Blevins, A. E., Diekema, D., Chiang, H. Y., Nair, R., & Perencevich, E. (2017). Incidence of Extended-Spectrum beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella* Infections in the United States: A Systematic Literature Review. *Infect Control Hosp Epidemiol, 38*(10), 1209-1215. doi:10.1017/ice.2017.156

Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. *Viruses, 10*(4), 205. doi:10.3390/v10040205

Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol Mol Biol Rev, 67*(4), 593-656. doi:10.1128/mmbr.67.4.593-656.2003

O'Neill, J. (2016). Tackling Drug-resistant Infections Globally: Final Report and Recommendations. *UK Government and Wellcome Trust*.

Pitout, J. D. (2012). Extraintestinal Pathogenic *Escherichia coli*: A Combination of Virulence with Antibiotic Resistance. *Front Microbiol, 3*, 9. doi:10.3389/fmicb.2012.00009

Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial Resistance in *Escherichia coli*. *Microbiol Spectr, 6*(4), 289-316. doi:10.1128/microbiolspec.ARBA-0026-2017

Prehm, P., Jann, B., Jann, K., Schmidt, G., & Stirm, S. (1976). On a Bacteriophage T3 and T4 Receptor Region within the Cell Wall Lipopolysaccharide of *Escherichia coli* B. *Journal of molecular biology, 101*(2), 277-281.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial Drug Resistance in Strains of *Escherichia coli* Isolated from Food Sources. *Rev Inst Med Trop Sao Paulo, 56*(4), 341-346. doi:10.1590/s0036-46652014000400012

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*: Cold spring harbor laboratory press. Cold Spring Harbor, USA.

Scanlan, P. D., Bischofberger, A. M., & Hall, A. R. (2017). Modification of *Escherichia coli*-Bacteriophage Interactions by Surfactants and Antibiotics *in vitro*. *FEMS Microbiol Ecol, 93*(1). doi:10.1093/femsec/fiw211

Shepherd, A. K., & Pottinger, P. S. (2013). Management of Urinary Tract Infections in the Era of Increasing Antimicrobial Resistance. *Med Clin North Am, 97*(4), 737-757, xii. doi:10.1016/j.mcna.2013.03.006

Tamaki, S., Sato, T., & Matsuhashi, M. (1971). Role of Lipopolysaccharides in Antibiotic Resistance and Bacteriophage Adsorption of *Escherichia coli* K-12. *J Bacteriol, 105*(3), 968-975.

Tritt, A., Eisen, J. A., Facciotti, M. T., & Darling, A. E. (2012). An Integrated Pipeline for *de novo* Assembly of Microbial Genomes. *PLoS One, 7*(9), e42304. doi:10.1371/journal.pone.0042304

Valério, N., Oliveira, C., Jesus, V., Branco, T., Pereira, C., Moreirinha, C., & Almeida, A. (2017). Effects of Single and Combined Use of Bacteriophages and Antibiotics to Inactivate *Escherichia coli*. *Virus research, 240*, 8-17.

Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the Interactions between *Escherichia coli* Receptors, LPS and OmpC, and Bacteriophage T4 Long Tail Fibers. *Microbiologyopen, 5*(6), 1003-1015. doi:10.1002/mbo3.384

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2—A Multiple Sequence Alignment Editor and Analysis Workbench. *Bioinformatics, 25*(9), 1189-1191. doi:10.1093/bioinformatics/btp033

Weidel, W. (1953, June 5th, 1953). *Phage Receptor Systems of Escherichia coli B.* Paper presented at the Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor, USA.

Westphal, O., & Jann, K. (1965). Bacterial Lipopolysaccharides. Methods in Carbohydrate Chemistry. In: Academic Press, Cambridge, USA.

World Health Organization. (2014). *Antimicrobial Resistance: Global Report on Surveillance*: World Health Organization. Geneva, Switzerland.

World Health Organization. (2015). Global Action Plan on Antimicrobial Resistance. In: World Health Organization. Geneva, Switzerland.

World Health Organization. (2016). *National Antimicrobial Resistance Surveillance Systems and Participation in the Global Antimicrobial Resistance Surveillance System (GLASS): A Guide to Planning, Implementation, and Monitoring and Evaluation*. Retrieved from

Yethon, J. A., Vinogradov, E., Perry, M. B., & Whitfield, C. (2000). Mutation of the Lipopolysaccharide Core Glycosyltransferase Encoded by waaG Destabilizes the Outer Membrane of *Escherichia coli* by Interfering with Core Phosphorylation. *J Bacteriol, 182*(19), 5620-5623. doi:10.1128/jb.182.19.5620-5623.2000

Yi, W., Liu, X., Li, Y., Li, J., Xia, C., Zhou, G., Zhang, W., Zhao, W., Chen, X., & Wang, P. G. (2009). Remodeling Bacterial Polysaccharides by Metabolic Pathway Engineering. *Proc Natl Acad Sci U S A, 106*(11), 4207-4212. doi:10.1073/pnas.0812432106

Yoon, S. H., Han, M. J., Jeong, H., Lee, C. H., Xia, X. X., Lee, D. H., Shim, J. H., Lee, S. Y., Oh, T. K., & Kim, J. F. (2012). Comparative Multi-omics Systems Analysis of *Escherichia coli* strains B and K-12. *Genome Biol, 13*(5), R37. doi:10.1186/gb-2012-13-5-r37

York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., & Albersheim, P. (1986). Isolation and Characterization of Plant Cell Walls and Cell Wall Components. In *Methods in enzymology* (Vol. 118, pp. 3-40): Elsevier. Amsterdam, Netherlands.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F. M., & Larsen, M. V. (2012). Identification of Acquired Antimicrobial Resistance Genes. *J Antimicrob Chemother, 67*(11), 2640-2644. doi:10.1093/jac/dks261

Zhong, Z., & Goodridge, L. (2020). *Characterization of Phage AR1 Resistant Escherichia coli O157:H7 Mutants*. Manuscript in preparation.

Connecting statement

In previous chapter, six *E. coli* B BIMs consistently showed mutations in gene *waaG*, which is associated with the addition of glucose residues to the inner core of *E. coli* B LPS. This genomic finding was also supported by the phenotypic characterizations, which confirmed the loss of T4-binding terminal glucose residues in the LPS outer core of the BIMs. This membrane modification sensitized the cells to various compounds, especially to the food grade surfactant SDS. Taking advantage of the hypersensitivity to SDS, we developed a novel strategy to reduce bacterial growth using a combination of phage T4 and sub-optimal amounts of SDS. Not only did this synergy successfully suppress the emergence of BIMs, but also achieved a 5-log bacterial reduction.

Building on Chapter III, we focused on BIMs derived from a pathogenic *E. coli* O157:H7 isolate in Chapter IV. The objective was to isolate *E. coli* O157:H7 BIMs using the T4-like phage AR1 and then characterize them using genomic and phenotypic approaches. These findings reveal the potential impacts of phage resistant *E. coli* O157:H7 mutants in foods, thus advancing the knowledge of using phage-based biocontrol agents as a food safety intervention.

Chapter IV

Bacteriophage-induced Lipopolysaccharide Truncations of Foodborne Pathogen *Escherichia coli* O157:H7 Mutants Increase Bacterial Cell Permeability and Enhance Biofilm Formation

Abstract

A number of phage-based antimicrobial products have been developed to target foodborne bacterial pathogens such as *Escherichia coli* (*E. coli*) O157:H7, which sicken millions of people through ingestion of contaminated foods. However, one issue of using these products is the emergence of bacteriophage insensitive mutants, which not only neutralize the antimicrobial efficacy of phages but also display altered physiological properties that may increase bacterial survivability in food matrices and pathogenicity to animal hosts. Here, we isolated four BIMs (ZZb1, ZZb2, ZZb3 and ZZb4) from *E. coli* O157:H7 (WT-O157) in the presence of the T4-like phage AR1 and characterized them using genomic and phenotypic approaches. Mutants ZZb1, ZZb3 and ZZb4 conferred resistance to phage AR1 by mutating one of the two AR1 receptors porin OmpC, while BIM ZZb2 produced severely truncated LPSs in which the binding site for phage AR1 is absent, due to a mutation in gene *hldE*. Modified outer membrane components permeabilized and sensitized the BIMs to different extents. The deep rough mutant ZZb2 showed the most variations in MIC values and exclusively displayed sensitivity to bile salt sodium cholate and hypersensitivity to SDS. Lastly, we compared the biofilm formation between the wildtype *E. coli* B, *E. coli* O157:H7 and their BIMs at different temperatures and salinities. When propagated at 37°C, BIMs with truncated LPS (ZZa0, ZZa1, ZZa2, ZZa3, ZZa4, ZZa5 and ZZb2) showed higher absorbance readings, suggesting higher biofilm production than the wildtypes.

4.1 Introduction

The recurrent foodborne diseases have necessitated the development of innovative strategies to suppress the growth of pathogenic bacteria in foods. With the increasing awareness of healthy diets, consumers also demand for not only effective but also natural and non-chemical control measures for food protection. Antimicrobial products formulated by phages isolated from various agricultural settings, such as soil, water, manure and foods fulfil these criteria as a green antimicrobial alternative in food industries (Greer, 2005). In addition to the sustainable sourcing process, additional advantage of phage biocontrol approaches include high specificity to target bacteria without perturbation of the normal microbiota (Goodridge & Abedon, 2003), no adverse or toxic effects to eukaryotic cells (S. T. Abedon et al., 2011) and limited impact on sensory properties of foods (Perera et al., 2015). These properties have positioned the phage-based biocontrol agents as a decent alternative for reducing harmful bacteria in foods. In fact, since the first phage cocktail ListShield™ was granted Generally Recognized As Safe (GRAS) status for direct use in various food products by the U.S. Food Drug Administration in 2006, a number of novel phage-based biocontrol agents has been developed to reduce different bacterial pathogens in food products such as deli meat and fresh produce (Moye et al., 2018).

The antimicrobial effect of these phage-based products may be constrained by bacteriophage insensitive mutants generated via various anti-phage mechanisms (Labrie et al., 2010). A common strategy employed by bacteria to resist phage involves alteration of phage specific receptors on the bacterial surface to avoid initial phage attachment. Modifications of such components, which play essential roles in sustaining bacterial growth, could lead to various physiological changes that may affect the host survival in food matrices and virulence after being ingested. Perhaps the most direct impact on BIMs with superficial alterations is the disrupted

membrane structure. Studies have repeatedly reported the association of modified membrane components with increased susceptibilities to antibiotics (Filippov et al., 2011; Chan et al., 2016; Fong et al., 2020; Zhong et al., 2020), provided that components like LPS and OMPs maintain the outer membrane barrier and mediate the transmembrane diffusion of these compounds (Delcour, 2009). Another fitness change commonly associated with membrane modifications is increased biofilm formation (Lacqua et al., 2006; Hosseinidoust et al., 2013). These studies illustrated that selective pressure caused by phage infection induce BIMs with membrane alterations and strong biofilm forming capability. Together, the above findings demonstrate that mutants acquired phage resistance by modifying membrane components may lead to physiological changes that affect bacterial survival and virulence in dynamic environments.

Shiga-toxin producing *E. coli* (STEC) strains, frequently represented by the serogroup O157, are one of the leading contributors to foodborne diseases in North America. Although the efficacy of phage-based antimicrobial products in reducing *E. coli* O157:H7 in foods such as beef products and fresh produce has been validated (O'Flynn et al., 2004; Sharma et al., 2009; Viazis et al., 2011; Carter et al., 2012), the impacts of emerged BIMs are not clearly elucidated. In this work, we generated four BIMs of *E. coli* O157:H7 920333 from the infection of the T4-like phage AR1 and characterized these BIMs using comparative genomic and phenotypic approaches. The genomes of BIMs were sequenced and examined for genetic mutations associated with phage resistance. Previous studies have shown that phage AR1 are able to use both OmpC and the terminal glucose of LPS (Figure 4.1) on the outer membrane of *E. coli* O157:H7 as receptors (S. Yu et al., 2000; Goodridge et al., 2003). Dissimilar mutations were found in either OmpC or LPS of BIMs, presumably resulting in changed membrane structure, which was also supported by the different membrane permeabilities to various substrates. In addition to changed membrane

permeabilities, one mutant with a severe LPS truncation showed a significant increase of biofilm formation, illustrating the variety of phage-induced fitness changes. These findings of phage resistance-associated physiological changes give insights into the microbial interaction of *E. coli* O157:H7 and phage AR1 and describe the potential impacts of phage-resistant mutants in foods and following ingestion.

4.2 Materials and Methods

4.2.1 Bacterial strain and growth condition

Wildtype *Escherichia coli* O157:H7 920333 (WT-O157), *Escherichia coli* B ATCC11303 (WT-B) and the derived BIMs in this study were cultured at 37 °C for 18 h in Luria-Bertani broth (LB; Sigma-Aldrich, St-Louis, Missouri, USA) in an orbital shaker at a speed of 225 rpm. The bacterial strains used in this study are listed in Table 4.1.

4.2.2 Bacteriophage preparation

Bacteriophage AR1 stock from our collection was used to prepare high titre phage stocks as described previously (Zhong et al., 2020). Briefly, approximately 10^4 PFU AR1 phage particles were added to LB molten agar (LB with 0.8% agar; Sigma-Aldrich, USA) containing 100 µL of wildtype *E. coli* O157:H7 overnight culture. The mixture was overlaid onto a pre-warmed LB agar plate (LB with 1.5% agar). After overnight incubation at 37 °C, the top agar containing phages was scraped off and submerged in lambda buffer to allow phage elution, followed by centrifugation at 6,000 g for 10 min at 4 °C. This crude phage lysate was further purified with DNase (Roche Diagnostics, Mannheim, Germany), RNase (Roche Diagnostics, Germany) and Proteinase K (Roche Diagnostics, Germany), then precipitated by polyethylene glycol 8000 (Fisher Scientific, Fair Lawn, New Jersey, USA) before centrifugation at 11,000 × g for 15 min at 4°C. Phage pellet was collected from the centrifuged lysate and re-suspended in SM buffer (v/v = 62.5/1). Residual

bacterial debris and precipitator were then removed by an equal volume of chloroform and centrifugation at 3,000 × g for 15 min at 4°C.

4.2.3 Bacteriophage insensitive mutant isolation and confirmation

Bacteriophage-insensitive mutants were generated as previously described (Zhong et al., 2020). Briefly, a bacterial lawn was formed by overlaying molten agar (LB with 0.8% agar; Sigma-Aldrich, USA) inoculated with 100ul of a wildtype *E. coli* O157:H7 overnight culture onto a LB agar plate (Sigma-Aldrich, USA). After the molten agar was solidified, 100 µL of each 10-fold serial diluted phage AR1 lysate (10^{-1} to 10^{-9}) was dispensed onto individual bacterial lawns and incubated at 37 °C overnight. Colonies that appeared within the phage clearing after incubation were considered as potential BIMs. Phage resistance of these candidates was further confirmed by a spot test as described by Clokie et al. (2009). Ten microliters of high titre phage AR1 stock (10^{10} PFU/mL) was spot-inoculated on the bacterial lawn populated with each putative BIM. The resistance to phage AR1 was confirmed by the lack of plaques on the bacterial lawn.

4.2.4 Phage adsorption measurements

The adsorption curves of phage AR1 to the wildtype and each BIM were determined as described by Kropinski (2009). Briefly, a mid-logarithm phase culture of the target strain was diluted into LB broth supplemented with 10 mM calcium chloride to give OD_{600} of 0.3, followed by the addition of phage AR1 at a multiplicity of infection (MOI) of 0.01. At certain timepoints (1, 2, 4, 7 and 10 min), a sample was taken from the mixture and transferred into a prechilled tube saturated with chloroform. The titre of free phages in each collected aliquot was then enumerated using the double agar overlay method. A negative control with the absence of bacteria was used to determine the initial phage titre which was then normalized to 100%. Each assay was performed

in duplicate and repeated at least three times. Phage adsorption rate constants were calculated using the following equation (1) (Krueger, 1931):

$$k = \frac{2.3}{\mathrm{B}t}\log\frac{P_0}{P}, (1)$$

where k represents the adsorption rate constant (mL/min); B stands for the inoculated bacterial load; and t is the time (min) required for a phage titre change from initial P_0 to final P.

4.2.5 Whole genome sequencing and bioinformatic analysis

Genomic DNA of the wildtype *E. coli* O157:H7 and BIMs were extracted from LB broth cultures after 37 °C overnight incubation using the E-Z 96 Tissue DNA Kit (Omega Bio-tek, Norcross GA, USA) following the manufacturer's instructions. Approximately 500 ng of genomic DNA was extracted and mechanically fragmented for 40 s by ultra-sonicator Covaris M220 (Covaris, Woburn MA, USA) using the default settings. Libraries were synthesized using the NEBNext Ultra II DNA library prep kit for Illumina (New England Biolabs, Ipswich MA, USA) according to the manufacturer's instructions and were sequenced using the EcoGenomics Analysis Platform on an Illumina® MiSeq sequencer with TruSeq 300 bps paired-end libraries and 30 × coverage giving approximately 75 contigs per genome.

The raw reads of sequenced strains were *de novo* assembled using the A5-miseq pipeline (Tritt et al., 2012). The sequence of the wildtype *E. coli* O157:H7 920333 was mapped as a reference for alignment and comparison to the BIMs. Retrieved genomes were then annotated by the Rapid Annotation using Subsystem Technology (RAST) service (Aziz et al., 2008). Single nucleotide polymorphism (SNP) analysis was performed using the Geneious software package. Gene sequences were aligned using the online platform Clustal Omega Multiple Sequence Alignment and visualized by Jalview version 2.11.0 (Waterhouse et al., 2009; Madeira et al., 2019).

4.2.6 LPS isolation, silver stain and glycosyl composition analysis

The LPS of *E. coli* O157:H7 920333 (WT-O157) and BIM ZZb2 were prepared following the phenol-water extraction procedure (Westphal & Jann, 1965). Briefly, approximately 1 g of bacterial cells was collected and resuspended into molecular-grade water. The same volume of preheated 90% phenol was added to extract LPS at 70 °C for 20 min. The mixture was then cooled down in ice, followed by centrifugation at 5,000 × g at 4 °C to promote phase separation. After the upper (aqueous) phase of the mixture was directly collected, extraction was repeated three times by mixing the same volume of water with the lower (organic) phase. The crude LPS extracts were then dialyzed through a 14,000 MWCO membrane, freeze-dried, and washed with 90% ethanol at 4 °C. Residual nucleic acids and proteins were removed by DNase (Roche Diagnostics, Germany), RNase (Roche Diagnostics, Germany) and proteinase K (Roche Diagnostics, Germany) at 37 °C for 12 h, followed by a 12 h incubation with Benzoase at 37 °C with mild agitation. The digests were further dialyzed at 4 °C against exchanges of dH_2O before ultracentrifugation at 100,000 × g at 4 °C for 18 h. The recovered LPSs were collected for deoxycholic acid polyacrylamide gel electrophoresis (DOC-PAGE) and glycosyl composition analysis.

Extracted LPS samples were analyzed by DOC-PAGE using an 18% acrylamide gel with deoxycholic acid buffer (21.7 g glycine, 4.5 g Tris base, and 2.5 g deoxycholic acid per liter), and visualized with either silver stain only or alcian blue/silver stain, as demonstrated by Muszyński et al. (2011). Briefly, the LPS samples were mixed with Laemmli buffer (Bio-Rad, Hercules, CA, USA) to make a 0.5 mg/mL solution. The gel was first equilibrated by pre-running at 30 mA current for 20 min at room temperature. Approximately 0.5-1μg of the LPS samples were added for PAGE separation using the same power settings and run until the front buffer reached the bottom of the gel. For silver staining, the gel was submerged overnight in a fixing solution (40%

ethanol w/v and 5% acetic acid v/v in dH_2O). For combined alcian blue/silver staining, the gel was submerged overnight in the same fixing solution supplemented with 0.005% alcian blue. Next, both gels were washed with water for 1 min to remove the fixing solution and then oxidized with a 0.7% sodium meta periodate solution for 10 min. After oxidation, the gels were washed five times with water and subsequently submerged in a 10% silver solution (Bio-Rad, USA) for 10 min before rinsing off the silver with water. Finally, the gels were treated with a developer solution (Bio-Rad, USA) until the LPS bands developed. The reaction was blocked with 5% glacial acetic.

In addition to visualization, LPS extracted from WT-O157 and ZZb2 were also analyzed by gas chromatography-mass spectrometry (GC-MS). The extracted LPSs were first depolymerized by incubating with 1 M HCl in methanol at 80 °C for 18 h as previously described (Santander et al., 2013). The monosaccharide methyl glycosides were then re-N-acetylated with acetic anhydride and pyridine in methanol (v/v/v = 1:1:1) at 80 °C for 30 min, followed by derivatization with Tri-Sil™ HTP Reagent (ThermoFisher, USA) at 80 °C for 30 min. The trimethylsilyl (TMS) derivatives were resolved on a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) interfaced to a 5975C mass spectrometry detector (Agilent Technologies, USA) using a Supelco Equity-1 fused silica capillary column (30 m x 0.25 mm I.D.). Temperature started at 80 °C for 2 min, then ramped to 140 °C at 20 °C/min with a 2-min hold, followed by a ramp at 2 °C/min to 200 °C and a final ramp at 30 °C/min to 250 °C, with a 5-min hold. This protocol was also used for the detection of unsaturated and hydroxylated fatty acids in LPSs (Bhat et al., 1994).

4.2.7 Minimum inhibitory concentration (MIC) assays

Minimum inhibitory concentration assays were conducted to compare the cell wall permeabilities of the wildtype strain and six BIMs to various compounds, i.e., kanamycin sulfate

(Sigma-Aldrich, USA), ampicillin (Sigma-Aldrich, USA), ethylenediaminetetraacetic acid (EDTA, ThermoFisher, USA), novobiocin (Sigma-Aldrich, USA), polymyxin B sulfate (ThermoFisher, USA), sodium cholate (ThermoFisher, USA) and SDS (ThermoFisher, USA), as previously described (Zhong et al., 2020). Briefly, MIC assays were carried out in culture tubes containing 5 mL of LB broth. Two-fold serial dilutions of kanamycin sulfate (from 64 to 1 μg/mL), ampicillin (from 64 to 1 μg/mL), novobiocin (from 200 to 1 μg/mL), polymyxin B sulfate (from 200 to 0.125 μg/mL), ethylenediaminetetraacetic acid (EDTA; from 200 to 1 mg/mL), sodium cholate sulfate (from 128 to 1 mg/mL) and SDS (from 200 to 1 mg/mL) were made in these tubes. Each tube was inoculated with 100 μL of overnight *E. coli* O157:H7 cultures and incubated with orbital shaking at 225 rpm at 37 °C for 18 h. A positive score was recorded if the culture was visibly turbid. The MIC of each isolate to a certain compound was determined based on the tube with the highest concentration and visibly clear appearance. Each trial was performed in duplicate and repeated in three individual experiments.

4.2.8 Sodium cholate permeability assays

The permeability of wildtype *E. coli* O157:H7 and the BIMs to sodium cholate were separately investigated by spread plating and bacterial cell enumeration. Briefly, bacterial isolates were freshly cultured in LB broth until OD_{600} reached 0.5 (approximately 10^8 CFU/mL). One hundred microliters of culture were used to inoculate 5 mL saline (0.85% NaCl) with and without 128 mg/mL sodium cholate. After mixing thoroughly, the inoculated saline solutions were serially diluted, followed by spread plating as the negative control, while the inoculated saline samples supplemented with sodium cholate were incubated at 37°C overnight before enumeration the next day. Three independent replicate experiments were performed.

4.2.9 Biofilm formation assays and phenotypic characterizations

Biofilm production was determined using the microtiter plate protocol described by Merritt et al. (2006). Briefly, bacterial isolates from -80°C stocks were freshly cultured in Luria-Bertani (LB; Sigma-Aldrich, USA) broth at 37°C for 18 h with orbital shaking at 250 rpm. Overnight cultures were then diluted at a ratio of 1:100 into fresh LB broth and modified LB with 1% NaCl. One hundred microliters of the diluted cultures were transferred into each well of 96-well microtiter plates, followed by incubation under various conditions. Wildtype *E. coli* B and its BIMs were incubated in modified LB broth with 1% NaCl at 37°C for 48 h, while *E. coli* O157:H7, and BIMs ZZb2 and ZZb4 were grown at 37, 22 and 12 ºC for 24, 48 and 120 h, respectively, in both LB with 1% NaCl and LB with no salt. Following the incubation, the liquid culture in the well was discarded and 100 µl of distilled water was dispensed into each well to gently remove any residual broth and planktonic cells. After rinsing the wells twice, 125 µl of 0.1% crystal violet (ThermoFisher, USA) solution was added into each well to stain the biofilm for 15 min, followed by removal from the wells before next step. The staining process was followed by another washing step as previously described to remove excessive crystal violet solution. Microtiter plates were left in the biosafety cabinet to dry overnight before solubilizing the crystal violet trapped in the biofilm with 200 µl of 30 % (v/v) acetic acid (ThermoFisher, USA). The absorbance of each well and the negative control with only acetic acid solution was measured at 600nm. The biofilm production for each bacterial strain was assessed in triplicate.

The production of biofilm from each bacterium was also phenotypically characterized using Calcofluor (fluorescent brightener 28). Bacterial isolates from frozen stocks were streak-plated on LB agar plates and incubated at 37°C for 24 h. A single colony of each strain was passaged and re-streaked onto a new LB plate supplemented with 200 µg/mL Calcofluor, followed

by incubation at 37 and 22 °C for 48 h, as well as at 12 ºC for 120 h. Bacteria grown on these plates were illuminated under a transilluminator model UVVIS-20 (Hoefer, Inc., Holliston, MA, USA) to measure fluorescence intensity, which indicates the exopolysaccharide production level (Bokranz et al., 2005) and phenotypically characterizes the biofilm formation as high, medium, low or none (Figure 4.8).

4.2.10 Statistical analysis

T-tests were used to detect differences in bacterial growth in the sodium cholate sensitivity assays and biofilm formation assays. Data were analyzed using Microsoft Excel and a difference was considered significant at $p < 0.05$.

4.3 Results

4.3.1 Isolation of bacteriophage insensitive *E. coli* O157:H7 mutants and adsorption assays

Four BIMs, i.e. ZZb1 (accession number JACBNR000000000), ZZb2 (accession number JACBNS000000000), ZZb3 (accession number JACBNT000000000) and ZZb4 (accession number JACBNU000000000) were isolated in the presence of 10^9 PFU of phage AR1 and confirmed for their resistance by spot tests. Phage attachments were evaluated in the adsorption assay (Figure 4.2). The rapid adsorption of AR1 to the WT-O157 was shown by the drastic decrease of free phages in the first minute, while recoveries of high numbers of free phages were consistent throughout all time points when phage AR1 were proliferated with the BIMs, suggesting the lack of phage attachment to these strains. Using the equation (1), the AR1 adsorption rate constant k of WT-O157 was 2.73×10^{-9} mL/min, in comparison to 1.38×10^{-10}, 2.78×10^{-10}, 1.73×10^{-10} and 9.26×10^{-11} mL/min of ZZb1, ZZb2, ZZb3 and ZZb4. The differences in adsorption rate constant demonstrate that phage would be at least 20 times less likely to attach to the BIMs

than to WT-O157. Together, these findings show that the isolated BIMs confer phage AR1 resistance through the mutations of components essential for phage adsorption.

4.3.2 Identification of genetic mutations contributing to the lack of phage adsorption

The lack of adsorption to the BIMs suggested that phage AR1 was not able to recognize and attach to the specific receptors OmpC and LPS on the bacterial surface. Using the WT-O157 sequence as reference, we compared the genes that are involved in the biosynthesis of these components and identified different mutations in the BIMs (Figure 4.3 and 4.4). For mutants ZZb1, ZZb3 and ZZb4, stop codons (TAA, TAG and TAA) were introduced to the respective *OmpC* sequence (Figure 4.3), causing nonsense mutations which prematurely terminate the OmpC translation in these three BIMs. Therefore, we argue that the failure of AR1 adsorption to these BIMs could be attributed to the *ompC* mutations which lead to either the complete lack of OmpC or altered OmpC to which AR1 cannot attach. On the other hand, a single point mutant (GAT to TAT) was found in ZZb2 gene *hldE*, causing an amino acid substitution (D18Y) in the HldE protein sequence (Figure 4.4). This bifunctional protein acts as a heptose 7-phosphate kinase and a heptose 1-phosphate adenyltransferase during the synthesis of ADP-L-glycero-β-D-manno-heptose in the LPS inner core (McArthur et al., 2005). Since these heptose residues in the inner core provide an essential base for extending the LPS core region, the mutation in *hldE* might therefore lead to severely shortened LPS molecules which do not have the terminal glucose for phage AR1 to bind.

4.3.3 Comparative LPS PAGE profiles and glycosyl composition analysis

To support the genetic finding that the *hldE* mutation of ZZb2 might lead to the production of heptoseless LPS, we compared the mass of LPS extracted from the WT-O157 and ZZb2 using DOC-PAGE and silver stain (Figure 4.6). In addition to one distinct band with a high molecular

weight (HMW), the LPS of WT-O157 developed a ladder pattern similar to the standard LPS of *Salmonella*, spreading across different molecular weights. In contrast, LPS extracted from ZZb2 only formed an intense and low molecular weight (LMW) band (line 3). These results indicate a severe change in the mass of ZZb2 LPS, possibly associated with the lack of O-chain polymer and even the core oligosaccharides.

The assumption that BIM ZZb2 carries a severely truncated LPS structure was further investigated in the comparative glycosyl composition analysis (Table 4.2 and Figure 4.7). In the glycosyl profile of WT-O157 LPS, all the main sugar components of the O-antigen (Glc, GalNAc, Rha4NAc and Fuc) and the core region (Hep, Glc, Gal and Kdo) were detected (Table 4.2). On the contrary, ZZb2 LPS only consisted of Kdo (77%) and GlcNAc (19.5%), as well as a trace amount of Man and Glc. The detected GlcNAc in both samples likely resulted from the conversion of GlcN in the lipid A backbone to GlcNAc during the re-N-acetylation step. Collectively, the absence of most sugar moieties in both O-antigen and the core region, as well as the significant increase in relative Kdo content confirmed that the biosynthesis or addition of heptose to the ZZb2 LPS inner core is missing. Together with the results from DOC-PAGE, we conclude that the lack of heptose in the ZZb2 LPS, due to the *hldE* mutation, aborts the extension of the remaining part of the core oligosaccharide and O-antigen repeats, thus conferring resistance to phage AR1, which uses terminal glucose in the outer core as a receptor.

In addition to glycosyl residues, the fatty acid profiles were also shown in the chromatograms (Figure 4.7). This comparative analysis indicated a similar fatty acid composition in both LPS samples. The major hydroxylated fatty acid of lipid A was (3-OH)14:0 and the main unsaturated fatty acid found were 14:0 and 16:0. The detections of small amounts of (3-OH)12:0,

(3-OH)13:0, 14:0 and 16:0 suggest a micro heterogeneity in the acylation of lipid A. However, the mole percentage of each fatty acid was not measured in this study.

4.3.4 Evaluation of bacterial membrane permeability

Both AR1 receptors OmpC and LPS on the outer membrane originally have indispensable roles for bacterial survival, i.e. mediating the exchange of nutrients required for sustaining growth and providing a physical barrier against the extracellular environment (Delcour, 2009). Phage-induced mutations in these elements may inevitably disrupt the outer membrane integrity and permeabilize the cell. Therefore, we measured the permeabilities of BIMs to various compounds, including kanamycin (aminoglycoside class antibiotic), ampicillin (penicillin class antibiotic), novobiocin (aminocoumarin class antibiotic), polymyxin B (polypeptide), EDTA (membrane permeabilizer), sodium cholate (bile salt) and SDS (food-grade surfactant), and compared them to that of the wildtype in the MIC assay (Table 4.3). Except for EDTA, WT-O157 and BIMs ZZb1 and ZZb3 showed similar permeabilities to most of the tested compounds. Meanwhile, the permeabilities of ZZb4 to ampicillin and kanamycin were two-fold higher than that of the parental strain. In contrast, BIM ZZb2 with the *hldE* mutation were more permeable than the WT-O157 to six out of seven tested compounds, especially to the food grade surfactant SDS. These results demonstrate that compared to the WT-O157, three BIMs with mutations in their OmpC only showed minor variations in membrane permeability, however, mutant ZZb2 with the *hldE* mutation was more susceptible to most tested substrates and showed hypersensitivity to SDS.

Since the different permeabilities to bile salt sodium cholate were not clearly distinguished among strains, an independent sodium cholate permeability assay was conducted (Figure 4.5). The recovered microbial load of all saline controls was consistently around 7 log total CFU, similar to the inoculation level. In the saline supplemented with sodium cholate, however, the microbial

loads of all BIMs were significantly reduced at least 0.6 log total CFU. In particular, BIM ZZb2 had a 2-log reduction, compared to the saline control, following incubation in sodium cholate (P<0.01). These findings indicate that BIMs with a disrupted membrane structure generally became more sensitive to sodium cholate than the wildtype and ZZb2 with abbreviated LPS became particularly susceptible to this bile salt in comparison to the wildtype and other three BIMs with OmpC mutation.

4.3.5 Biofilm formation assays and phenotypic characterizations

In addition to membrane permeability, phage-induced modifications in outer membrane structures may also affect the secretion of extracellular polymeric substances of phage-resistant *E. coli* mutants, and therefore affect biofilm development. In a preliminary study, we examined and compared the biofilm production of the parental strain *E. coli* B (WT-B) to its six BIMs (Figure 4.9), which lost the terminal glucose moieties in LPS in exchange for phage T4 resistance. All mutants showed significantly higher absorbance than the wildtype, suggesting that these BIMs might produce more biofilm than the WT-B.

Next, we compared the biofilm formation of wildtype *E. coli* O157:H7 to that of two BIMs ZZb2 and ZZb4, which possess phage resistance-associated modifications in HldE and OmpC, respectively (Figure 4.10 and 4.11). Generally, biofilms were produced by all strains in every tested condition and the productions were weaker when cells were propagated with NaCl. While BIM ZZb4 cultured in LB supplemented with NaCl showed either lower or similar OD_{600} readings to the wildtype, this mutant consistently displayed higher absorbance than the parental strain in the absence of NaCl, suggesting that the additional salt might have an impact on the biofilm formation of ZZb4. On the other hand, the absorbance readings of BIM ZZb2 were constantly lower than WT-O157 when grown at 12 and 22°C. When the temperature was elevated to 37°C,

however, the measured OD_{600} values of ZZb2 were significantly higher than the wildtype and ZZb4, indicating the unique biofilm forming capability of ZZb2 in this condition.

In addition, calcofluor characterizations also delineate the differences in biofilm development phenotypically (Table 4.4 and Figure 4.8). The WT-O157 grown on LB plates was characterized as having none to low fluorescence levels at all temperature and salinity conditions (Table 4.4). Mutant ZZb4 produced strongest fluorescent signals at 37°C and displayed similar fluorescence intensities on LB plates with and without NaCl supplement. On the contrary, the salinity of growth culture plays a significant role in the fluorescence intensity of ZZb2. When grown in LB plates supplemented with NaCl, this mutant only showed none to low fluorescence. However, ZZb2 produced medium to high fluorescence levels on LB plates without NaCl, despite the weak growth at 12 and 22 °C. Consistent to ZZb4, the fluorescence intensity of ZZb2 peaked when propagated at 37°C, implying that the growth temperature plays a role in biofilm formation of these mutants.

To sum up, six *E. coli* B BIMs with the same LPS truncation due to gene *waaG* mutations likely produced more biofilm at 37°C than the parental strain. All three strains WT-O157, ZZb2 and ZZb4 displayed none to low fluorescence intensities when cultured on LB plates supplemented with NaCl (Table 4.4), supportive to the relatively low absorbance readings reported in the biofilm formation assay (Figure 4.10). Consistently, when propagated in culture without NaCl at 37°C, BIM ZZb2 exhibited the highest absorbance reading (Figure 4.11) and fluorescence intensity (Table 4.4), collectively suggesting that this phage-resistant *E. coli* O157:H7 mutant, which bears a phage-induced severe LPS truncation, is a more robust biofilm former than the wildtype and BIMs with intact LPSs. Together with the *E. coli* B findings, we conclude that phage-induced LPS truncation increases the biofilm production of *E. coli* BIMs grown at 37°C.

4.4 Discussion

Formation of bacteriophage insensitive mutants can reduce the effectiveness of commercial phage preparation against foodborne pathogens like *E. coli* O157:H7. However, the physiological changes of these BIMs are largely unknown. In this work, we isolated four *E. coli* O157:H7 mutants insensitive to phage AR1 infection. These BIMs showed diverse alterations in essential membrane components (either OmpC or LPS) for AR1 attachment and therefore prevented the initial adsorption as shown in Figure 4.2. The identified *hldE* mutation of ZZb2 was further demonstrated by silver stain and GC-MS, suggesting that this mutant carries deeply truncated LPS that only contain Kdo and lipid A. As one of the resulting fitness changes, all BIMs experienced increased permeability to at least one tested antimicrobial compound. In particular, not only did ZZb2 show higher susceptibilities than the WT-O157 to most compounds, but it also had decreased MICs to novobiocin, sodium cholate and SDS as compared to other BIMs with OmpC mutations. Another phage resistance-associated fitness change of *E. coli* BIMs was illustrated by the biofilm formation assays. Our results showed that the biofilm production of *E. coli* O157:H7 BIMs were greatly influenced by environmental factors e.g. temperature and salinity, and agreed with the findings in previous studies (Beloin et al., 2008; Rossi et al., 2018). When grown at 37°C, *E. coli* BIMs with phage-induced LPS truncations consistently showed higher absorbance readings, suggesting higher biofilm production than the wildtypes.

Originally isolated from the manure of adult cows, the T4-like phage AR1 is known for its specificity to *E. coli* O157:H7 strains (Ronner & Cliver, 1990). Studies have thoroughly delineated phage AR1 morphology, genomic organization and phage receptors on the *E. coli* surface (S. Yu et al., 2000; Goodridge et al., 2003; Liao et al., 2011). The T4-like phages are a diverse group of phages belonging to the *Myoviridae* family that infect evolutionary distant

bacteria and share genetic homologies and morphological similarities with the well-studied archetypical phage of the group, T4 (Ackermann & Krisch, 1997). The T4-like phages possess relatively large dsDNA genomes that vary widely in size (~160–250 kb) and genetic composition. They contain host-like functions, including nucleotide metabolism and a DNA replisome. Due to their virulent lifestyle, they exhibit different evolutionary constraints than their bacterial hosts or temperate phages. In addition, the T4-encoded recombination machinery (Karam & Drake, 1994) may generate a high degree of evolutionary diversity, via both homologous and non-homologous recombination between this phage genome and that of their bacterial hosts or other phages. Therefore, the characteristics of the T4-like genome, its mechanism of replication, and the interactions with cellular hosts make the T4-like phages an attractive model for the generation of bacteriophage insensitive mutants and the study of their resulting phage resistance associated changes.

Mutations that caused defective OmpC biogenesis of BIMs ZZb1, ZZb3 and ZZb4 were responsible for the lack of phage AR1 adsorption to these mutants. The hollow β-barrel structure of OmpC consists of 16 antiparallel strands connecting by short turns on the periplasmic side and long loops on the extracellular ends. By comparing to the proposed *E. coli* OmpC structural model (Baslé et al., 2006), we noticed that the introduced stop codon in the OmpC sequences of both ZZb1 and ZZb3 was located near the sequence that encodes the loop 3 of OmpC. Unlike other loops extending on the extracellular side, the constriction loop 3, connecting strand 5 with strand 6, distinctly folds inwardly and constricts the channel at halfway the height (Baslé et al., 2006). In addition, a previous study by Washizaki et al. (2016) suggested that loop 4 is the binding site in OmpC for T4-like phages. Therefore, the premature termination of OmpC biosynthesis of ZZb1 and ZZb3 might lead to the absence of phage-binding site on loop 4, and thus conferring resistance

to phage AR1. On the other hand, the stop codon in ZZb4 *ompC* terminated the translation of β16 strand, suggesting that the resulting OmpC might likely still be functional but with conformational changes that conferred phage AR1 resistance.

In addition to OmpC, previous studies also illustrated that successful phage AR1 infection to *E. coli* O157:H7 also required the presence of terminal glucose in the LPS core region, as AR1 failed to infect both *waaJ* and *waaG E. coli* O157:H7 mutants (S. Yu et al., 2000; Goodridge et al., 2003). Mutant ZZb2 is a deep rough mutant with severely truncated LPSs through a single amino acid substitution in the protein HldE (D18Y). Three-dimensional protein structure assessment showed that the substituted tyrosine at position 18 overlaps with the solvent-accessible surface of HldE, suggesting that this radical replacement may alter the protein structure by causing steric hindrance (Supplemental Figure 4.1). Instead of the terminal glucose, BIM ZZb2 aborts the synthesis of heptose in the LPS inner core to confer resistance to phage AR1. This might be explained by the finding that the short tail fibers of T4-like phages can bind to the heptose of LPS for irreversible attachment (Riede, 1987). Together with the findings of the glycosyl composition assay, we conclude that ZZb2 carrying this mutated HldE produced heptoseless LPS in which the phage AR1-binding glucose is absent.

As demonstrated in the MIC assay (Table 4.3), the loss of the LPS core region significantly permeabilized ZZb2 to various compounds. *E. coli* outer membrane structure is maintained by the lateral force between LPS molecules. This crosslinking interaction is a result of the negatively charged phosphate groups in the core region and lipid A binding to divalent cations. Phage-induced truncations in LPS core region inevitably reduce these negatively charged phosphorylation groups, and thus permeabilizing the bacterial membrane due to the weakened lateral strength (Nikaido, 2003). As a result, mutant ZZb2 with deep LPS truncation displayed lowered MICs to most of the

tested substrates, especially to SDS. Similarly, the hypersensitivity to this food grade surfactant was observed in the *E. coli* B BIMs whose LPS were truncated upon phage T4 infection (Zhong et al., 2020). In addition, deep rough BIM ZZb2 also exhibited higher susceptibility to the bile salt sodium cholate. This finding agreed with a previous study showing that the lack of heptose in the LPS increased the bile sensitivity of phage resistant *E. coli* K-12 mutants (Wilkinson et al., 1972). Additional studies have demonstrated the relevance of bacterial LPS complexity in bile tolerance, illustrating that long extensive O-antigen chains increased bile resistance (Crawford et al., 2012), while the loss of O-antigen or LPS core truncations sensitized the bacteria to bile (Picken & Beacham, 1977; Gunn, 2000).

Secreted biofilm matrix plays different roles in bacterial survival, such as promoting colonization of special niches in the human body and protecting the embedded bacteria from desiccation, toxic molecules and recognition by the host immune system (Beloin et al., 2008). In biofilm formation assays at 37°C (Figures 4.9 to 4.11), all BIMs with LPS truncations consistently showed relative higher absorbance, suggesting better biofilm forming ability than their respective wildtypes. All of the six *E. coli* B BIMs, they produce LPS lacking terminal glucose in the outer core due to the T4 phage-induced *waaG* mutations. However, the putative enhanced biofilm formation of these *waaG* mutants is contradictory to the findings by Beloin et al. (2006). This group constructed an uropathogenic *E. coli* 536 strain *waaG* mutant, compared its biofilm formation in microfermentors to that of the wildtype *E. coli* 536 strain and reported that the *waaG* mutant displayed normal bacterial growth *in vitro* but completely abolished biofilm secretion. This contrast could be a consequence of using different *E. coli* strains, growth methods and methods for biofilm measurements. It is not advisable to compare the biofilm formation data obtained from two different methods, given that each biofilm quantification method has its advantages and

inherent limitations (Azeredo et al., 2017). For example, the crystal violet assay used in the current study is commonly used for biofilm quantification, but the relationship between absorbance readings and the actual biomass of the biofilm has not been established yet. Therefore, the slightly higher absorbance readings of *E. coli* B BIMs could only suggest that these mutants have more tendency to produce biofilm than the WT-B.

When grown at 37°C, however, the differences in absorbance readings between WT-O157 and LPS-truncated BIM ZZb2 were much more significant. This association of increased biofilm secretion and HldE mutation was previously shown by Nakao et al. (2012). Among a series of *E. coli* LPS mutants, a HldE mutant exhibited a similar phenotype as a deep rough *waaC* mutant, showing stronger surface hydrophobicity, increased auto-aggregation and enhanced biofilm formation. They also showed that the enhanced biofilm development of HldE mutant is dependent on the accumulation of extracellular DNA that escaped the cytoplasm through the leaky outer membrane. Together, these results show that T4-like phage treatment against *E. coli* O157:H7 mutant select for BIMs with severely truncated LPS and enhanced biofilm production.

In conclusion, phage AR1 insensitive mutants appeared by the modifications of either OmpC or LPS. These modifications directly led to membrane disruption and sensitized the cells to various extents. All *E. coli* mutants with LPS truncation showed higher biofilm forming potential, suggesting a fitness change that might affect bacterial virulence and needs to be considered in the design of phage-based biocontrol agents.

Table 4.1: Wildtype *Escherichia coli* strains and their derived bacteriophage insensitive mutants used in this chapter

Strain	Phage resistance	Gene involved in phage resistance	Affected receptor	Source
Wildtype *E. coli* O157:H7 920333 (WT-O157)				Personal strain collection of Dr. Lawrence Goodridge
E. coli O157:H7 BIM ZZb1	AR1	*ompC*	OmpC	This study
E. coli O157:H7 BIM ZZb2	AR1	*hldE*	LPS	This study
E. coli O157:H7 BIM ZZb3	AR1	*ompC*	OmpC	This study
E. coli O157:H7 BIM ZZb4	AR1	*ompC*	OmpC	This study
Wildtype *E. coli* B ATCC11303 (WT-B)				Purchased from America Type Culture Collection
E. coli B BIM ZZa0	T4	*waaG*	LPS	Zhong et al. (2020)
E. coli B BIM ZZa1	T4	*waaG*	LPS	Zhong et al. (2020)
E. coli B BIM ZZa2	T4	*waaG*	LPS	Zhong et al. (2020)
E. coli B BIM ZZa3	T4	*waaG*	LPS	Zhong et al. (2020)
E. coli B BIM ZZa4	T4	*waaG*	LPS	Zhong et al. (2020)
E. coli B BIM ZZa5	T4	*waaG*	LPS	Zhong et al. (2020)

Table 4.2: Glycosyl composition of LPSs isolated from wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutant ZZb2

Strain	Glycosyl Residue [mol %]								
	Fuc[1]	Rha4NAc	Man	Gal	Glc	Hep	Kdo	GalNAc	GlcNAc[2]
WT-O157	18.1	6.2	n.d.	1.3	49.5	7.1	4.0	5.4	8.3
ZZb2	n.d.	n.d.	1.3	n.d.	2.3	n.d.	77.0	n.d.	19.5

1. The abbreviations are as follow: Rha4NAc, N-Acetylperosamine (4-amino-4,6-dideoxy-α-D-mannopyranose); Kdo, 3-Deoxy-D-manno-oct-2-ulosonic acid; Rib, Ribose; Rha, Rhamnose; Fuc, Fucose; Man, Mannose; Gal, Galactose; Glc, Glucose; Hep, mannoheptose; GalNAc, N-acetyl-galactosamine; GlcNAc, N- acetyl-glucosamine; n.d., non-detected. The LPS of *E. coli* O157:H7 contains both N-acetyl glucosamine (in the O-chain) and glucosamine (in the lipid A).
2. The applied derivatization method specifically re-N-acetylation converts GlcN to GlcNAc. Therefore, GlcNAc likely represents the GlcN in the lipid A.

Table 4.3: Minimum inhibitory concentration assays for wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutants

Compounds	WT-O157	ZZb1	ZZb2	ZZb3	ZZb4
Mutation		OmpC	HldE	OmpC	OmpC
Ampicillin (ug/ml)	4	4	2	4	2
Kanamycin (ug/ml)	32	32	16	32	16
Novobiocin (ug/ml)	100	100	25	100	100
Polymyxin B (µg/mL)	0.125	0.125	0.125	0.125	0.125
EDTA (mg/ml)	25	12.5	12.5	12.5	12.5
Sodium cholate (mg/ml)	>128	>128	128	>128	>128
SDS (mg/ml)	>200	>200	0.125	>200	>200

Table 4.4: Bacterial fluorescence intensity on LB plates supplemented with calcofluor

Strain	1% NaCl			No NaCl		
	12ºC	22ºC	37ºC	12ºC	22ºC	37ºC
WT-O157	-/-/+[1]	-/-/+	-/-/+	-/-/+	-/-/+	-/-/+
ZZb2	-/-/+[2]	(-/+/+)	+/+/+	(++/++/++)	(+++/+++/+++)	+++/+++/+++
ZZb4	+/+/++	+/+/+	+/++/++	-/+/+	-/-/+	++/++/++

[1]. The levels of intensity are indicated as none (-), low (+), medium (++) and high (+++). The results of three independent replicates are separated by a slash (/).
[2]. Parentheses indicate strains with poor bacterial growth.

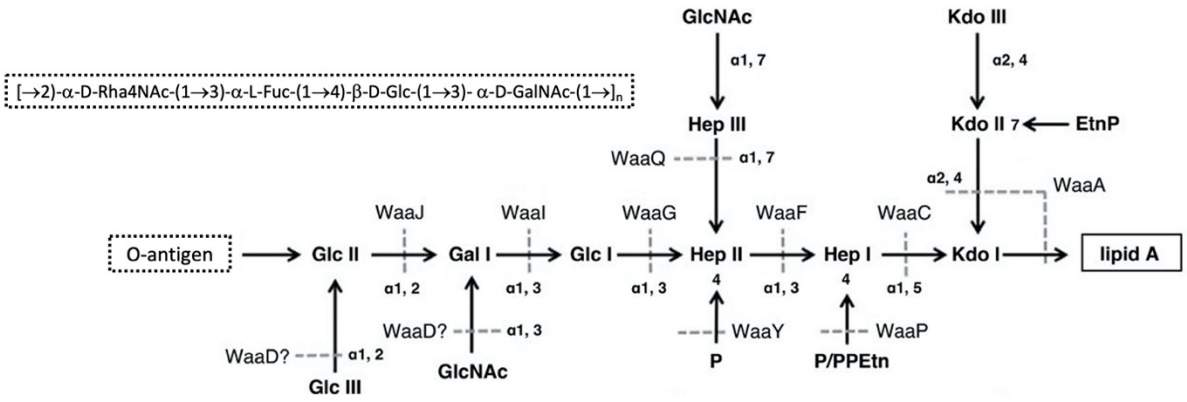

Figure 4.1. Complete lipopolysaccharide structure of wildtype *E. coli* O157:H7 (WT-O157). The O-antigen is boxed with dot lines and its sugar moieties are listed above. Horizontal and vertical arrows indicate the main backbone and branches, respectively. Dash lines represent the reactions catalyzed by glycosyltransferases in waa operon. The abbreviations are as follows: Rha4NAc, N-Acetylperosamine (4-amino-4,6-dideoxy-α-D-mannopyranose); Fuc, fucose; Glc, glucose; GalNAc, N-acetyl-galactosamine; Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; P, phosphate; P/PPEtn, phosphate or 2- aminoethyl diphosphate; and EtnP, ethanolamine phosphate. This figure was adapted from the publication of Washizaki et al. (Washizaki et al., 2016).

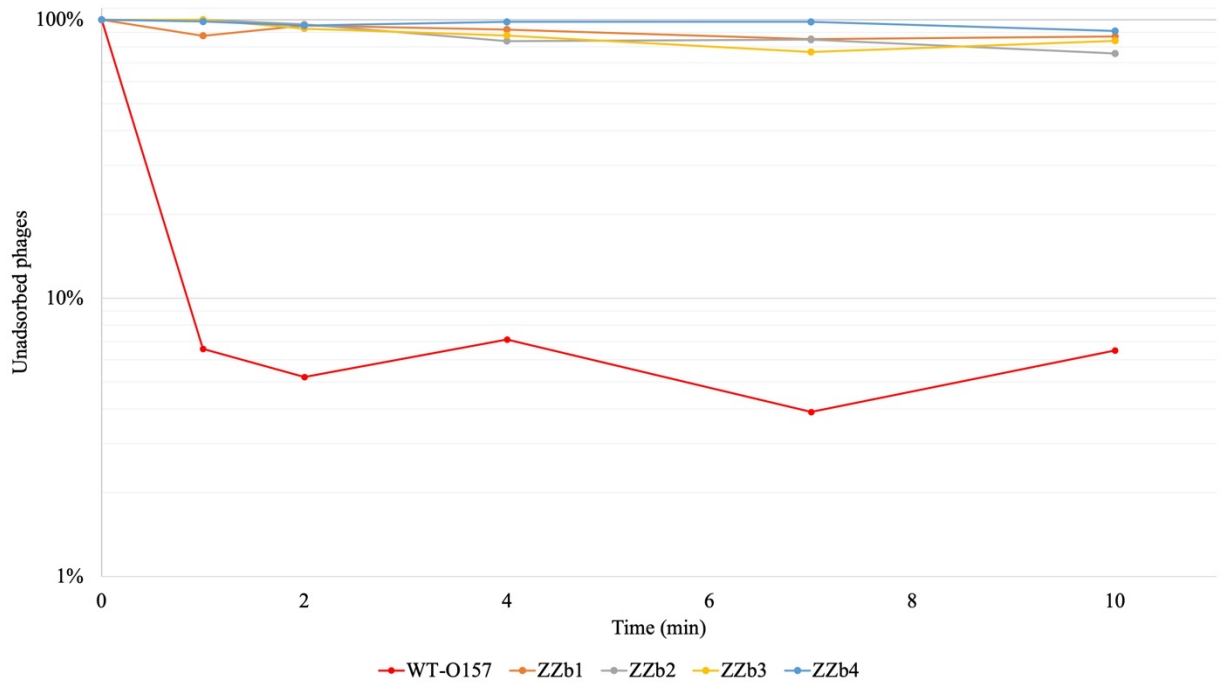

Figure 4.2. Adsorption kinetics of phage AR1 to wildtype *Escherichia coli* O157:H7 (WT-O157) and four isolated bacteriophage insensitive mutants, shown as free phage percentage. Each data point was generated using the average results from three independent experiments.

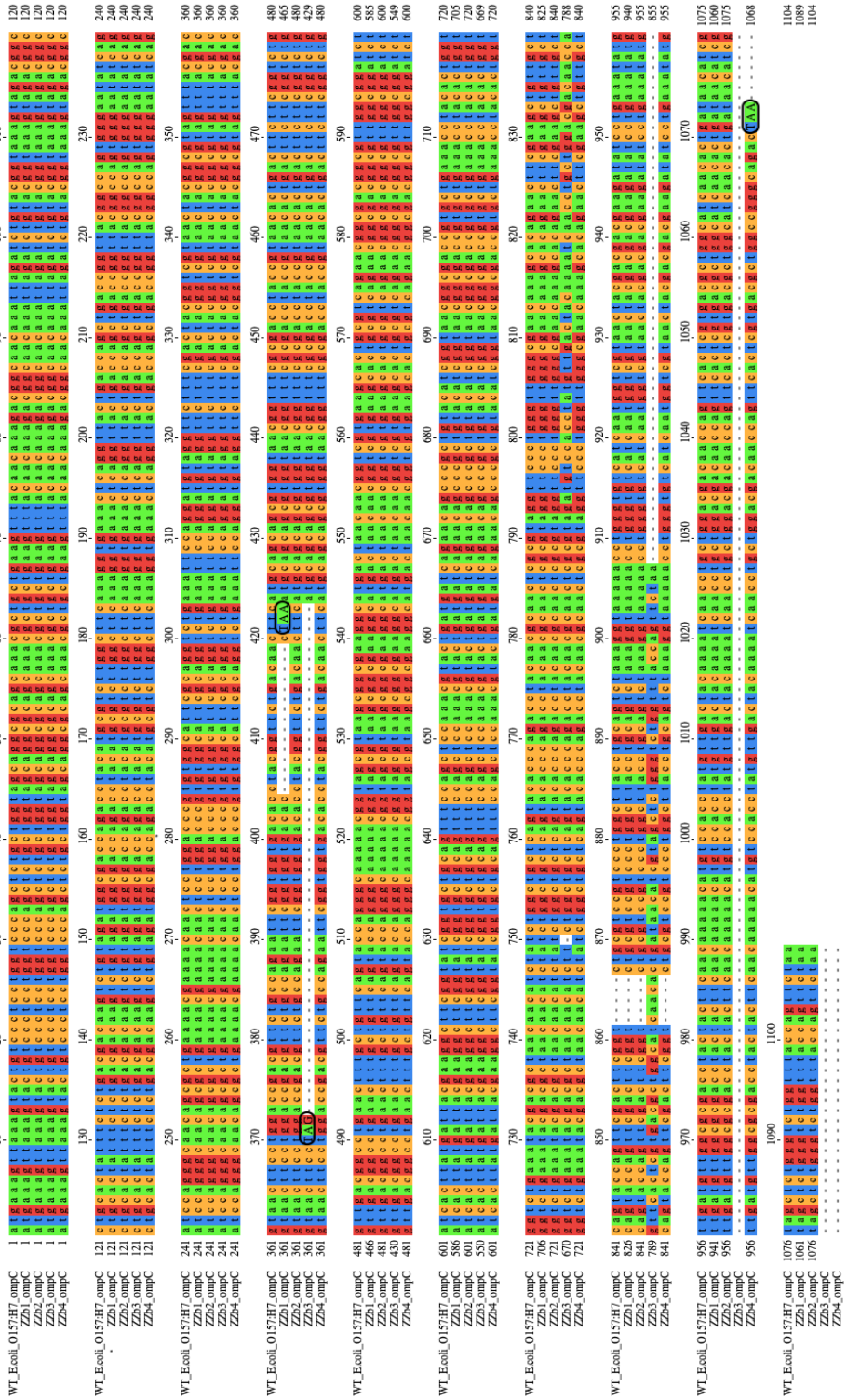

Figure 4.3. Nucleic acid sequences of OmpC of wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutants. Nonsense mutation introduced in mutants ZZb1, ZZb3 and ZZb4 are highlighted with a black box. Sequences were aligned using Clustal Omega and visualized by Jalview. Nucleotides are colored as green (adenine), blue (thymine), red (guanine) and orange (cytosine). Gap is indicated by a dash.

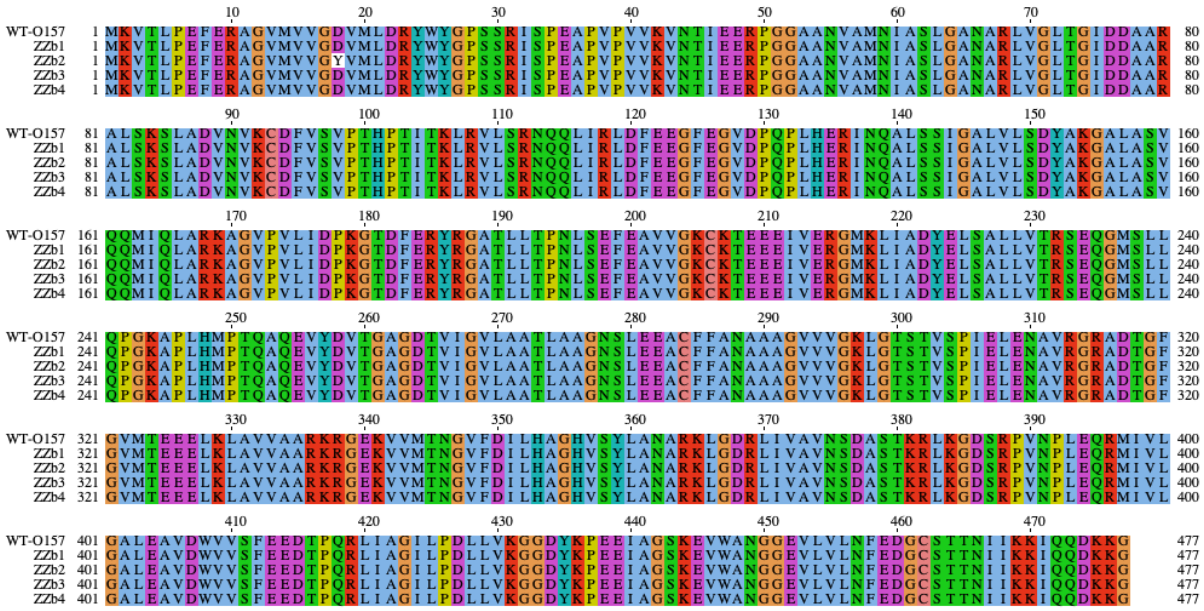

Figure 4.4. Amino acid sequences of HldE of wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutants. One amino acid substitution (D18Y) was identified in the sequence of mutant ZZb2. Sequences were aligned using Clustal Omega and visualized by Jalview. Amino acids were colored by the Clustal X scheme (orange: G; yellow: P; pink: C; red: K and R; magenta: E and D; cyan: H and Y; green: S, T, N and Q; blue: A, I, L, M, F, W and V). Gap is indicated by a dash.

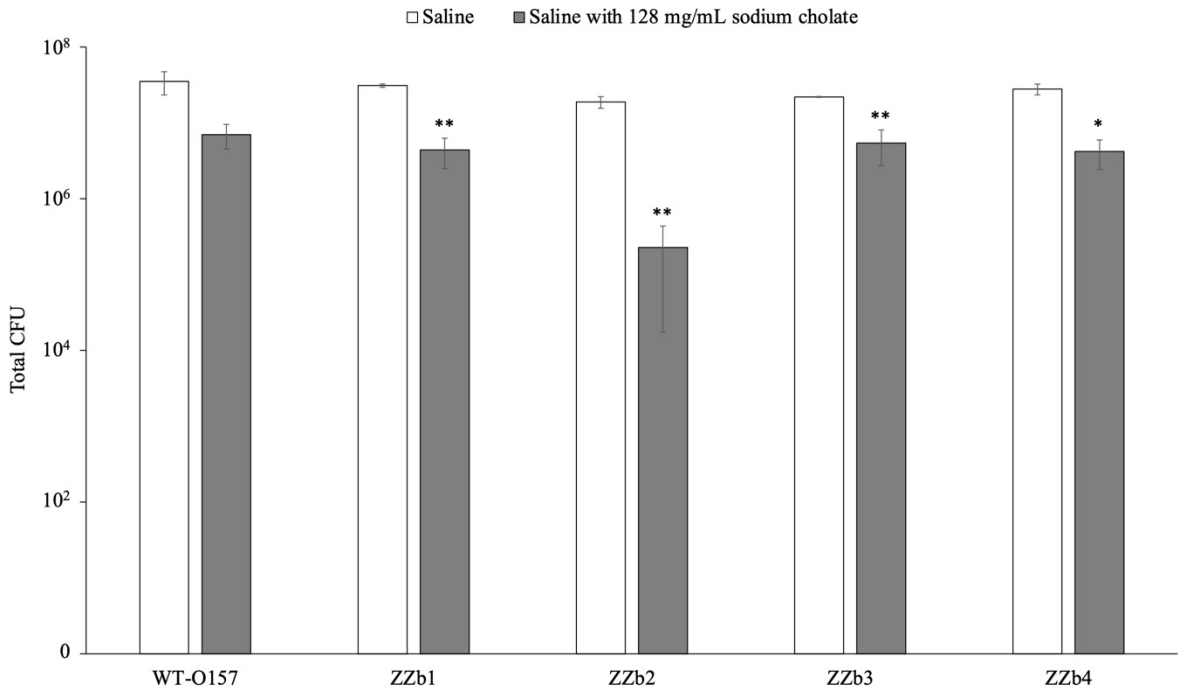

Figure 4.5. Comparative sensitivity assay of wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutants to sodium cholate. The mean values of three replicate trials are showed with error bars representing standard deviation. Statistical analysis was performed using t-test. *P<0.05 and **P<0.01, against the microbial loads in saline without sodium cholate.

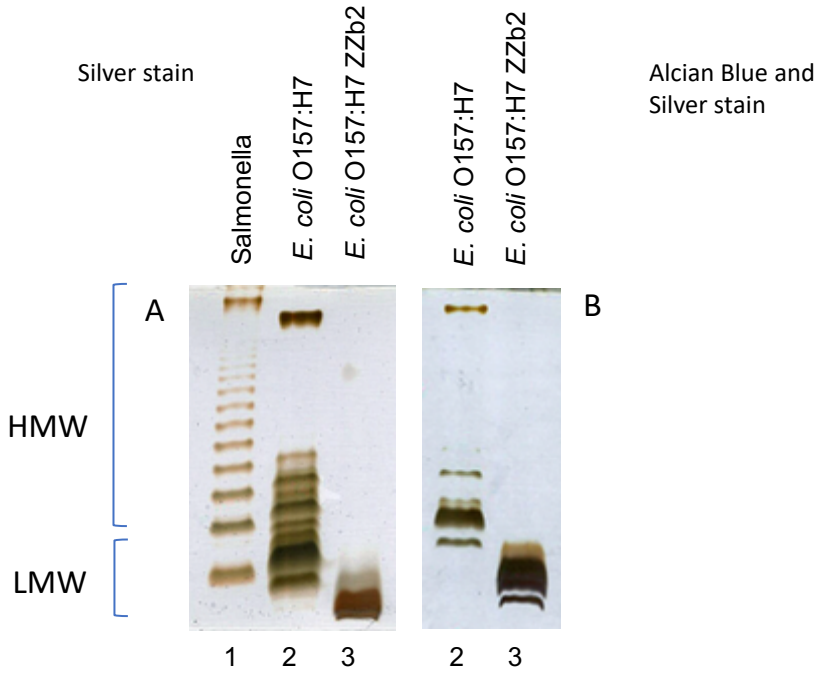

Figure 4.6. Visualizations of LPS from wildtype *Escherichia coli* O157:H7 (line 2) and bacteriophage insensitive mutant ZZb2 (line 3), and the standard LPS from *Salmonella enterica* serotype *Typhimurium* (line 1). The samples were stained with silver (Panel A) and with the combination of alcian blue and silver (Panel B). HMW, high molecular weight; LMW, low molecular weight.

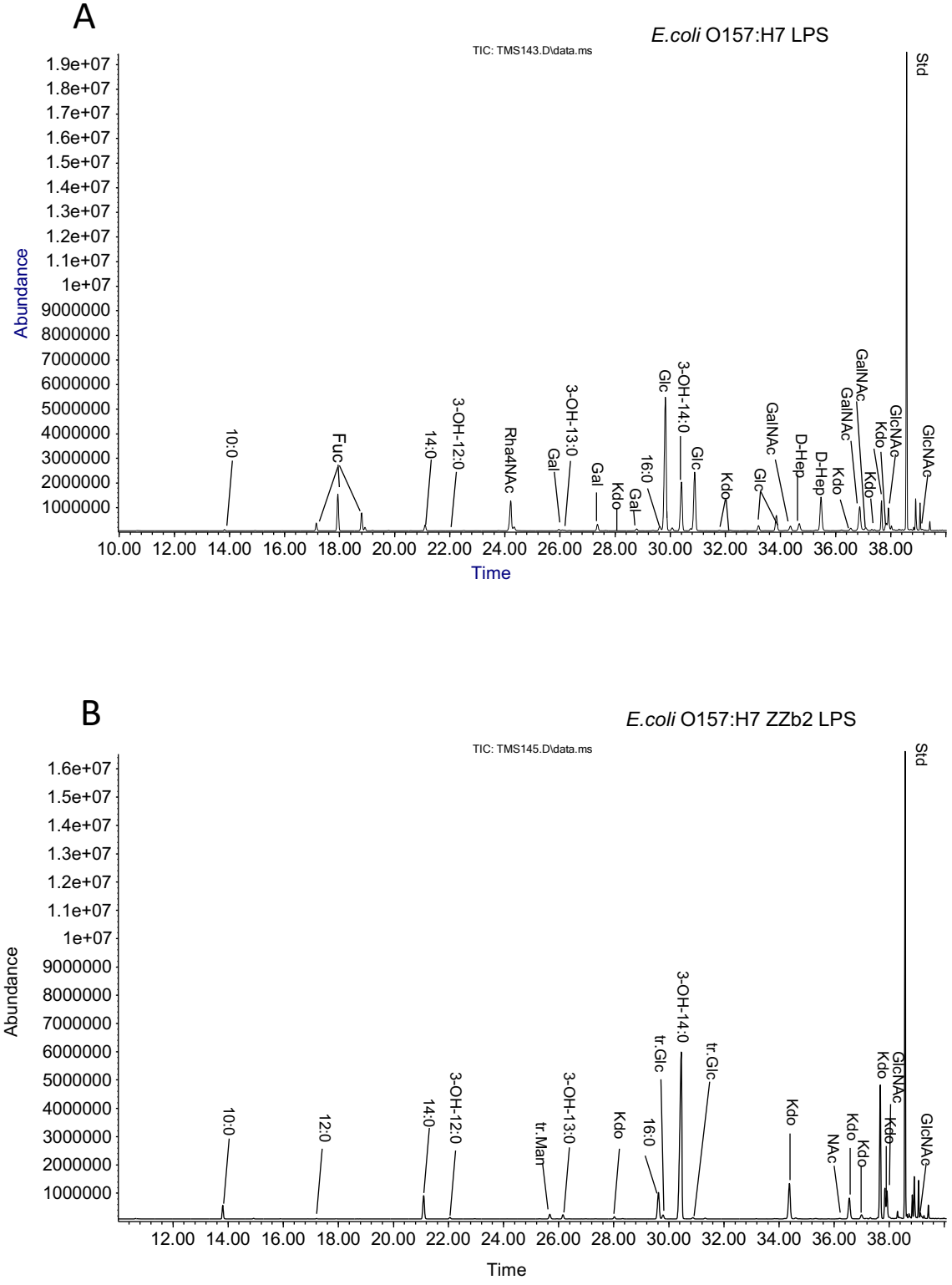

Figure 4.7. Glycosyl and fatty acid composition analysis of LPS isolated from wildtype *Escherichia coli* O157:H7 (A), and bacteriophage insensitive mutant ZZb2 (B).

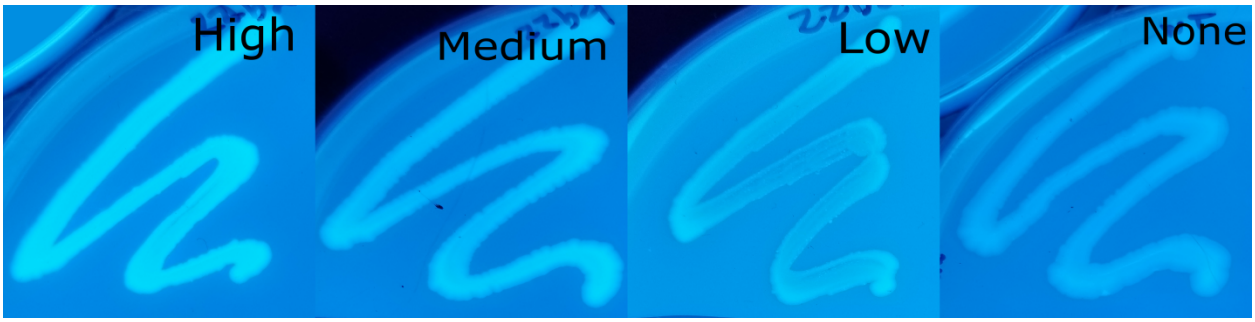

Figure 4.8. Representative images of high, medium, low and none levels of fluorescence in bacterial streaks on LB media plates supplemented with calcofluor.

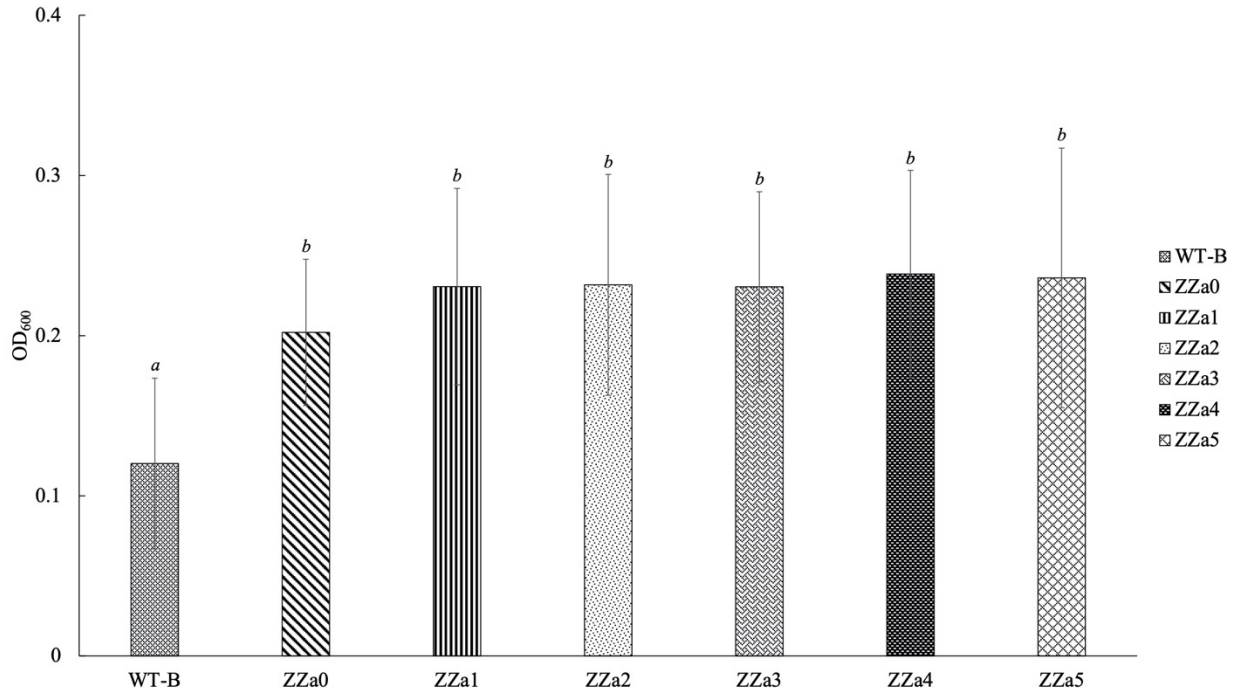

Figure 4.9. Biofilm formation of wildtype *Escherichia coli* B (WT-B) and six derived BIMs after incubation in modified LB broth with 1 % NaCl at 37°C for 48 h. The means and standard deviations of at least three independent replicates are shown. Values labeled with different letters differ significantly according to t-test (*P*-value <0.01).

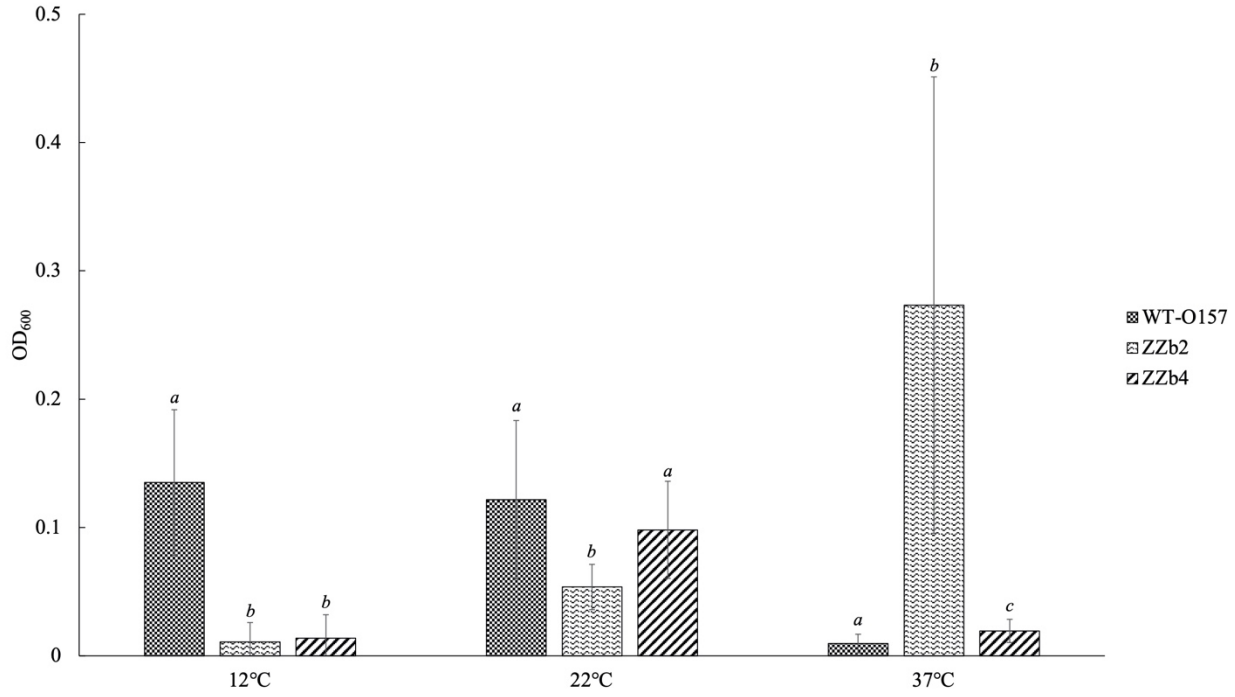

Figure 4.10. Biofilm formation of wildtype *E. coli* O157:H7 (WT-O157), bacteriophage insensitive mutants ZZb2 and ZZb4 after incubation in modified LB broth with 1 % NaCl at 12, 22 and 37°C for 120, 48 and 24 h, respectively. The means and standard deviations of at least three independent replicates are shown. Values labeled with different letters differ significantly according to t-test (*P*-value <0.01).

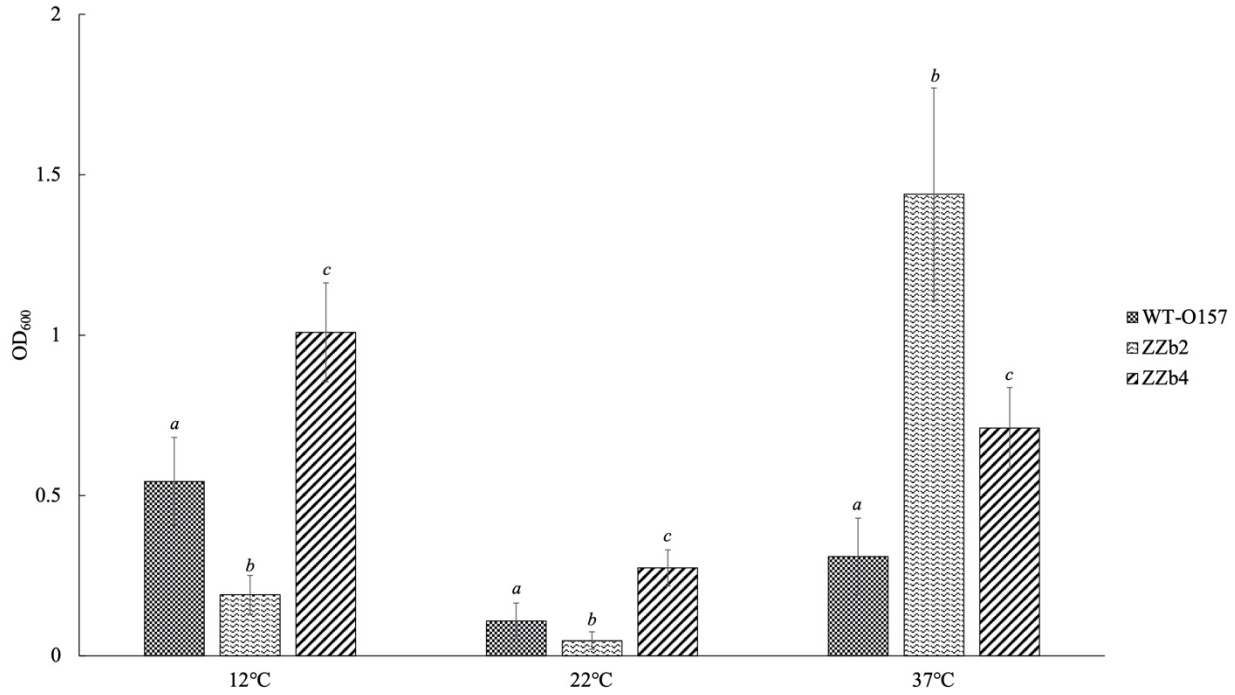

Figure 4.11. Biofilm formation of wildtype *E. coli* O157:H7 (WT-O157), bacteriophage insensitive mutants ZZb2 and ZZb4 after incubation in LB broth at 12, 22 and 37°C for 120, 48 and 24 h, respectively. The means and standard deviations of at least three independent replicates are shown. Values labeled with different letters differ significantly according to t-test (P-value <0.01).

4.5 References

Abedon, S. T., Kuhl, S. J., Blasdel, B. G., & Kutter, E. M. (2011). Phage treatment of human infections. *Bacteriophage, 1*(2), 66-85. doi:10.4161/bact.1.2.15845

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., Desvaux, M., Di Bonaventura, G., Hébraud, M., & Jaglic, Z. (2017). Critical review on biofilm methods. *Critical reviews in microbiology, 43*(3), 313-351.

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., & Kubal, M. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC genomics, 9*(1), 75.

Baslé, A., Rummel, G., Storici, P., Rosenbusch, J. P., & Schirmer, T. (2006). Crystal structure of osmoporin OmpC from *E. coli* at 2.0 Å. *Journal of molecular biology, 362*(5), 933-942.

Beloin, C., Michaelis, K., Lindner, K., Landini, P., Hacker, J., Ghigo, J.-M., & Dobrindt, U. (2006). The transcriptional antiterminator RfaH represses biofilm formation in *Escherichia coli*. *Journal of bacteriology, 188*(4), 1316-1331.

Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In *Bacterial Biofilms* (pp. 249-289): Springer-Verlag Berlin Heidelberg. Berlin, Germany.

Bhat, U. R., Forsberg, L. S., & Carlson, R. W. (1994). Structure of Lipid A Component of *Rhizobium leguminosarum* bv. *phaseoli* Lipopolysaccharide. . *J Biol Chem, 269*(20), 14402-14410.

Bokranz, W., Wang, X., Tschäpe, H., & Römling, U. (2005). Expression of cellulose and curli fimbriae by *Escherichia coli* isolated from the gastrointestinal tract. *Journal of Medical Microbiology, 54*(12), 1171-1182.

Carter, C. D., Parks, A., Abuladze, T., Li, M., Woolston, J., Magnone, J., Senecal, A., Kropinski, A. M., & Sulakvelidze, A. J. B. (2012). Bacteriophage cocktail significantly reduces *Escherichia coli* O157: H7 contamination of lettuce and beef, but does not protect against recontamination. *2*(3), 178-185.

Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas aeruginosa*. *Sci Rep, 6*, 26717. doi:10.1038/srep26717

Clokie, M. R., Kropinski, A. M., & Lavigne, R. (2009). *Bacteriophages*: Springer. Berlin, Germany.

Crawford, R. W., Keestra, A. M., Winter, S. E., Xavier, M. N., Tsolis, R. M., Tolstikov, V., & Bäumler, A. J. (2012). Very long O-antigen chains enhance fitness during *Salmonella*-induced colitis by increasing bile resistance. *PLoS Pathog, 8*(9), e1002918.

Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta, 1794*(5), 808-816. doi:10.1016/j.bbapap.2008.11.005

Filippov, A. A., Sergueev, K. V., He, Y., Huang, X. Z., Gnade, B. T., Mueller, A. J., Fernandez-Prada, C. M., & Nikolich, M. P. (2011). Bacteriophage-resistant mutants in *Yersinia pestis*: identification of phage receptors and attenuation for mice. *PLoS One, 6*(9), e25486. doi:10.1371/journal.pone.0025486

Fong, K., Mu, K., Rheault, J.-G., Levesque, R. C., Kitts, D. D., Delaquis, P., Goodridge, L., & Wang, S. (2020). Bacteriophage-Insensitive Mutants of Antimicrobial-Resistant *Salmonella* Enterica are Altered in their Tetracycline Resistance and Virulence in Caco-2 Intestinal Cells. *International journal of molecular sciences, 21*(5), 1883.

Goodridge, L., & Abedon, S. T. (2003). Bacteriophage biocontrol and bioprocessing: application of phage therapy to industry. *SIM news, 53*(6), 254-262.
Goodridge, L., Gallaccio, A., & Griffiths, M. W. (2003). Morphological, host range, and genetic characterization of two coliphages. *Appl Environ Microbiol, 69*(9), 5364-5371. doi:10.1128/aem.69.9.5364-5371.2003
Greer, G. G. (2005). Bacteriophage Control of Foodborne Bacteria. *Journal of Food Protection, 68*(5), 1102-1111.
Gunn, J. S. (2000). Mechanisms of bacterial resistance and response to bile. *Microbes and infection, 2*(8), 907-913.
Hosseinidoust, Z., Tufenkji, N., & van de Ven, T. G. (2013). Formation of biofilms under phage predation: considerations concerning a biofilm increase. *Biofouling, 29*(4), 457-468.
Kropinski, A. M. (2009). Measurement of the Rate of Attachment of Bacteriophage to Cells. In *Bacteriophages* (pp. 151-155): Springer. Berlin, Germany.
Krueger, A. P. (1931). The Sorption of Bacteriophage by Living and Dead Susceptible Bacteria. *J Gen Physiol, 14*(4), 493-516. doi:10.1085/jgp.14.4.493
Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat Rev Microbiol, 8*(5), 317-327. doi:10.1038/nrmicro2315
Lacqua, A., Wanner, O., Colangelo, T., Martinotti, M. G., & Landini, P. (2006). Emergence of biofilm-forming subpopulations upon exposure of *Escherichia coli* to environmental bacteriophages. *Applied and environmental microbiology, 72*(1), 956-959.
Liao, W. C., Ng, W. V., Lin, I. H., Syu, W. J., Liu, T. T., & Chang, C. H. (2011). T4-Like genome organization of the *Escherichia coli* O157:H7 lytic phage AR1. *J Virol, 85*(13), 6567-6578. doi:10.1128/JVI.02378-10
Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. *Nucleic Acids Res, 47*(W1), W636-W641. doi:10.1093/nar/gkz268
McArthur, F., Andersson, C. E., Loutet, S., Mowbray, S. L., & Valvano, M. A. (2005). Functional analysis of the glycero-manno-heptose 7-phosphate kinase domain from the bifunctional HldE protein, which is involved in ADP-L-glycero-D-manno-heptose biosynthesis. *Journal of bacteriology, 187*(15), 5292-5300.
Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2006). Growing and analyzing static biofilms. *Current protocols in microbiology*, 1B. 1.1-1B. 1.17.
Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. *Viruses, 10*(4), 205. doi:10.3390/v10040205
Muszyński, A., Laus, M., Kijne, J. W., & Carlson, R. W. (2011). Structures of the lipopolysaccharides from Rhizobium leguminosarum RBL5523 and its UDP-glucose dehydrogenase mutant (exo5). *Glycobiology, 21*(1), 55-68.
Nakao, R., Ramstedt, M., Wai, S. N., & Uhlin, B. E. (2012). Enhanced biofilm formation by *Escherichia coli* LPS mutants defective in Hep biosynthesis. *PLoS One, 7*(12), e51241. doi:10.1371/journal.pone.0051241
Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol Mol Biol Rev, 67*(4), 593-656. doi:10.1128/mmbr.67.4.593-656.2003
O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Appl Environ Microbiol, 70*(6), 3417-3424. doi:10.1128/AEM.70.6.3417-3424.2004

Perera, M. N., Abuladze, T., Li, M., Woolston, J., & Sulakvelidze, A. (2015). Bacteriophage cocktail significantly reduces or eliminates Listeria monocytogenes contamination on lettuce, apples, cheese, smoked salmon and frozen foods. *Food microbiology, 52*, 42-48.

Picken, R., & Beacham, I. (1977). Bacteriophage-resistant mutants of *Escherichia coli*K12. Location of receptors within the lipopolysaccharide. *Microbiology, 102*(2), 305-318.

Ronner, A. B., & Cliver, D. O. (1990). Isolation and Characterization of a Coliphage Specific for *Escherichia coli* 0157: H7. *Journal of Food Protection, 53*(11), 944-947.

Rossi, E., Cimdins, A., Lüthje, P., Brauner, A., Sjöling, Å., Landini, P., & Römling, U. (2018). "It'sa gut feeling"–*Escherichia coli* biofilm formation in the gastrointestinal tract environment. *Critical reviews in microbiology, 44*(1), 1-30.

Santander, J., Martin, T., Loh, A., Pohlenz, C., Gatlin III, D. M., & Curtiss III, R. (2013). Mechanisms of intrinsic resistance to antimicrobial peptides of Edwardsiella ictaluri and its influence on fish gut inflammation and virulence. *Microbiology, 159*(Pt 7), 1471.

Sharma, M., Patel, J. R., Conway, W. S., Ferguson, S., & Sulakvelidze, A. (2009). Effectiveness of bacteriophages in reducing *Escherichia coli* O157: H7 on fresh-cut cantaloupes and lettuce. *Journal of Food Protection, 72*(7), 1481-1485.

Tritt, A., Eisen, J. A., Facciotti, M. T., & Darling, A. E. (2012). An Integrated Pipeline for *de novo* Assembly of Microbial Genomes. *PLoS One, 7*(9), e42304. doi:10.1371/journal.pone.0042304

Viazis, S., Akhtar, M., Feirtag, J., Brabban, A., & Diez-Gonzalez, F. (2011a). Isolation and characterization of lytic bacteriophages against enterohaemorrhagic *Escherichia coli*. *Journal of applied microbiology, 110*(5), 1323-1331.

Viazis, S., Akhtar, M., Feirtag, J., & Diez-Gonzalez, F. (2011b). Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. *Food Microbiol, 28*(1), 149-157. doi:10.1016/j.fm.2010.09.009

Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the Interactions between *Escherichia coli* Receptors, LPS and OmpC, and Bacteriophage T4 Long Tail Fibers. *Microbiologyopen, 5*(6), 1003-1015. doi:10.1002/mbo3.384

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2—A Multiple Sequence Alignment Editor and Analysis Workbench. *Bioinformatics, 25*(9), 1189-1191. doi:10.1093/bioinformatics/btp033

Westphal, O., & Jann, K. (1965). Bacterial Lipopolysaccharides. Methods in Carbohydrate Chemistry. In: Academic Press, Cambridge, USA.

Wilkinson, R., Jun, P. G., & Stocker, B. (1972). Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. *Microbiology, 70*(3), 527-554.

Yu, S., Ko, K., Chen, C., Chang, Y., & Syu, W. (2000). Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1, Which Specifically Infects*Escherichia coli* O157: H7. *Journal of bacteriology, 182*(21), 5962-5968.

Zhong, Z., Emond-Rheault, J.-G., Bhandare, S., Lévesque, R., & Goodridge, L. (2020). Bacteriophage-Induced Lipopolysaccharide Mutations in *Escherichia coli* Lead to Hypersensitivity to Food Grade Surfactant Sodium Dodecyl Sulfate. *Antibiotics, 9*(9), 552.

Connecting statement

In the last two chapters, we studied phage resistant *E. coli* mutants and revealed that these BIMs developed phage resistance to T4-like phages by modifying their outer membrane components (LPS and OMPs) to which phages attach. These phage resistance-related alterations destabilized the bacterial membrane integrity and resulted in various fitness changes. At the cost of phage resistance, these changed physiological properties showed various impacts on bacterial survivability and they possibly affect the bacterial virulence in human gut.

In this chapter, we continued the comparison of physiological changes between the wildtypes and their respective BIMs using a high throughput phenotypic assay. The objective of Chapter V was to characterize and compare the metabolic profiles of the wildtypes and mutants, as well as crosslink to metabolic differences to KEGG pathways derived from the bacterial genomes.

Chapter V

Characterizing Metabolic Changes of Bacteriophage Insensitive *Escherichia coli* Mutants using a Pheno-genomic Approach

Abstract

In Gram-negative bacteria, bacteriophages attach to their hosts via protein and carbohydrate receptors on the outer membrane. Bacteria respond by mutating these receptors to avoid phage infection. These mutations may lead to changes in metabolizing various compounds. However, these phage resistance-induced metabolism changes of BIMs are still not fully understood. Herein, we characterized the metabolic features of three BIMs (ZZa3, ZZb2 and ZZb4) previously isolated from *E. coli* B (WT-B) and *E. coli* O157:H7 (WT-O157) by means of the BIOLOG Phenotypic Microarrays and compared them to that of their respective ancestral strains. The high-throughput phenotyping data were analyzed by an integrated genomic-phenomic program called DuctApe. The results showed that each BIM has varied metabolic changes as compared to their wildtype. *E. coli* B mutant ZZa3 became more sensitive to osmotic and pH variation but showed enhanced metabolism of carbon sources. Various compounds, including dipeptides, N-acetyl-neuraminic acid (Neu5Ac), D-glucose 6-phosphate and D-serine were differently used by the wildtype *E. coli* O157:H7 and both BIMs ZZb2 and ZZb4. Deep rough ZZb2 exhibited a great amount metabolic changes and uniquely showed elevated the tolerance to two common food preservatives sodium chloride (NaCl) and sodium lactate, and to acidic pressure. Overall, this study provided insights of the complex metabolic modifications that occur in phage resistant mutants and revealed their potential impacts on food safety.

5.1 Introduction

Many studies have demonstrated the variety of phage resistance-associated fitness changes that affect bacterial virulence (Smith & Huggins, 1982, 1983; Le et al., 2014), sensitivity to antibiotics (Chan et al., 2016; Fong et al., 2020; Zhong et al., 2020), biofilm formation (Nesper et al., 2001; Mills et al., 2010; X. Liu et al., 2017), and nutrient uptake (Charbit et al., 1988; Benz et al., 1992). However, the impact of phage resistance-associated membrane modifications on bacterial metabolic properties has yet to be studied. Bacterial metabolism refers to a substantial series of biochemical reactions that allow the cell to grow, function, replicate and survive in an appropriate environment by uptake and utilization of accessible compounds. These nutrients have to cross the bacterial membrane before being metabolized by the cell. Therefore, the phage-induced modifications of outer membrane components, which mediate the nutrient influx and provide a physical barrier for the cell, disrupt the membrane structure and likely change the bacterial metabolism of various substrates.

As one of the major contributors to global foodborne disease burden, pathogen *E. coli* O157:H7 strains are the target of numerous phage-based biocontrol agents (Kudva et al., 1999; O'Flynn et al., 2004; Lu & Breidt, 2015). *E. coli* O157:H7 BIMs that emerged from these applications may possess various phenotypic changes that affect their susceptibility or tolerance to compounds found within dynamic niches like foods. Using BIOLOG Phenotypic Microarrays (PM), we studied the overall metabolic profile of *E. coli* O157:H7 (WT-O157) and compared it to that of its derived BIMs ZZb2 and ZZb4, which have mutations in the LPS and OmpC, respectively. Parallelly, the metabolic discrepancies between a non-pathogenic *E. coli* B strain (WT-B) and its BIM ZZa3, which also has truncated LPS but different from that of ZZb2, were investigated. The metabolic profiles of these isolates were determined by the metabolic intensities of essential

nutrient sources (carbon, nitrogen, phosphorus and sulfur) under dissimilar growth conditions (osmolarity and pH) over a period of 72 h. Lastly, the phenotypic data were quantified and crosslinked to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (available from https://www.genome.jp/kegg/) derived from the bacterial whole genome sequences by means of an integrated genomic-phenomic platform called DuctApe. These findings elucidate the global dissimilarities of nutrient uptake and tolerance to environmental factors between *E. coli* wildtype and their T4-like BIMs, providing insights of bacterial metabolic adaptation to phage infection.

5.2 Materials and Methods

5.2.1 Bacterial strain and growth conditions

Wildtype *Escherichia coli* O157:H7 920333 (WT-O157), *Escherichia coli* B ATCC11303 (WT-B) and BIMs ZZa3, ZZb2 and ZZb4 were cultured at 37 °C for 18 h in Luria-Bertani broth (LB; Sigma-Aldrich, USA) in an orbital shaker at a speed of 225 rpm. The bacterial strains used in this study are listed in Table 5.1.

5.2.2 BIOLOG Phenotypic Microarray (PM) experiments

The BIOLOG PM assay, comprising a total of 960 unique tests (details of each well and its constituent can be found at: https://www.biolog.com/wp-content/uploads/2020/04/00A-042-Rev-C-Phenotype-MicroArrays-1-10-Plate-maps.pdf), was performed to determine the bacterial physiological fingerprints of the wildtypes and BIMs based on their metabolism of different organic substrates. Three *E. coli* O157:H7 strains (WT-O157, ZZb2 and ZZb4) in this study and two *E. coli* B strains (WT-B and ZZa3) described previously (Zhong et al., 2020) were tested to compare their cellular phenotypes using PM1 to PM10 microplates (Biolog, Inc., Hayward, California, USA).

The preparation of different inoculating fluids (IF-0 and IF-10) and Biolog Redox Dye mix A (100x) and inoculation of PM plates were conducted following the BIOLOG PM protocol for *E. coli* and other Gram-negative bacteria. All PM plates were inoculated with cell suspension of 100 µL per well. Bacterial strains were streaked from frozen stocks and propagated on fresh Tryptic Soy Agar (ThermoFisher, USA) plates overnight at 37°C. Cells were then sub-cultured onto a new plate and grown overnight for three to five times successively to stabilize the phenotype. Isolated colonies were transferred from the streaked plates using a sterile swab and suspend into a tube containing 16 ml of IF-0 solution to achieve a transmittance (T) of 42% in the BIOLOG turbidimeter. Fifteen ml of this cell suspension was diluted in 75 ml of IF-0 + dye mix to make 85% T. Twenty-two ml of this suspension was directly used to inoculate PM1 and PM2, which measure carbon utilization. Next, 145.2 ml of sodium pyruvate was added to 66 ml of the 85% T suspension (2.2 mg/ml) as a carbon source and then used to inoculate PM3 to PM8, which evaluate the metabolism of nitrogen, phosphorus and sulfur sources. Lastly, 600 µL of the 85% T suspension was further diluted into 120 ml of IF-10 before inoculating into PM9 and PM10, which represent different osmolytes and pH conditions. All PM plates were incubated at 37°C for 72 h in an OmniLog instrument during which time the metabolism activity in each well was monitored continuously based on the color development. This analysis was performed in duplicate for each strain.

5.2.3 KEGG database analysis of metabolic pathways

To compare the metabolic differences between the two WT strains and their derived BIMs, the duplicate output data from BIOLOG was averaged and analyzed using the DuctApe software suite version 0.18.2 (Galardini et al., 2014). Briefly, comparative genomic and phenotypic analysis of WT and derived BIMs were conducted using dgenome and dphenome modules, respectively.

First, the dgenome module maps the bacterial sequences to the KEGG database in an attempt to identify putative metabolic pathways in the genomes. The dphenome module computes an activity value (AV) indicating the metabolic intensity for each well of the PM plates and reports the significantly different metabolism (AV ≥ 2.5). Dphenome also generates an illustrative activity ring to depict the comparative metabolic profiles in six categories: carbon sources (PM1 and PM2), nitrogen sources (PM3), phosphorus and sulfur sources (PM4), nutrient supplements (PM5), nitrogen peptide sources (PM6, PM7 and PM8), osmolytes (PM9 and pH (PM10). Lastly, the dape module crosslinks the determining genome-derived KEGG metabolic pathways (Aoki-Kinoshita & Kanehisa, 2007) to the substrates that showed significant metabolic differences between tested strains, providing insight into the determinants of the observed phenotypic variability (cut-off: AV ≥ 2).

5.3 Results

5.3.1 Comparative metabolic profile analysis using BIOLOG Phenotypic Microarrays

5.3.1.1 Altered metabolic features of phage insensitive *E. coli* B mutant

In a previous study, we isolated a phage T4 resistant *E. coli* B mutant ZZa3 with a truncated LPS and destabilized outer membrane integrity, due to its mutation in gene *waaG* (Zhong et al., 2020). In comparison to its parental *E. coli* B strain, BIM ZZa3 showed lower percentages of active reactions (AV ≥ 3) in most PM plates (Figure 5.1), suggesting that this mutant has fewer active metabolism and is less adaptable under osmotic and pH stress than the wildtype. The largest difference was observed in PM7 where WT-B effectively metabolized 92 out of 96 nitrogen peptide sources (91%), while only 77% of these compounds were actively used by ZZa3. The reduced metabolic capabilities of this mutant compared to the wildtype were also reiterated in PM3 (50% vs 35%), PM4 (70% vs 57%), PM6 (91% vs 77%), PM8 (75% vs 65%), PM9 (40% vs 35%)

and PM10 (71% vs 56%). However, neither strains showed metabolic activities in PM5 and when both bacteria were exposed to the carbon sources in PM1 and PM2, mutant ZZa3 (72% and 9%, respectively) were more active in metabolizing these compounds as carbon sources than the parental strain (64% and 8%, respectively). Together, this comparative metabolic profiling indicates that except for carbon sources, *E. coli* B mutants experience decreased abilities in utilizing various substrates, possibly due to their disrupted membrane structures caused by the LPS truncation.

Next, the AVs of the 960 tested phenotypes of both strains were then presented and compared in two concentric activity ring plots (Figure 5.2 and Appendix Table S5.1). In Figure 5.2A, the two circles composed of radical strips represent the overall metabolic profiles of WT-B and ZZa3, respectively. Individual metabolic activity comparisons revealed that WT-B was capable of metabolizing 56 substrates that ZZa3 cannot use (AV=0); and surprisingly, ZZa3 exclusively showed metabolic activities when incubated with 22 substrates, even although most of the substrate only had a low AV of 0.5.

Using the WT-B as the reference strain, we identified seven phenotypic tests that showed significantly different metabolism (AV $\geq$ 2.5) (Figure 5.2B and Table 5.2). While WT-B maintained active metabolism (AV=3), mutant ZZa3 was completely inactive under the osmotic stress caused by 5% sodium sulfate (AV=0). Meanwhile, the low to zero metabolism of amino acids alanine, asparagine and serine in low acid conditions (pH 4.5), as well as methionine, valine and phenylalanine intensively at pH 9.5 suggest that phage resistant ZZa3 is less capable of decarboxylating and deaminating these amino acids under acidic and basic conditions, respectively.

5.3.1.2 Altered metabolic features of phage insensitive *E. coli* O157:H7 mutants

By challenging the host WT-O157 with phage AR1, we isolated two BIMs ZZb2 and ZZb4 with gene mutations involved in the biosynthesis of LPS and OmpC, respectively (Chapter IV). All AVs of the 960 phenotypic tests for the wildtypes and BIMs were listed (Appendix Table S5.2) and compared to that of WT-O157 (Figures 5.3 and 5.4). In Figure 5.3, all three strains showed low activities in metabolizing carbon sources in PM2 (8%) and nutrient supplements in PM5 (0%). The active metabolic rates of WT-O157 in PM1 (65%) and PM4 (63%) were higher than that of both BIMs. While BIM ZZb4 had a higher active metabolism percentage in PM6 (61%), BIM ZZb2 was the most active isolate in metabolizing nitrogen sources [PM3 (34%)], peptide nitrogen sources [PM7 (55%), PM8 (50%) and PM9 (55%)] and under various pH conditions [PM10 (72%)]. The active metabolism percentages of ZZb4 in many PM plates were similar to that of WT-O157, except for PM6, PM7 and PM10, indicating that these two strains had similar activity levels when metabolizing carbon, nitrogen, and phosphorus and sulfur sources, but behaved differently when exposed to peptide nitrogen sources and various pH conditions. On the other hand, compared to the wildtype, BIM ZZb2 was more active in using nitrogen and peptide nitrogen sources, as well as metabolizing in various pH and osmolarity conditions. Together, these findings illustrated that most of the metabolic changes of *E. coli* O157:H7 BIMs with different membrane modifications were associated with the catabolism of peptide nitrogen and tolerance in different conditions.

To further investigate the metabolic profiles of WT-O157 and BIMs, the activity values in the 960 phenotypic tests were compared in three concentric ring plots (Figure 5.4). In Figure 5.4A, individual comparison between WT-O157 and ZZb4 revealed 96 tested substrates that were only utilized by either WT-O157 (53) or ZZb4 (43). The discrepancy between the wildtype and ZZb2 was even more considerable. WT-O157 was able to grow on 49 substrates that ZZb2 cannot use, while ZZb2 exclusively showed metabolic activities in 117 substrates that WT-O157 metabolism

was not detected. Moreover, when comparing the three strains together, the deep rough mutant ZZb2 uniquely grew on 97 substrates, suggesting that there might be a relationship between severe LPS truncation and higher metabolic potential.

The metabolic activities of BIM ZZb4 were different from WT-O157 in 9 phenotypic tests (Table 5.3 and Figure 5.4B). Most of these differences were associated with dipeptide metabolism and in low acid conditions. Mutant ZZb4 was able to actively use dipeptides Met-Pro, Trp-Asp, Trp-Glu, Tyr-Glu and Pro-Ser as nitrogen sources, while almost none of them could be used by the parental strain. In addition, growth of ZZb4 at pH 4.5 was not detected, thus resulting in the absent decarboxylation of glycine and hydroxy-L-proline at the same pH. N-acetyl-neuraminic acid (Neu5Ac) was also used differently. BIM ZZb4 was able to metabolize this compound as a carbon source at the highest activity level (AV=4), while WT-O157 only showed low metabolic activity (AV=1.5).

Comparing the metabolic activities of the wildtype and BIM ZZb2, we identified a total of 61 phenotypic tests that show metabolic differences across all PM plates (Table 5.4 and Figure 5.4B). Firstly, the metabolism of glutamic acid, α-ketobutyric acid, α-hydroxybutyric acid, mucic acid, Gly-Glu, N-acetyl-D-mannosamine, D-psicose, pectin and D-lactic acid methyl ester as carbon sources by ZZb2 were mostly either reduced or terminated. In contrast, with only a few exceptions, ZZb2 showed higher metabolic activities than the wildtype when grown onto nitrogen, phosphorus, sulfur and dipeptide nitrogen sources. Enhanced tolerance of ZZb2 in high osmotic conditions established by common food preservatives NaCl and sodium lactate was also observed. Lastly, altered amino acid decarboxylation and deamination abilities of ZZb2 were observed at pH 4.5 and 9.5, respectively.

5.3.2 Crosslinking phenotypic observations to genome-derived metabolic pathways

Using the dape module of DuctApe, we integrated the results gathered from the BIOLOG phenotypic tests to the metabolic networks derived from the bacterial genomes using the KEGG database.

In a comparison of WT-B and ZZa3, the different deamination of amino acids methionine and phenylalanine at pH 9.5 were found to be involved in a total of five KEGG pathways (Figure 5.5). Specifically, both amino acids were weakly metabolized by ZZa3 and associated with the aminoacyl-tRNA biosynthesis pathway (KEGG map 00970), thus indicating that the synthesis of L-methionyl tRNA and L-phenylalanyl tRNA might be one of the altered metabolic features of phage-resistant *E. coli* B mutants. In addition, low methionine catabolism of ZZa3 was observed in the cysteine and methionine metabolism pathway (KEGG map 00270), while the inability to use phenylalanine was associated with phenylpropanoid biosynthesis (KEGG map 00940), phenylalanine metabolism (KEGG map 00360) and tropane, piperidine and pyridine alkaloid biosynthesis (KEGG map 00960).

Five substrates differently used by WT-O157 and ZZb4 were found to be linked to the discrepancies involving a total of 9 metabolic pathways (Figure 5.6). These phenotypic differences are associated with using Neu5Ac as carbon source, D-serine as nitrogen source, D-glucose 6-phosphate and D-glucose 1-phossphate as phosphorous sources, and metabolizing sarcosine at 6% NaCl. The metabolism of these substrates is mainly related to O-antigen nucleotide sugar biosynthesis (KEGG map: 00541), amino sugar and nucleotide sugar metabolism (KEGG map: 00520), starch and sucrose metabolism (KEGG map: 00500) and glycine, serine and threonine metabolism (KEGG map: 00260). The increased activities of BIM ZZb4 metabolizing Neu5Ac, D-glucose 6-phosphate and D-glucose 1-phophate are related to most of the identified pathways,

while the decreased metabolic activities of D-serine and sarcosine at pH 9.5 are linked to the glycine, serine and threonine metabolism pathway (KEGG map: 00260) and arginine and proline metabolism pathway (KEGG map: 00330).

On the other hand, relevant metabolic differences between WT-O157 and ZZb2 were shown and crosslinked to a total of 26 KEGG pathways (Figure 5.7). The most striking difference was the deamination of phenethylamine at pH 9.5, as ZZb2 showed the highest metabolic intensity, and the wildtype was completely inactive in phenylalanine metabolism (KEGG map: 00360). In contrast, no activity of ZZb2 was detected when grown on another amino acid cadaverine at pH 9.5, while WT-O157 actively metabolized this amino acid in the glutathione metabolism (KEGG map: 00480). Furthermore, the increased ability of ZZb2 to use D-glucosamine as a nitrogen source is related to the amino sugar and nucleotide sugar metabolism pathway (KEGG map: 00520), which is also associated with the varied metabolism of pectin, N-acetyl-D-glucosamine (GlcN), Neu5Ac and N-acetyl-D-mannosamine. For the rest of the compounds, the results demonstrated that the deep rough mutant ZZb2 gained abilities to use GlcN, Neu5Ac, L-methionine sulfoxide, L-methionine, phosphorylcholine with 6% NaCl, guanosine, D-glucose 6-phosphate and 2-aminoethlphosphonate, but showed decreased metabolic activities when exposed to pectin, 2-ketobutyric acid, 2-hydroxybutyric acid, L-glutamic acid, N-acetyl-D-mannosamine, D-serine and thiosulfate. Interestingly, the metabolism of Neu5Ac as a carbon source, D-serine as a nitrogen source and D-glucose 6-phosphate as a phosphorous source of both ZZb2 and ZZb4 were consistently different from that of the wildtype (Figures 5.6 and 5.7), suggesting them as common phage-induced metabolic changes of *E. coli* O157:H7 BIMs. Finally, among the 18 substrates differentially metabolized and linked to metabolic pathways (Figure 5.7), six carbon source compounds are involved in 19 out of 26 KEGG pathways, supporting the claim that the

discrepancies in using carbon sources are the major metabolic change of deep rough *E. coli* O157:H7 mutants.

5.4 Discussion

Understanding the altered metabolism of BIMs has a profound impact on applying phage-based antimicrobial products against foodborne bacterial pathogens. In this study, we assessed the overall metabolic profile of phage resistant *E. coli* mutants and identified the changed phenotypes for bacterial adaptation to phage predation. By linking these phenotypic features to genomic information, we were able to reveal putative metabolic networks that might be subject to change upon phage infections. These findings are of great importance since they shed light on the complex metabolic changes related to phage resistance and offer insight into the physiological status of BIMs.

As shown in Table 5.2, BIM ZZa3 consistently displayed lower activities in seven phenotypic tests in PM9 and PM10, suggesting that this mutant is less tolerant than the wildtype in certain pH and osmolarity conditions. Mutant ZZa3 possesses a truncated LPS and is susceptible to the osmotic stress caused by 5% sodium sulfate, which is a common food additive (FDA, 2017a; Food Standard Agency, 2020). Increased sensitivity to this compound might be attributed to the disrupted membrane structure caused by LPS abbreviation. Previous studies showed that the integrity of LPS is essential for the biogenesis of OMPs (Nikaido, 2005; Arunmanee et al., 2016), which have been implicated in osmoregulation (Forst et al., 1988; Poolman et al., 2004).

Among the six amino acids differently used by the *E. coli* B strains, the varied deamination of methionine and phenylalanine at pH 9.5 were both linked to aminoacyl-tRNA biosynthesis (KEGG map: 00970) (Figure 5.5). This is not surprising because the lack of catabolizing these amino acids will inevitably affect the synthesis of methionyl-tRNA and phenylalanyl-tRNA.

Together, the negatively affected amino acid amination and decarboxylation demonstrate that BIM ZZa3 may be less adaptable than the wildtype in basic and acidic environments.

Similarly, *E. coli* o157:H7 mutant ZZb4 also showed reduced decarboxylation activity at pH 4.5 (Table 5.3). Although this discrepancy might be simply explained by the fact that ZZb4 cannot grow at this pH, three out of 35 decarboxylation tests showed high metabolic activity (AV=3) at pH 4.5 (Appendix Table S5.2). Further experiments are needed to address these exceptions. In addition, when compared to the wildtype, BIM ZZb4, which contains a mutation in OmpC, was superior at using five dipeptides as nitrogen sources and Neu5Ac as a carbon source (Table 5.3). As a source of nutrients, these substrates must be able to travel through the bacterial outer and cytoplasmic membranes. Dipeptide passage through the inner membrane of *E. coli* requires the presence of the dipeptide permease (dpp) locus which comprises an operon of dppABCDE encoding dipeptide-binding proteins (DBPs) and transporter membrane subunits (Abouhamad & Manson, 1994). In this study, no genetic differences involved in this ABC transporter located at the cytoplasmic membrane was observed between WT-O157 and ZZb4. On the other hand, peptide permeation through the outer membrane relies on porins like OmpF and OmpC, and alterations in these protein channels was associated with changed peptide permeation (Andrews & Short, 1985). Therefore, we reason that the mutation of ZZb4 in response to phage infection may subsequently permeabilize the cell to dipeptides Met-Pro, Trp-Asp, Trp-Glu, Tyr-Glu and Pro-Ser. Lastly, the increased metabolism of the carbon source Neu5Ac might also be a result of the OmpC alteration. Although this sialic acid would be transported more efficiently through the specific channel NanC when both OmpC and OmpF are absent, the passage of Neu5Ac through OmpC and OmpF was also observed in laboratory conditions (Condemine et al., 2005;

Wirth et al., 2009). Therefore, the facilitated uptake of Neu5Ac might be a result of the altered OmpC channel of ZZb4, allowing active metabolism as a carbon source.

In addition to Neu5Ac, four other substrates differently used by WT-O157 and ZZb4 were crosslinked to KEGG pathways in the combined heatmap (Figure 5.6). Phosphorus is essential for bacterial growth as an element of energy compounds (ATP), nucleic acid and membrane phospholipid. Under phosphate starvation conditions, porin PhoE is preferably expressed to facilitate the diffusion of anionic phosphate-containing nutrients (Nikaido, 2005). However, in the presence of excessive phosphate esters such as D-glucose 6-phosphate and D-glucose 1-phosphate, *E. coli* transports phosphates across the outer membrane via porins OmpC and OmpF (Korteland et al., 1982; Wolschendorf et al., 2007). Therefore, the altered OmpC of ZZb4, which presumably allowed increased permeation of several dipeptides and Neu5Ac, might result in the elevated metabolism of these two phosphates.

Interestingly, the increased metabolism of various dipeptidesr, Neu5Ac, D-glucose 6 phosphate were also observed in BIM ZZb2 (Figure 5.7), which produces truncated LPS, but not an altered OmpC. However, deep rough mutants like ZZb2 may have significant reduction (up to 60%) in outer membrane protein content (Schnaitman & Klena, 1993; Nikaido, 2005; Pagnout et al., 2019), which may lead to substantial membrane destabilization and enhanced permeability. Together, we argue that the observed phenotypic differences might be at least partially caused by changes in the integrity of outer membrane structure.

The metabolic profile of BIM ZZb2 differs from that of WT-O157 even more significantly (Table 5.4 and Figure 5.7). The metabolic activities of this deep rough mutant generally outcompeted that of the parental strain in nitrogen, phosphorus, sulfur and most dipeptide sources (Table 5.4 and Figure 5.7). For example, the two amino sugars D-glucosamine and N-acetyl-

glucosamine were better utilized by ZZb2 as nitrogen sources, even though the metabolism of these compounds as a carbon source were similar between the two strains (Appendix Table S5.2). These findings suggest that both strains were able to transport these amino sugars through the membrane via the phosphotransferase (PTS) system and convert them to glucosamine-6-phosphate (Tchieu et al., 2001), but ZZb2 might be better at deaminating the resulting glucosamine-6-phosphate to ammonia, possibly due to enhanced expression of enzyme NagB and other regulators (Altamirano et al., 1987; Álvarez-Añorve et al., 2009; Rodionova et al., 2017; Rodionova et al., 2018). One interesting exception was that none of the BIMs was able to use D-serine as a nitrogen source (Figures 5.6, 5.7 and S2). This consistent lack of metabolism might imply a common metabolic defect of *E. coli* O157:H7 BIMs likely resulting from the differently expressed ammonia-lyase DsdA, which catalyzes D-serine into pyruvate and ammonia (Bloom & McFALL, 1975). In addition, sulfur-containing molecules L-methionine and L-methionine sulfoxide were better used by ZZb2 than the wildtype as a sulfur source. The uptake of these molecules largely depend on the high affinity MetNIQ methionine uptake system (Kadner & Watson, 1974; Kadner, 1977), and as shown in the cysteine and methionine pathway (KEGG map: 00270), the following catabolism of these two compounds require an oxidoreductase to convert L-methionine sulfoxide to L-methionine and a S-adenosyltransferase to catalyze L-methionine to S-adenosyl-L-methionine, which can be used as a source of sulfur. Since no genetic differences were found involved in these reactions, additional studies are thereby required.

Unlike metabolism of other sources, BIM ZZb2 had a lower metabolizing activity on carbon sources than WT-O157. Nevertheless, this finding is contradicted by the finding of another BIM ZZa3 which also has truncated LPSs but showed higher metabolic activity than its parental strain in PM1 and PM2 (Figure 5.1). This discrepancy might be explained by the different levels

of LPS truncation. For the *E. coli* B mutant ZZa3, *waaG* mutations caused the removal of two terminal glucose in the LPS outer core (Zhong et al., 2020). However, the phage-induced HldE mutation of ZZb2 led to a heptoseless LPS structure, meaning that carbohydrate residues of O-antigen repeats, outer core and most of the inner core are completely absent. Therefore, the different severities of LPS truncation might explain the discrepancies between two BIMs with altered LPS structures to some extent.

Furthermore, BIM ZZb2 showed an elevated tolerance to osmotic pressure caused by high concentration of food preservatives NaCl and sodium lactate (Table 5.4). It was previously shown that addition of 6% NaCl in cucumber fermentation was able to achieve 5-log reduction of STEC strains (Dupree et al., 2019), and the growth of *E. coli* O157:H7 strains in ground beef was further delayed by an increase of sodium lactate concentration (maximum level allowed is 4.8% [21 CFR 184.1768]) (Hwang & Juneja, 2011; FDA, 2017a). However, the active metabolism under conditions of 6% NaCl and 12% sodium lactate in BIM ZZb2 would render these food safety hurdles ineffective in controlling the growth of this mutant. The elevated tolerance to osmotic stress possibly linked to one of the physiological changes observed in ZZb2: namely the enhanced biofilm production (Chapter IV). This fitness alteration, due to the HldE mutation (Nakao et al., 2012), allows this mutant to be a stronger biofilm former than the WT-O157, which may explain the ability of ZZb2 to survive under osmotic stress. In addition, this feature might also play a role in the increased decarboxylation activities in low acid conditions. Unlike other mutants, BIM ZZb2 was more capable of decarboxylating certain amino acids at pH 4.5 than its parental strain. It is reasonable to assume that the enhanced biofilm formation of ZZb2 protects the cells from acid stress, thus allowing amino acid decarboxylation.

In conclusion, Phenotypic Microarrays provided an overview of changed phenotypes for the three BIMs and the genomic-phenomic approach offered an evaluation of the metabolic pathways putatively involved in the adaptations to phage predation. *E. coli* B BIM ZZa3 became more sensitive to osmotic and pH fluctuation than the wildtype and these increased sensitives might further affect the amino acid metabolism and tRNA biosynthesis. On the other hand, *E. coli* O157:H7 mutant ZZb4 appeared to be intolerant to pH 4.5 and more competitive in metabolizing dipeptides and Neu5Ac as compared to WT-O157. Lastly, the deep rough mutant ZZb2 displayed a great number of metabolic differences, including the less active metabolism of carbon sources, increased capabilities in using dipeptides as well as the elevated tolerance under osmotic pressure and pH variation. Further studies such as comparative transcriptomics analysis may provide more details to interpret the results. So far, these findings demonstrate the complexity of phage resistance-associated metabolic modifications, advancing the understanding of phage resistant *E. coli* physiological properties and their potential impacts on food safety.

Table 5.1: Wildtype *Escherichia coli* strains and their derived bacteriophage insensitive mutants used in this chapter

Strain	Phage resistance	Gene involved in phage resistance	Affected receptor	Source
Wildtype *E. coli* O157:H7 920333 (WT-O157)				Personal strain collection of Dr. Lawrence Goodridge
E. coli O157:H7 BIM ZZb2	AR1	*hldE*	LPS	From Chapter IV
E. coli O157:H7 BIM ZZb4	AR1	*ompC*	OmpC	From Chapter IV
Wildtype *E. coli* B ATCC11303 (WT-B)				Purchased from America Type Culture Collection
E. coli B BIM ZZa3	T4	*waaG*	LPS	Zhong et al. (2020)

Table 5.2: Different metabolism of wildtype *Escherichia coli* B (WT-B) and bacteriophage insensitive mutant ZZa3

Plate	Well	Chemical	Mode of action	Co. ID*	WT-B	ZZa3
PM09	D08	5% Sodium Sulfate	osmotic sensitivity	C13199	3	0
PM10	B02	pH 4.5 + L-Alanine	pH, decarboxylase	C00041	3	0.5
PM10	B04	pH 4.5 + L-Asparagine	pH, decarboxylase	C00152	4	1
PM10	C04	pH 4.5 + L-Serine	pH, decarboxylase	C00065	3.5	1
PM10	F01	pH 9.5 + L-Methionine	pH, deaminase	C00073	4	1.5
PM10	F02	pH 9.5 + L-Phenylalanine	pH, deaminase	C00079	3	0
PM10	F08	pH 9.5 + L-Valine	pH, deaminase	C00183	3	0.5

*. Compound ID in the KEGG database.

Table 5.3: Different metabolism of wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutant ZZb4

Plate	Well	Chemical	Mode of action	Co. ID*	WT-O157	ZZb4
PM02	B02	N-Acetyl-Neuraminic acid	C-Source, carboxylic acid	C00270	1.5	4
PM07	C06	Met-Pro	N-Source, peptide		0	3
PM07	F08	Trp-Asp	N-Source, peptide	C02871	0	3
PM07	F09	Trp-Glu	N-Source, peptide		0	3
PM07	G07	Tyr-Glu	N-Source, peptide		0	3
PM08	D04	Pro-Ser	N-Source, peptide		0.5	3
PM10	A03	pH 4.5	pH, growth at 4.5		3	0
PM10	B08	pH 4.5 + Glycine	pH, decarboxylase	C00037	3	0
PM10	C09	pH 4.5 + Hydroxy-L-Proline	pH, decarboxylase	C01015	2.5	0

*. Compound ID in the KEGG database.

Table 5.4: Different metabolism of wildtype *Escherichia coli* O157:H7 (WT-O157) and bacteriophage insensitive mutant ZZb2

Plate	Well	Chemical	Mode of action	Co. ID*	WT-O157	ZZb2
PM01	B12	L-Glutamic acid	C-Source, amino acid	C00025	3	0.5
PM01	D07	α-Ketobutyric acid	C-Source, carboxylic acid	C00109	3	0.5
PM01	E07	α-Hydroxybutyric acid	C-Source, carboxylic acid	C05984	4	1.5
PM01	F08	Mucic acid	C-Source, carboxylic acid	C01807	3	0
PM01	G01	Gly-Glu	C-Source, amino acid		3	0
PM01	G08	N-Acetyl-D-Mannosamine	C-Source, carbohydrate	C00645	4	1.5
PM01	H05	D-Psicose	C-Source, carbohydrate	C06468	3	0
PM02	A12	Pectin	C-Source, polymer	C00714	3	0
PM02	F01	D-Lactic acid Methyl Ester	C-Source, ester		3.5	0
PM02	G02	L-Alaninamide	C-Source, amide		0	2.5
PM03	E08	D-Glucosamine	N-Source, other	C00329	0.5	4
PM03	E11	N-Acetyl-D-Glucosamine	N-Source, other	C00140	0	3
PM03	E12	N-Acetyl-D-Galactosamine	N-Source, other	C01074	0	4
PM03	G07	D, L-α-Amino-N-Butyric acid	N-Source, other	C02261	2.5	0
PM04	B01	Thiophosphate	P-Source, inorganic		4	1.5
PM04	C09	Cytidine 3`-Monophosphate	P-Source, organic	C05822	0.5	4
PM04	C12	Cytidine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00941	0.5	3
PM04	G07	L-Methionine	S-Source, organic	C00073	0	2.5
PM04	G08	D-Methionine	S-Source, organic	C00855	0	2.5
PM04	G09	Gly-Met	S-Source, organic		0	2.5
PM04	G10	N-Acetyl-D, L-Methionine	S-Source, organic	C02712	0	2.5
PM04	G11	L-Methionine Sulfoxide	S-Source, organic	C02989	0	2.5
PM06	B11	Arg-Leu	N-Source, peptide	C05035	3.5	0.5
PM06	C01	Arg-Met	N-Source, peptide		4	0.5
PM06	C06	Arg-Val	N-Source, peptide		3	0.5
PM07	B04	Lys-Trp	N-Source, peptide	C02732	0	3
PM07	C04	Met-Met	N-Source, peptide		3	0
PM07	C06	Met-Pro	N-Source, peptide		0	3.5
PM07	C07	Met-Trp	N-Source, peptide	C02732	0.5	3
PM07	D06	Pro-Gln	N-Source, peptide		0	3
PM07	D07	Pro-Gly	N-Source, peptide		0.5	3

Table 5.4 continued: Significantly different metabolism between WT-O157 and ZZb2

Plate	Well	Chemical	Mode of action	Co. ID*	WT-O157	ZZb2
PM07	D09	Pro-Leu	N-Source, peptide	C05035	0	4
PM07	D11	Pro-Pro	N-Source, peptide		0	3
PM07	E03	Ser-His	N-Source, peptide		0.5	3
PM07	E06	Ser-Phe	N-Source, peptide		0	3
PM07	E08	Ser-Ser	N-Source, peptide		4	1
PM07	F05	Thr-Pro	N-Source, peptide		1	4
PM07	F08	Trp-Asp	N-Source, peptide	C02871	0	3
PM07	F09	Trp-Glu	N-Source, peptide		0	3
PM07	G03	Trp-Trp	N-Source, peptide	C02732	0.5	3
PM07	G07	Tyr-Glu	N-Source, peptide		0	3
PM08	B04	His-Glu	N-Source, peptide		0	3
PM08	D04	Pro-Ser	N-Source, peptide		0.5	3.5
PM09	B03	6% NaCl + N, N Dimethyl Glycine	osmolyte, dimethylglycine	C01026	0	3
PM09	B09	6% NaCl + Phosphorylcholine	osmolyte, phosphorylcholine	C00588	0.5	3
PM09	C02	6% NaCl + L-Proline	osmolyte, proline	C00148	0.5	3
PM09	C03	6% NaCl + N-Acetyl-L-Glutamine	osmolyte, acetyl glutamine		0.5	3
PM09	C05	6% NaCl + γ -Amino-N-Butyric acid	osmolyte, γ-amino butyric acid		0.5	3
PM09	F04	4% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0.5	3
PM09	F05	5% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3
PM09	F08	8% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3
PM09	F12	12% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3
PM10	B01	pH 4.5	pH, decarboxylase control		0	3
PM10	B02	pH 4.5 + L-Alanine	pH, decarboxylase	C00041	0	2.5
PM10	B07	pH 4.5 + L-Glutamine	pH, decarboxylase	C00064	3	0
PM10	B09	pH 4.5 + L-Histidine	pH, decarboxylase	C00135	0	2.5
PM10	C02	pH 4.5 + L-Phenylalanine	pH, decarboxylase	C00079	0	2.5
PM10	C12	pH 4.5 + L-Homoserine	pH, decarboxylase	C00263	0	2.5
PM10	G02	pH 9.5 + L-Norleucine	pH, deaminase	C01933	3.5	0
PM10	G05	pH 9.5 + Cadaverine	pH, deaminase	C01672	3.5	0
PM10	G08	pH 9.5 + β -Phenylethylamine	pH, deaminase	C05332	0	4

*. Compound ID in the KEGG database.

Figure 5.1. High metabolic activity comparisons between wildtype *Escherichia coli* B (WT-B; darkred) and bacteriophage insensitive mutant ZZa3 (pink) in each PM plates. Radar plots indicate the percentage of wells that showed intense metabolic activities (AV ≥ 3). Each radial strip corresponds to a single PM plate. PM categories: Carbon sources (PM1, PM2), nitrogen sources (PM3), phosphorus and sulfur sources (PM4), nutrient supplements (PM5), peptide nitrogen sources (PM6, PM7, PM8), osmolytes (PM9) and pH (PM10).

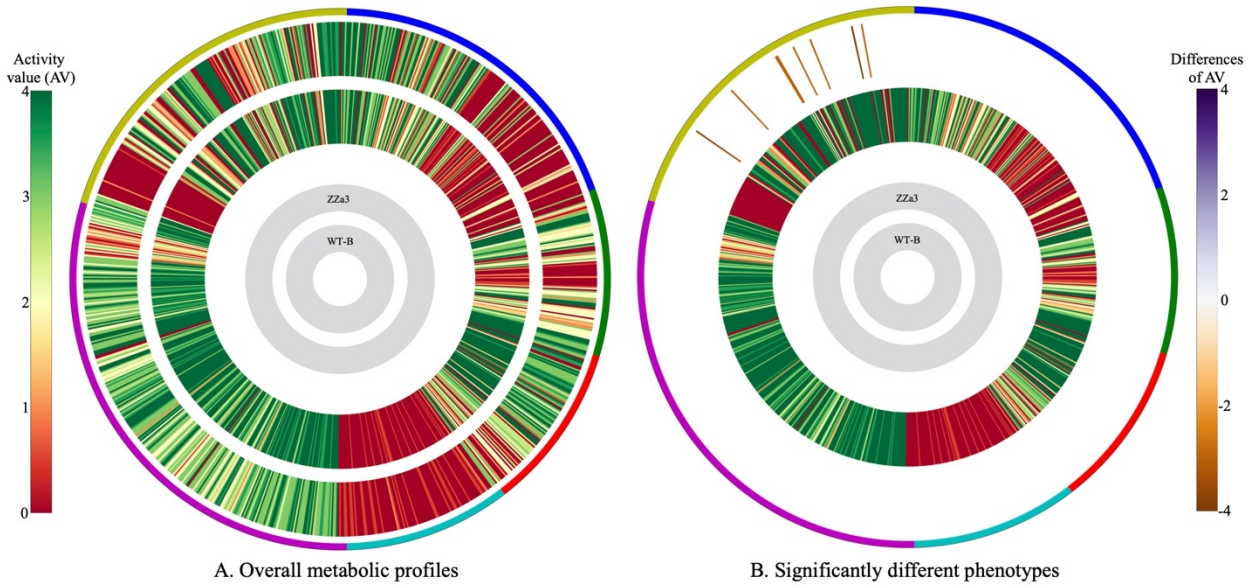

Figure 5.2. Comparative Phenotypic Microarray analysis of wildtype *Escherichia coli* B (WT-B; inner ring) and bacteriophage insensitive mutant ZZa3 (outer ring). The external line surrounding the concentric circles is color-coded according to one of the six tested categories: carbon source (blue); nitrogen source (green), phosphate and sulfur substrates (red) nutrient supplements (cyan), nitrogen peptide sources (purple), osmolytes and pH (light green). (A) Overall metabolic profile. The AV of each phenotypic test is represented by a strip colored from red (low activity) to green (high activity). (B) Significantly different phenotypes. Strips in the outer ring represent the phenotypic tests that show a significant difference (AV $\geq$ 2.5) and are colored by the AV difference when comparing to the reference strain WT-B.

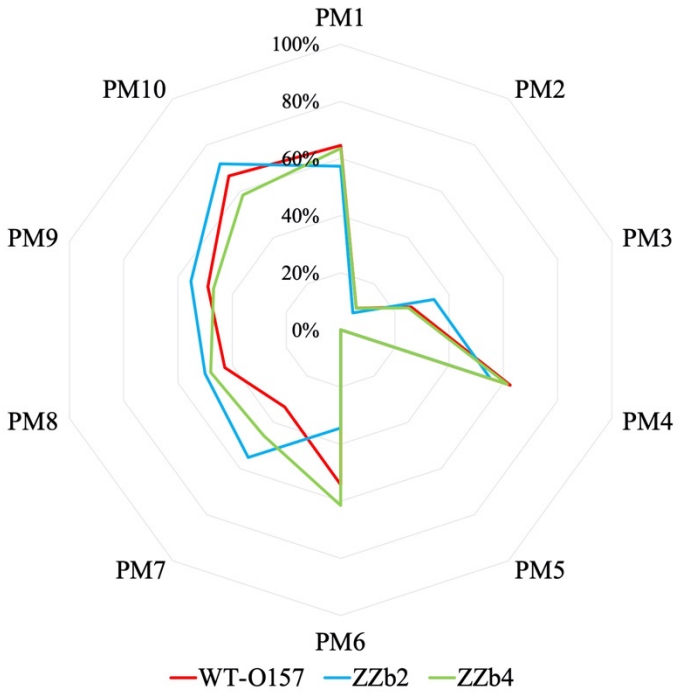

Figure 5.3. High metabolic activity comparisons between wildtype *E. coli* O157:H7 (WT-O157; red), bacteriophage insensitive mutants ZZb2 (blue) and ZZb4 (green) in each PM plates. Radar plots indicate the percentage of wells that showed intense metabolic activities (AV ≥ 3). Each radial strip corresponds to a single PM plate. PM categories: Carbon sources (PM1, PM2), nitrogen sources (PM3), phosphorus and sulfur sources (PM4), nutrient supplements (PM5), peptide nitrogen sources (PM6, PM7, PM8), osmolytes (PM9) and pH (PM10).

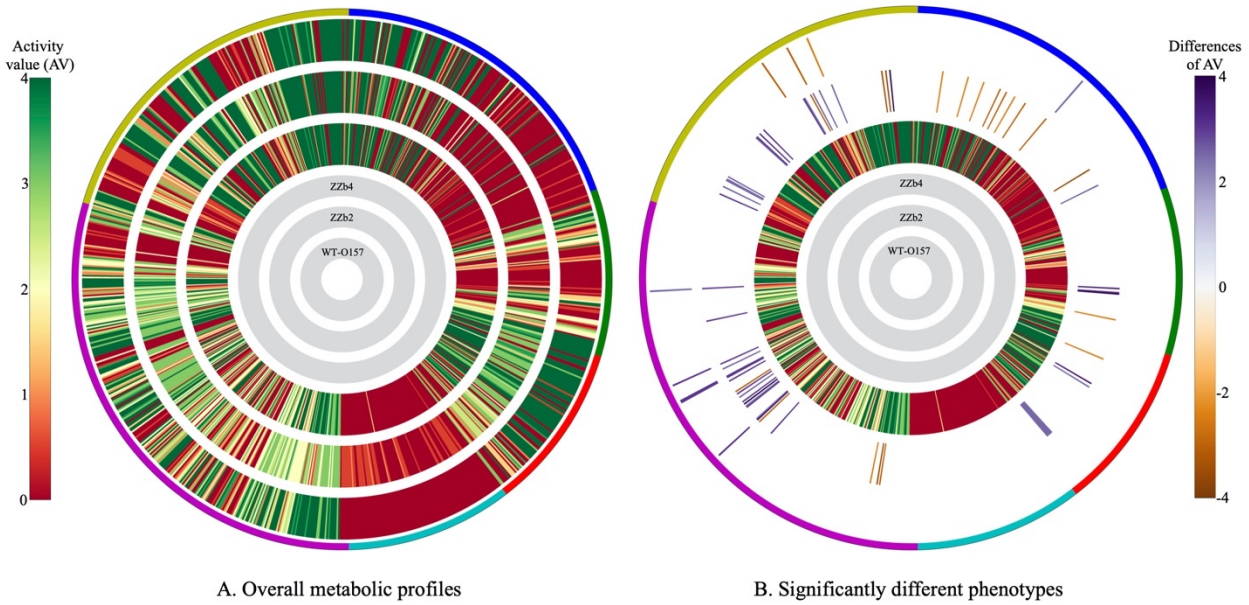

Figure 5.4. Comparative metabolism analysis of wildtype *E. coli* O157:H7 (WT-O157; inner ring), bacteriophage insensitive mutants ZZb2 (middle ring) and ZZb4 (outer ring). The external line surrounding the concentric circles is color-coded according to one of the six tested categories: carbon source (blue); nitrogen source (green), phosphate and sulfur substrates (red) nutrient supplements (cyan), nitrogen peptide sources (purple), osmolytes and pH (light green). (A) Overall metabolic profile. The AV of each phenotypic test is represented by a strip colored from red (low activity) to green (high activity). (B) Significantly different phenotypes. Strips in the outer ring represent the phenotypic tests that show a significant difference (AV $\geq$ 2.5) and are colored by the AV difference when comparing to the reference strain WT-O157.

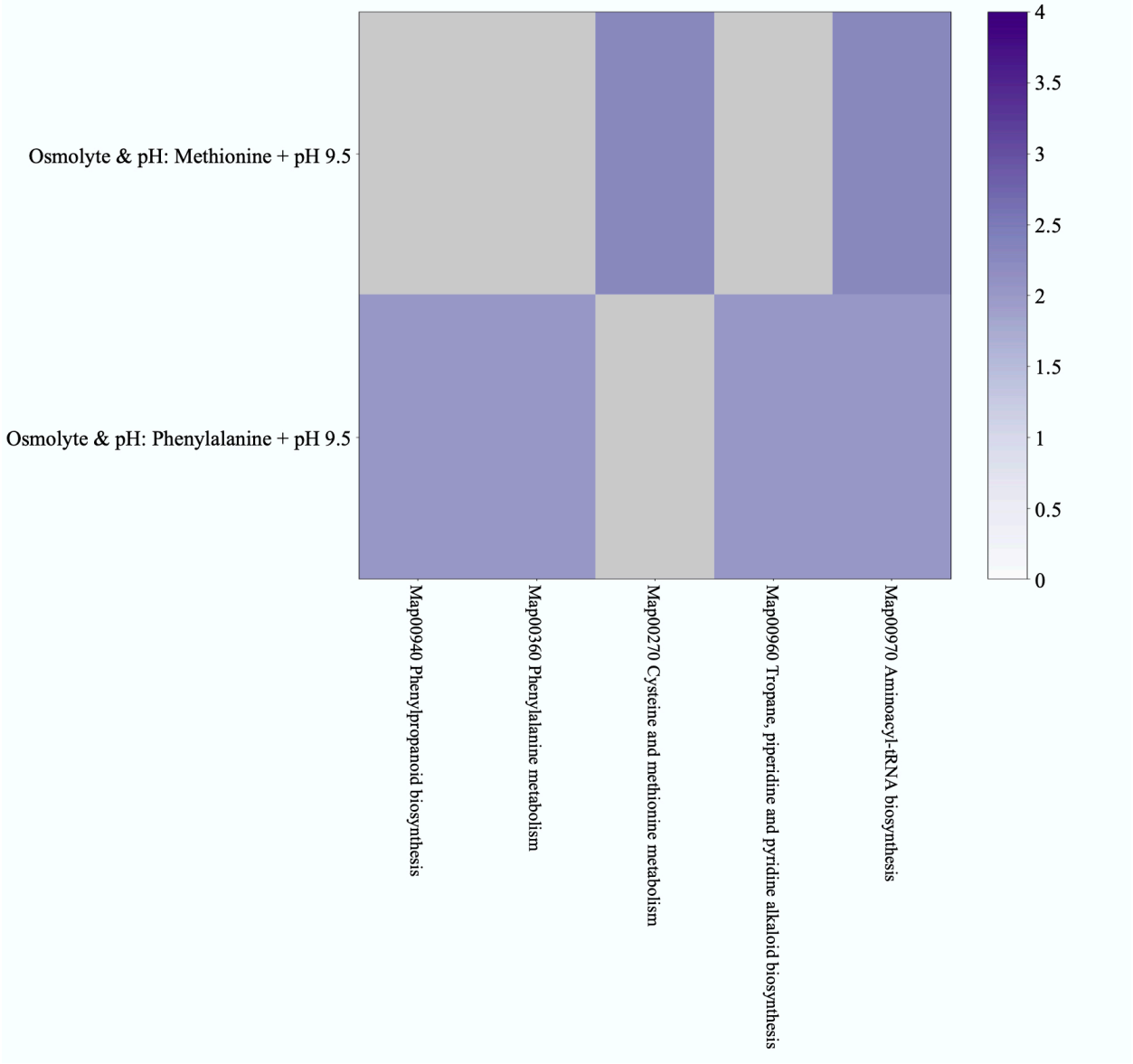

Figure 5.5. Combined genome/phenome variability heatmap of wildtype *Escherichia coli* B (WT-B) and bacteriophage insensitive mutant ZZa3 (AV≥2). Substrates with significantly different phenotypes are reported on Y axis with their mode of action. The genome derived KEGG pathways are shown on X axis. The involvement of a compound in a specific pathway is highlighted by purple blocks which are colored according to the magnitude of variability.

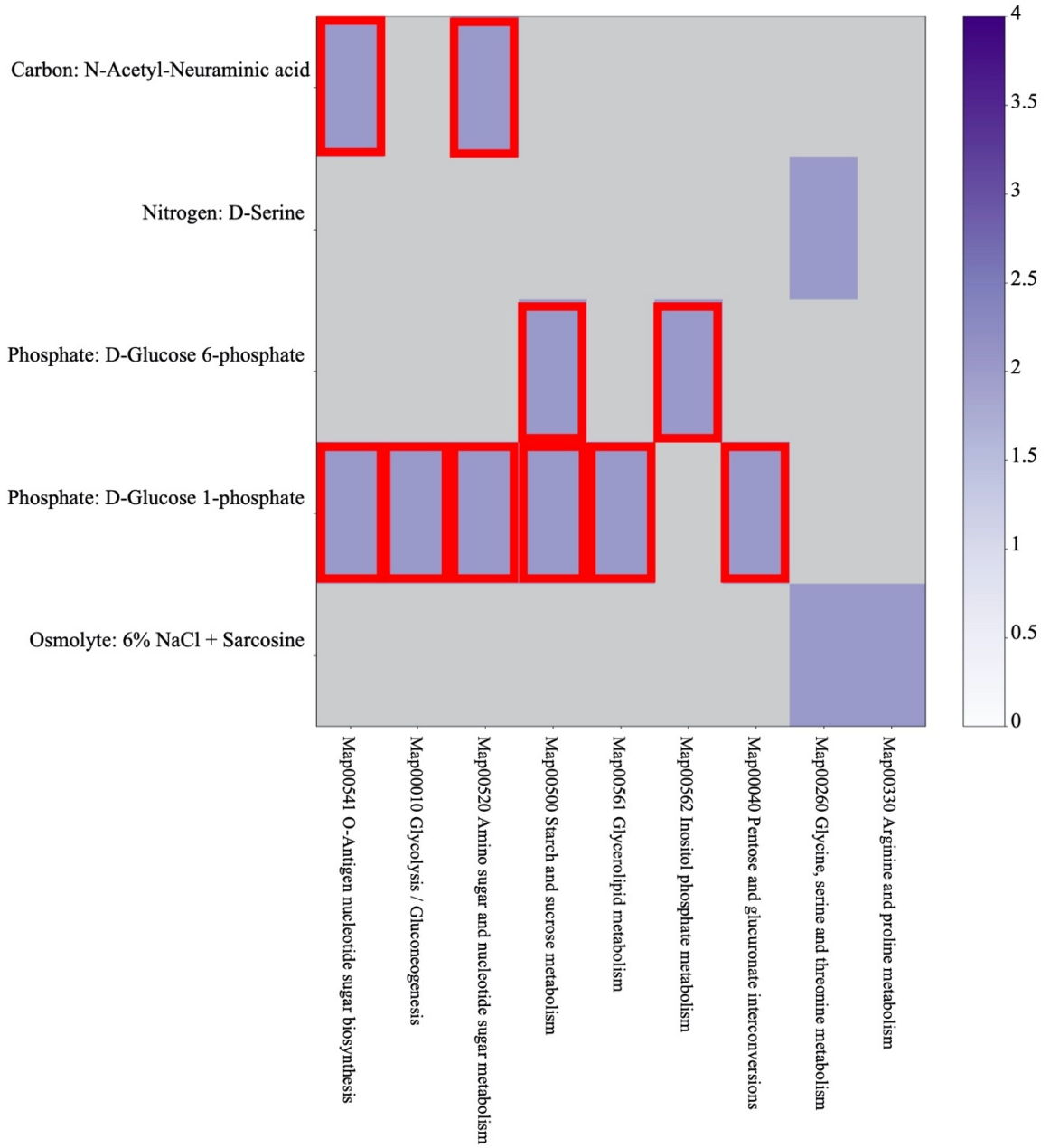

Figure 5.6. Combined genome/phenome variability of wildtype *E. coli* O157:H7 (WT-O157) and bacteriophage insensitive mutant ZZb4 (AV≥2). Substrates with significantly different phenotypes are reported on Y axis with their mode of action. The genome derived KEGG pathways are shown on X axis. The involvement of a compound in certain specific pathway is highlighted by purple blocks which are colored according to the magnitude of variability. Red-border squares indicate BIM ZZb4 as the dominant strain.

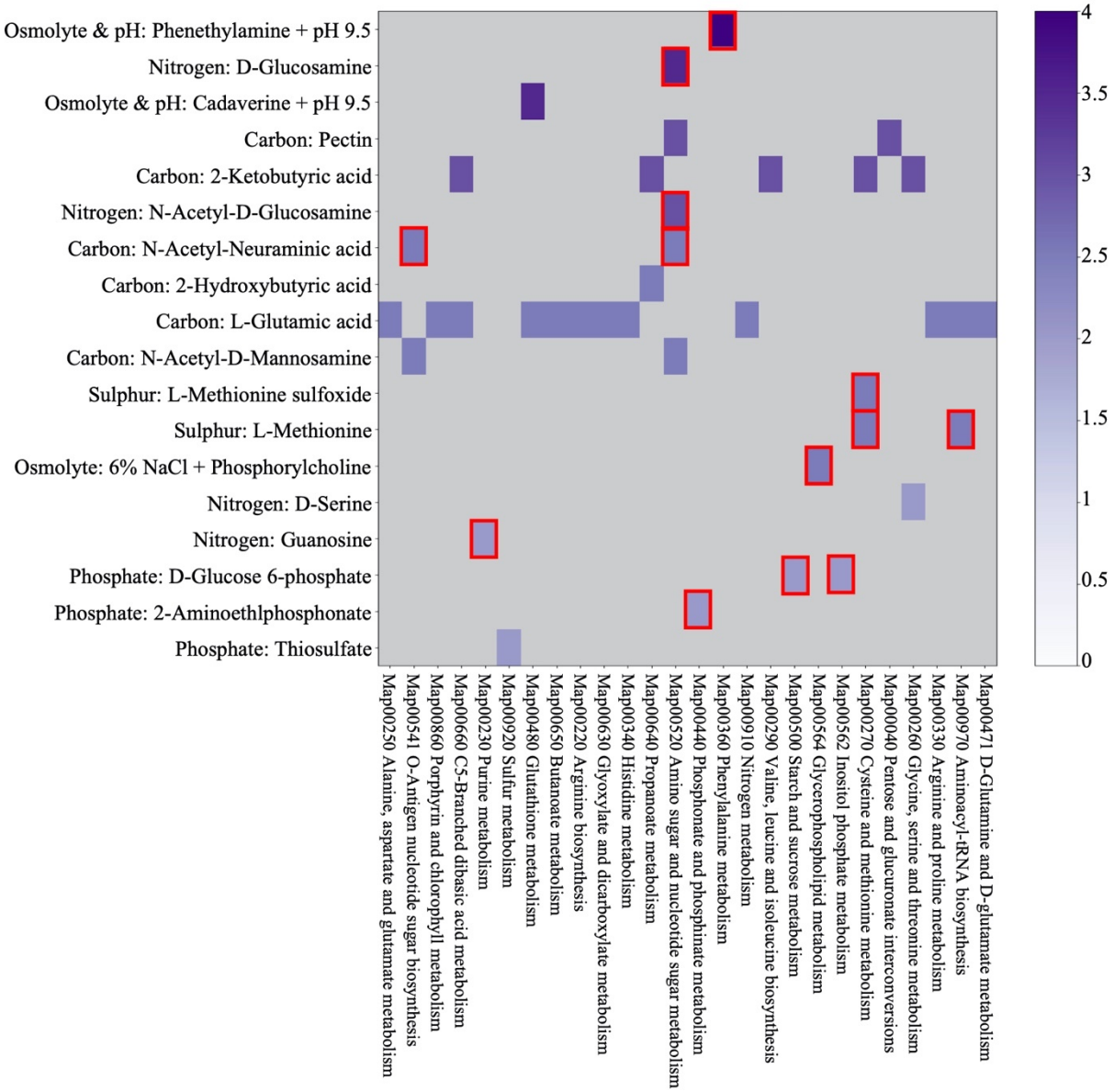

Figure 5.7. Combined genome/phenome variability of wildtype *E. coli* O157:H7 (WT-O157) and bacteriophage insensitive mutant ZZb2 (AV≥2). Substrates with significantly different phenotypes are reported on Y axis with their mode of action. The genome derived KEGG pathways are shown on X axis. The involvement of a compound in certain specific pathway is highlighted by purple blocks which are colored according to the magnitude of variability. Red-border squares indicate BIM ZZb2 as the dominant strain.

5.5 References

Abedon, S. T., Kuhl, S. J., Blasdel, B. G., & Kutter, E. M. (2011). Phage treatment of human infections. *Bacteriophage, 1*(2), 66-85. doi:10.4161/bact.1.2.15845

Abedon, S. T. (2019). Phage-antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? *Antibiotics, 8*(4), 182.

Abouhamad, W. N., & Manson, M. D. (1994). The dipeptide permease of Escherichia coli closely resembles other bacterial transport systems and shows growth-phase-dependent expression. *Molecular microbiology, 14*(5), 1077-1092.

Ackermann, H. W., & Krisch, H. M. (1997). A catalogue of T4-type bacteriophages. *Arch Virol, 142*(12), 2329-2345. doi:10.1007/s007050050246

Ackermann, H. W. (2003). Bacteriophage observations and evolution. *Res Microbiol, 154*(4), 245-251. doi:10.1016/S0923-2508(03)00067-6

Albesa-Jove, D., Giganti, D., Jackson, M., Alzari, P. M., & Guerin, M. E. (2014). Structure-Function Relationships of Membrane-associated GT-B Glycosyltransferases. *Glycobiology, 24*(2), 108-124. doi:10.1093/glycob/cwt101

Aldick, T., Bielaszewska, M., Zhang, W., Brockmeyer, J., Schmidt, H., Friedrich, A. W., Kim, K. S., Schmidt, M. A., & Karch, H. (2007). Hemolysin from Shiga toxin-negative *Escherichia coli* O26 strains injures microvascular endothelium. *Microbes and infection, 9*(3), 282-290.

Aldick, T., Bielaszewska, M., Uhlin, B. E., Humpf, H. U., Wai, S. N., & Karch, H. (2009). Vesicular stabilization and activity augmentation of enterohaemorrhagic *Escherichia coli* haemolysin. *Molecular microbiology, 71*(6), 1496-1508.

Alisky, J., Iczkowski, K., Rapoport, A., & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *Journal of Infection, 36*(1), 5-15.

Allos, B. M., Moore, M. R., Griffin, P. M., & Tauxe, R. V. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. In: The University of Chicago Press.

Altamirano, M. M., Mulliert, G., & Calcagno, M. (1987). Sulfhydryl groups of glucosamine-6-phosphate isomerase deaminase from Escherichia coli. *Archives of biochemistry and biophysics, 258*(1), 95-100.

Álvarez-Añorve, L. I., Bustos-Jaimes, I., Calcagno, M. L., & Plumbridge, J. (2009). Allosteric regulation of glucosamine-6-phosphate deaminase (NagB) and growth of Escherichia coli on glucosamine. *Journal of bacteriology, 191*(20), 6401-6407.

Alvarez-Uria, G., Gandra, S., Mandal, S., & Laxminarayan, R. (2018). Global Forecast of Antimicrobial Resistance in Invasive Isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Int J Infect Dis, 68*, 50-53. doi:10.1016/j.ijid.2018.01.011

Amor, K., Heinrichs, D. E., Frirdich, E., Ziebell, K., Johnson, R. P., & Whitfield, C. (2000). Distribution of core oligosaccharide types in lipopolysaccharides from Escherichia coli. *Infect Immun, 68*(3), 1116-1124. doi:10.1128/iai.68.3.1116-1124.2000

Andrews, J. C., & Short, S. A. (1985). Genetic analysis of Escherichia coli oligopeptide transport mutants. *Journal of bacteriology, 161*(2), 484-492.

Aoki-Kinoshita, K. F., & Kanehisa, M. (2007). Gene annotation and pathway mapping in KEGG. In *Comparative Genomics* (pp. 71-91): Springer.

Arunmanee, W., Pathania, M., Solovyova, A. S., Le Brun, A. P., Ridley, H., Baslé, A., van den Berg, B., & Lakey, J. H. (2016). Gram-negative trimeric porins have specific LPS

binding sites that are essential for porin biogenesis. *Proceedings of the National Academy of Sciences, 113*(34), E5034-E5043.
Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., Desvaux, M., Di Bonaventura, G., Hébraud, M., & Jaglic, Z. (2017). Critical review on biofilm methods. *Critical reviews in microbiology, 43*(3), 313-351.
Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., & Kubal, M. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC genomics, 9*(1), 75.
Bach, S., McAllister, T., Veira, D., Gannon, V., & Holley, R. (2002). Transmission and control of *Escherichia coli* O157:H7—a review. *Canadian journal of animal science, 82*(4), 475-490.
Bai, X., Hu, B., Xu, Y., Sun, H., Zhao, A., Ba, P., Fu, S., Fan, R., Jin, Y., & Wang, H. (2016). Molecular and phylogenetic characterization of non-O157 Shiga toxin-producing *Escherichia coli* strains in China. *Frontiers in cellular and infection microbiology, 6*, 143.
Bartual, S. G., Otero, J. M., Garcia-Doval, C., Llamas-Saiz, A. L., Kahn, R., Fox, G. C., & van Raaij, M. J. (2010). Structure of the bacteriophage T4 long tail fiber receptor-binding tip. *Proc Natl Acad Sci U S A, 107*(47), 20287-20292. doi:10.1073/pnas.1011218107
Baslé, A., Rummel, G., Storici, P., Rosenbusch, J. P., & Schirmer, T. (2006). Crystal structure of osmoporin OmpC from E. coli at 2.0 Å. *Journal of molecular biology, 362*(5), 933-942.
Beef Cattle Research Council. (2014). In-plant Mitigation of Pathogens. Retrieved from http://www.beefresearch.ca/research-topic.cfm/in-plant-mitigation-of-pathogens-12?language=. Accesed date:[Septermber 4th, 2020]
Beloin, C., Michaelis, K., Lindner, K., Landini, P., Hacker, J., Ghigo, J.-M., & Dobrindt, U. (2006). The transcriptional antiterminator RfaH represses biofilm formation in Escherichia coli. *Journal of bacteriology, 188*(4), 1316-1331.
Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In *Bacterial Biofilms* (pp. 249-289): Springer-Verlag Berlin Heidelberg. Berlin, Germany.
Benz, R., Francis, G., Nakae, T., & Ferenci, T. (1992). Investigation of the selectivity of maltoporin channels using mutant LamB proteins: mutations changing the maltodextrin binding site. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1104*(2), 299-307.
Bergh, O., BØrsheim, K. Y., Bratbak, G., & Heldal, M. (1989). High Abundance of Viruses Found in Aquatic Environments. *Nature, 340*(6233), 467.
Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host Receptors for Bacteriophage Adsorption. *FEMS Microbiol Lett, 363*(4). doi:10.1093/femsle/fnw002
Bhat, U. R., Forsberg, L. S., & Carlson, R. W. (1994). Structure of Lipid A Component of *Rhizobium leguminosarum* bv. *phaseoli* Lipopolysaccharide. . *J Biol Chem, 269*(20), 14402-14410.
Bielaszewska, M., Fell, M., Greune, L., Prager, R., Fruth, A., Tschäpe, H., Schmidt, M. A., & Karch, H. (2004). Characterization of cytolethal distending toxin genes and expression in Shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups. *Infection and immunity, 72*(3), 1812-1816.
Bielaszewska, M., Köck, R., Friedrich, A. W., Von Eiff, C., Zimmerhackl, L. B., Karch, H., & Mellmann, A. (2007). Shiga Toxin-mediated Hemolytic Uremic Syndrome: Time to Change the Diagnostic Paradigm? *PLoS One, 2*(10), e1024.

Bielaszewska, M., Rüter, C., Kunsmann, L., Greune, L., Bauwens, A., Zhang, W., Kuczius, T., Kim, K. S., Mellmann, A., & Schmidt, M. A. (2013). Enterohemorrhagic *Escherichia coli* Hemolysin Employs Outer Membrane Vesicles to Target Mitochondria and Cause Endothelial and Epithelial Apoptosis. *PLoS Pathog, 9*(12), e1003797.

Blanco, M., Blanco, J., Mora, A., Dahbi, G., Alonso, M., González, E., Bernárdez, M., & Blanco, J. (2004). Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). *Journal of Clinical Microbiology, 42*(2), 645-651.

Bloom, F. R., & McFALL, E. (1975). Isolation and characterization of D-serine deaminase constitutive mutants by utilization of D-serine as sole carbon or nitrogen source. *Journal of bacteriology, 121*(3), 1078-1084.

Boerlin, P., McEwen, S. A., Boerlin-Petzold, F., Wilson, J. B., Johnson, R. P., & Gyles, C. L. (1999). Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology, 37*(3), 497-503.

Bokranz, W., Wang, X., Tschäpe, H., & Römling, U. (2005). Expression of cellulose and curli fimbriae by Escherichia coli isolated from the gastrointestinal tract. *Journal of Medical Microbiology, 54*(12), 1171-1182.

Bondy-Denomy, J., & Davidson, A. R. (2014). When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. *Journal of Microbiology, 52*(3), 235-242.

Bonyadian, M., Moshtaghi, H., & Mohamadtaghipour, L. (2017). Antibiotic Resistance of Verotoxigenic *Escherichia coli* Isolated from Vegetables. *Biological Journal of Microorganism, 5*(20), 9-16.

Brenner, D. J., Fanning, G., Steigerwalt, A., Ørskov, I., & Ørskov, F. (1972). Polynucleotide sequence relatedness among three groups of pathogenic Escherichia coli strains. *Infection and immunity, 6*(3), 308-315.

Brenner, S. (1955). The Adsorption of Bacteriophages by Sensitive and Resistant Cells of *Escherichia coli* strain B. *Proc R Soc Lond B Biol Sci, 144*(914), 93-99. doi:10.1098/rspb.1955.0036

Brockmeyer, J., Aldick, T., Soltwisch, J., Zhang, W., Tarr, P. I., Weiss, A., Dreisewerd, K., Müthing, J., Bielaszewska, M., & Karch, H. (2011). Enterohaemorrhagic *Escherichia coli* haemolysin is cleaved and inactivated by serine protease EspPα. *Environmental Microbiology, 13*(5), 1327-1341.

Brunder, W., Schmidt, H., & Karch, H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology, 142*(11), 3305-3315.

Brunder, W., Schmidt, H., & Karch, H. (1997). EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Molecular microbiology, 24*(4), 767-778.

Burland, V., Shao, Y., Perna, N. T., Plunkett, G., Blattner, F. R., & Sofia, H. J. (1998). The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic acids research, 26*(18), 4196-4204.

Buzby, J. C., & Roberts, T. (2009). The economics of enteric infections: human foodborne disease costs. *Gastroenterology, 136*(6), 1851-1862.

Caliezi, C., Wuillemin, W., Zeerleder, S., Redondo, M., Eisele, B., & Hack, C. (2000). C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacological reviews, 52*(1), 91-112.

Canadian Food Inspection Agency. Permitted Synonyms for Food Additives Table. Retrieved from https://www.inspection.gc.ca/food-label-requirements/labelling/industry/food-additives/permitted-synonyms/eng/1369857665232/1369857767799. Accesed date:[April 16, 2020]

Capparelli, R., Nocerino, N., Iannaccone, M., Ercolini, D., Parlato, M., Chiara, M., & Iannelli, D. (2010). Bacteriophage therapy of Salmonella enterica: a fresh appraisal of bacteriophage therapy. *J Infect Dis, 201*(1), 52-61. doi:10.1086/648478

Carlton, R. M. (1999). Phage therapy: past history and future prospects. *Arch Immunol Ther Exp (Warsz), 47*(5), 267-274.

Carter, C. D., Parks, A., Abuladze, T., Li, M., Woolston, J., Magnone, J., Senecal, A., Kropinski, A. M., & Sulakvelidze, A. J. B. (2012). Bacteriophage cocktail significantly reduces Escherichia coli O157: H7 contamination of lettuce and beef, but does not protect against recontamination. *2*(3), 178-185.

Casjens, S. (2003). Prophages and bacterial genomics: what have we learned so far? *Molecular microbiology, 49*(2), 277-300.

CDC. (2020a). E. coli homepage. Retrieved from https://www.cdc.gov/ecoli/index.html. Accesed date:[September 4th, 2020]

CDC. (2020b). Salmonella Hompage. Retrieved from https://www.cdc.gov/salmonella/index.html#:~:text=with%20backyard%20poultry-,Get%20tips%20to%20help%20protect%20you,family%20from%20a%20Salmonella%20infection.&text=CDC%20estimates%20Salmonella%20bacteria%20cause,the%20United%20States%20every%20year. Accesed date:[September 4th, 2020]

CDC. (2020c). Shigell-Shigellosis Fast Facts. Retrieved from https://www.cdc.gov/shigella/fast-facts.html. Accesed date:[September 4th, 2020]

Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas aeruginosa*. *Sci Rep, 6*, 26717. doi:10.1038/srep26717

Chang, V., Chen, L. Y., Wang, A., & Yuan, X. (2010). The Effect of Lipopolysaccharide Core Structure Defects on Transformation Efficiency in Isogenic *Escherichia coli* BW25113 rfaG, rfaP, and rfaC Mutants. *J ExpMicrobiol Immunol (JEMI), 14*, 101-107.

Charbit, A., Gehring, K., Nikaido, H., Ferenci, T., & Hofnung, M. (1988). Maltose transport and starch binding in phage-resistant point mutants of maltoporin. Functional and topological implications. *J Mol Biol, 201*(3), 487-496. doi:10.1016/0022-2836(88)90630-4

Chart, H., Row, B., Threlfall, E. J., & Ward, L. R. (1989). Conversion of Salmonella enteritidis phage type 4 to phage type 7 involves loss of lipopolysaccharide with concomitant loss of virulence. *FEMS Microbiol Lett, 51*(1), 37-40. doi:10.1016/0378-1097(89)90073-6

Chatterjee, S., & Rothenberg, E. (2012). Interaction of bacteriophage l with its E. coli receptor, LamB. *Viruses, 4*(11), 3162-3178. doi:10.3390/v4113162

Chung, W., Siu, J., & Tanner, K. (2005). Roles of lipopolysaccharide and the outer membrane protein OmpC on bacteriophage T4 infectivity in Escherichia coli. *J. Exp. Microbiol. Immunol, 7*, 62-67.

Clarke, S. C. (2001). Diarrhoeagenic *Escherichia coli*—an emerging problem? *Diagnostic microbiology and infectious disease, 41*(3), 93-98.

Clokie, M. R., Kropinski, A. M., & Lavigne, R. (2009). *Bacteriophages*: Springer. Berlin, Germany.

Comeau, A. M., Tetart, F., Trojet, S. N., Prere, M. F., & Krisch, H. M. (2007). Phage-Antibiotic Synergy (PAS): Beta-lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. *PLoS One, 2*(8), e799. doi:10.1371/journal.pone.0000799

Condemine, G., Berrier, C., Plumbridge, J., & Ghazi, A. (2005). Function and expression of an N-acetylneuraminic acid-inducible outer membrane channel in Escherichia coli. *Journal of bacteriology, 187*(6), 1959-1965.

Confer, A. W., & Ayalew, S. (2013). The OmpA family of proteins: roles in bacterial pathogenesis and immunity. *Vet Microbiol, 163*(3-4), 207-222. doi:10.1016/j.vetmic.2012.08.019

Coombes, B. K., Wickham, M. E., Mascarenhas, M., Gruenheid, S., Finlay, B. B., & Karmali, M. A. (2008). Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. *Applied and environmental microbiology, 74*(7), 2153-2160.

Council of Canadian Academies. (**2019**). *When Antibiotics Fail. The Expert Panel on the Potential Socio-Economic Impacts of Antimicrobial Resistance in Canada*. Retrieved from

Coutinho, P. M., Deleury, E., Davies, G. J., & Henrissat, B. (2003). An Evolving Hierarchical Family Classification for Glycosyltransferases. *J Mol Biol, 328*(2), 307-317. doi:10.1016/s0022-2836(03)00307-3

Crawford, R. W., Keestra, A. M., Winter, S. E., Xavier, M. N., Tsolis, R. M., Tolstikov, V., & Bäumler, A. J. (2012). Very long O-antigen chains enhance fitness during Salmonella-induced colitis by increasing bile resistance. *PLoS Pathog, 8*(9), e1002918.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic Escherichia coli. *Clin Microbiol Rev, 26*(4), 822-880. doi:10.1128/CMR.00022-13

Currie, C. G., & Poxton, I. R. (1999). The lipopolysaccharide core type of Escherichia coli O157:H7 and other non-O157 verotoxin-producing E. coli. *FEMS Immunol Med Microbiol, 24*(1), 57-62. doi:10.1111/j.1574-695X.1999.tb01265.x

d'Herelle, F. (1918). Sur le rôle du microbe filtrant bactériophage dans la dysentérie bacillaire. *CR Acad Sci, 167*, 970.

Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta, 1794*(5), 808-816. doi:10.1016/j.bbapap.2008.11.005

Deresinski, S. (2009). Bacteriophage therapy: exploiting smaller fleas. *Clinical Infectious Diseases, 48*(8), 1096-1101.

Destoumieux-Garzón, D., Duquesne, S., Peduzzi, J., Goulard, C., Desmadril, M., Letellier, L., Rebuffat, S., & Boulanger, P. (2005). The iron–siderophore transporter FhuA is the receptor for the antimicrobial peptide microcin J25: role of the microcin Val11–Pro16 β-hairpin region in the recognition mechanism. *Biochemical Journal, 389*(3), 869-876.

Doughty, S., Sloan, J., Bennett-Wood, V., Robertson, M., Robins-Browne, R. M., & Hartland, E. L. (2002). Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. *Infection and immunity, 70*(12), 6761-6769.

Duckworth, D. H. (1976). " Who discovered bacteriophage?". *Bacteriological reviews, 40*(4), 793.

Dunne, M., Hupfeld, M., Klumpp, J., & Loessner, M. J. (2018). Molecular Basis of Bacterial Host Interactions by Gram-Positive Targeting Bacteriophages. *Viruses, 10*(8), 397. doi:10.3390/v10080397

Dupree, D. E., Price, R. E., Burgess, B. A., Andress, E. L., & Breidt, F. (2019). Effects of sodium chloride or calcium chloride concentration on the growth and survival of Escherichia coli O157: H7 in model vegetable fermentations. *Journal of Food Protection, 82*(4), 570-578.

EFSA, & ECDC. (2019). The European Union one health 2018 zoonoses report. *EFSA Journal, 17*(12), e05926.

El Haddad, L., Harb, C. P., Gebara, M. A., Stibich, M. A., & Chemaly, R. F. (2019). A Systematic and Critical Review of Bacteriophage Therapy Against Multidrug-resistant ESKAPE Organisms in Humans. *Clin Infect Dis, 69*(1), 167-178. doi:10.1093/cid/ciy947

Endriss, F., & Braun, V. (2004). Loop deletions indicate regions important for FhuA transport and receptor functions in Escherichia coli. *J Bacteriol, 186*(14), 4818-4823. doi:10.1128/JB.186.14.4818-4823.2004

Farfan, M. J., Cantero, L., Vidal, R., Botkin, D. J., & Torres, A. G. (2011). Long polar fimbriae of enterohemorrhagic *Escherichia coli* O157:H7 bind to extracellular matrix proteins. *Infection and immunity, 79*(9), 3744-3750.

Faruque, S. M., & Mekalanos, J. J. (2012). Phage-bacterial interactions in the evolution of toxigenic Vibrio cholerae. *Virulence, 3*(7), 556-565.

FDA. (2017a). CFR-code of federal regulations title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=186.1797&SearchTerm=sodium%20sulfate#:~:text=(a)%20sodium%20sulfate%20(Na2SO4,No.&text=It%20is%20prepared%20by%20the,used%20for%20dry%20food%20packaging. Accesed date:[December 4th, 2020]

FDA. (2017b). Retail food protection: Employee health and personal hygiene handbook. Retrieved from https://www.fda.gov/media/77065/download. Accesed date:[Septerber 3rd, 2020]

Feige, U., & Stirm, S. (1976). On the structure of the Escherichia coli C cell wall lipopolysaccharide core and on its øX174 receptor region. *Biochemical and biophysical research communications, 71*(2), 566-573.

Filippov, A. A., Sergueev, K. V., He, Y., Huang, X. Z., Gnade, B. T., Mueller, A. J., Fernandez-Prada, C. M., & Nikolich, M. P. (2011). Bacteriophage-resistant mutants in Yersinia pestis: identification of phage receptors and attenuation for mice. *PLoS One, 6*(9), e25486. doi:10.1371/journal.pone.0025486

Fong, K., Mu, K., Rheault, J.-G., Levesque, R. C., Kitts, D. D., Delaquis, P., Goodridge, L., & Wang, S. (2020). Bacteriophage-Insensitive Mutants of Antimicrobial-Resistant Salmonella Enterica are Altered in their Tetracycline Resistance and Virulence in Caco-2 Intestinal Cells. *International journal of molecular sciences, 21*(5), 1883.

Food and Drug Administration. Code of Federal Regulations Title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.822. Accesed date:[Apr 16, 2020]

Food Standard Agency. (2020). Approved Additives and their E Numbers. *Food Standard Agency, London.* Retrieved from https://www.food.gov.uk/business-guidance/approved-additives-and-e-numbers. Accesed date:[December 4th, 2020]

Forst, S., Delgado, J., Ramakrishnan, G., & Inouye, M. (1988). Regulation of ompC and ompF expression in Escherichia coli in the absence of envZ. *J Bacteriol, 170*(11), 5080-5085. doi:10.1128/jb.170.11.5080-5085.1988

Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., & Spode, A. (2011). Epidemic profile of Shiga-toxin–producing *Escherichia coli* O104:H4 outbreak in Germany. *New England Journal of Medicine, 365*(19), 1771-1780.

Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B., & Knutton, S. (1998). Enteropathogenic and enterohaemorrhagic Escherichia coli: more subversive elements. *Molecular microbiology, 30*(5), 911-921.

Fuller, C. A., Pellino, C. A., Flagler, M. J., Strasser, J. E., & Weiss, A. A. (2011). Shiga toxin subtypes display dramatic differences in potency. *Infection and immunity, 79*(3), 1329-1337.

Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Los, M., Thomas, M. S., & Wegrzyn, G. (2002). The Cell Surface Protein Ag43 Facilitates Phage Infection of *Escherichia coli* in the Presence of Bile Salts and Carbohydrates. *Microbiology, 148*(Pt 5), 1533-1542. doi:10.1099/00221287-148-5-1533

Galardini, M., Mengoni, A., Biondi, E. G., Semeraro, R., Florio, A., Bazzicalupo, M., Benedetti, A., & Mocali, S. (2014). DuctApe: a suite for the analysis and correlation of genomic and OmniLog Phenotype Microarray data. *Genomics, 103*(1), 1-10. doi:10.1016/j.ygeno.2013.11.005

Gao, L., Hu, J., Zhang, X., Wei, L., Li, S., Miao, Z., & Chai, T. (2015). Application of Swine Manure on Agricultural Ffields Contributes to Extended-spectrum Beta-lactamase-producing *Escherichia coli* Spread in Tai'an, China. *Front Microbiol, 6*, 313. doi:10.3389/fmicb.2015.00313

Ghosh, C., Sarkar, P., Issa, R., & Haldar, J. (2019). Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance. *Trends Microbiol, 27*(4), 323-338. doi:10.1016/j.tim.2018.12.010

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int J Food Microbiol, 134*(1-2), 37-45. doi:10.1016/j.ijfoodmicro.2009.05.021

Gill, J., & Abedon, S. T. (2003). Bacteriophage ecology and plants. *APSnet Feature*, 1-17.

Gilmore, B. F. (2012). Bacteriophages as anti-infective agents: recent developments and regulatory challenges. *Expert review of anti-infective therapy, 10*(5), 533-535.

Girgis, H. S., Liu, Y., Ryu, W. S., & Tavazoie, S. (2007). A comprehensive genetic characterization of bacterial motility. *PLoS Genet, 3*(9), 1644-1660. doi:10.1371/journal.pgen.0030154

Goerke, C., Koller, J., & Wolz, C. (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. *Antimicrob Agents Chemother, 50*(1), 171-177. doi:10.1128/AAC.50.1.171-177.2006

Goodridge, L., & Abedon, S. T. (2003). Bacteriophage biocontrol and bioprocessing: application of phage therapy to industry. *SIM news, 53*(6), 254-262.

Goodridge, L., Gallaccio, A., & Griffiths, M. W. (2003). Morphological, host range, and genetic characterization of two coliphages. *Appl Environ Microbiol, 69*(9), 5364-5371. doi:10.1128/aem.69.9.5364-5371.2003

Greer, G. G. (2005). Bacteriophage Control of Foodborne Bacteria. *Journal of Food Protection, 68*(5), 1102-1111.

Grys, T. E., Siegel, M. B., Lathem, W. W., & Welch, R. A. (2005). The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. *Infection and immunity, 73*(3), 1295-1303.

Gunn, J. S. (2000). Mechanisms of bacterial resistance and response to bile. *Microbes and infection, 2*(8), 907-913.

Hammad, A. (1998). Evaluation of alginate-encapsulated Azotobacter chroococcum as a phage-resistant and an effective inoculum. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 38*(1), 9-16.

Hanlon, G. W., Denyer, S. P., Olliff, C. J., & Ibrahim, L. J. (2001). Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through Pseudomonas aeruginosa biofilms. *Appl. Environ. Microbiol., 67*(6), 2746-2753.

Hatfull, G. F., & Hendrix, R. W. (2011). Bacteriophages and their genomes. *Current opinion in virology, 1*(4), 298-303.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., Praet, N., Bellinger, D. C., de Silva, N. R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F. J., Devleesschauwer, B., & World Health Organization Foodborne Disease Burden Epidemiology Reference, G. (2015). World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Med, 12*(12), e1001923. doi:10.1371/journal.pmed.1001923

Heiman, K. E., Mody, R. K., Johnson, S. D., Griffin, P. M., & Gould, L. H. (2015). *Escherichia coli* O157 outbreaks in the United States, 2003–2012. *Emerging infectious diseases, 21*(8), 1293.

Heller, K., & Braun, V. (1982). Polymannose O-antigens of Escherichia coli, the binding sites for the reversible adsorption of bacteriophage T5+ via the L-shaped tail fibers. *J Virol, 41*(1), 222-227.

Ho, T. D., Davis, B. M., Ritchie, J. M., & Waldor, M. K. (2008). Type 2 secretion promotes enterohemorrhagic *Escherichia coli* adherence and intestinal colonization. *Infection and immunity, 76*(5), 1858-1865.

Hobbs, Z., & Abedon, S. T. (2016). Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. *FEMS microbiology letters, 363*(7).

Hoffman, S., Maculloch, B., & Batz, M. (2015). *Economic burden of major foodborne illnesses acquired in the United States*. Retrieved from

Hosseinidoust, Z., Tufenkji, N., & van de Ven, T. G. (2013). Formation of biofilms under phage predation: considerations concerning a biofilm increase. *Biofouling, 29*(4), 457-468.

Hu, B., Margolin, W., Molineux, I. J., & Liu, J. (2015). Structural remodeling of bacteriophage T4 and host membranes during infection initiation. *Proceedings of the National Academy of Sciences, 112*(35), E4919-E4928.

Hussein, H. (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *Journal of animal science, 85*(suppl_13), E63-E72.

Hwang, C.-A., & Juneja, V. (2011). Effects of salt, sodium pyrophosphate, and sodium lactate on the probability of growth of Escherichia coli O157: H7 in ground beef. *Journal of Food Protection, 74*(4), 622-626.

International Agency for Research on Cancer. (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. In *Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances*.
Janka, A., Bielaszewska, M., Dobrindt, U., & Karch, H. (2002). Identification and distribution of the enterohemorrhagic *Escherichia coli* factor for adherence (efa1) gene in sorbitol-fermenting *Escherichia coli* O157:H−. *International Journal of Medical Microbiology, 292*(3-4), 207-214.
Jeong, H., Barbe, V., Lee, C. H., Vallenet, D., Yu, D. S., Choi, S. H., Couloux, A., Lee, S. W., Yoon, S. H., Cattolico, L., Hur, C. G., Park, H. S., Segurens, B., Kim, S. C., Oh, T. K., Lenski, R. E., Studier, F. W., Daegelen, P., & Kim, J. F. (2009). Genome sequences of Escherichia coli B strains REL606 and BL21(DE3). *J Mol Biol, 394*(4), 644-652. doi:10.1016/j.jmb.2009.09.052
Kadner, R. J., & Watson, W. J. (1974). Methionine transport in Escherichia coli: physiological and genetic evidence for two uptake systems. *Journal of bacteriology, 119*(2), 401-409.
Kadner, R. J. (1977). Transport and utilization of D-methionine and other methionine sources in Escherichia coli. *Journal of bacteriology, 129*(1), 207-216.
Karam, J. D., & Drake, J. W. (1994). *Molecular Biology of Bacteriophage* (Vol. 4): American Society for Microbiology. Washington DC, USA.
Karmali, M. A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., Isaac-Renton, J., Clark, C., Rahn, K., & Kaper, J. B. (2003). Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *Journal of Clinical Microbiology, 41*(11), 4930-4940.
Karmali, M. A., Gannon, V., & Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet Microbiol, 140*(3-4), 360-370. doi:10.1016/j.vetmic.2009.04.011
Kasman, L. M., Kasman, A., Westwater, C., Dolan, J., Schmidt, M. G., & Norris, J. S. (2002). Overcoming the Phage Replication Threshold: A Mathematical Model with Implications for Phage Therapy. *J Virol, 76*(11), 5557-5564. doi:10.1128/jvi.76.11.5557-5564.2002
Kaufmann, A., Stierhof, Y. D., & Henning, U. (1994). New outer membrane-associated protease of Escherichia coli K-12. *J Bacteriol, 176*(2), 359-367. doi:10.1128/jb.176.2.359-367.1994
Kazi, M., & Annapure, U. S. (2016). Bacteriophage biocontrol of foodborne pathogens. *J Food Sci Technol, 53*(3), 1355-1362. doi:10.1007/s13197-015-1996-8
Killmann, H., Videnov, G., Jung, G., Schwarz, H., & Braun, V. (1995). Identification of receptor binding sites by competitive peptide mapping: phages T1, T5, and phi 80 and colicin M bind to the gating loop of FhuA. *J Bacteriol, 177*(3), 694-698. doi:10.1128/jb.177.3.694-698.1995
Kim, M., & Ryu, S. (2012). Spontaneous and transient defence against bacteriophage by phase-variable glucosylation of O-antigen in S almonella enterica serovar T yphimurium. *Molecular microbiology, 86*(2), 411-425.
Kirk, M. D., Angulo, F. J., Havelaar, A. H., & Black, R. E. (2017). Diarrhoeal disease in children due to contaminated food. *Bulletin of the World Health Organization, 95*(3), 233.
Kita, E., Yunou, Y., Kurioka, T., Harada, H., Yoshikawa, S., Mikasa, K., & Higashi, N. (2000). Pathogenic Mechanism of Mouse Brain Damage Caused by Oral Infection with Shiga Toxin-Producing *Escherichia coli* O157:H7. *Infection and immunity, 68*(3), 1207-1214.

Koebnik, R., Locher, K. P., & Van Gelder, P. (2000). Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol Microbiol, 37*(2), 239-253. doi:10.1046/j.1365-2958.2000.01983.x

Kondou, Y., Kitazawa, D., Takeda, S., Tsuchiya, Y., Yamashita, E., Mizuguchi, M., Kawano, K., & Tsukihara, T. (2005). Structure of the central hub of bacteriophage Mu baseplate determined by X-ray crystallography of gp44. *Journal of molecular biology, 352*(4), 976-985.

Konowalchuk, J., Speirs, J., & Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. *Infection and immunity, 18*(3), 775-779.

Koo, H. L., Jiang, Z.-D., Brown, E., Garcia, C., Qi, H., & DuPont, H. L. (2008). Coliform contamination of vegetables obtained from popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Clinical Infectious Diseases, 47*(2), 221-221.

Korteland, J., Tommassen, J., & Lugtenberg, B. (1982). PhoE protein pore of the outer membrane of Escherichia coli K12 is a particularly efficient channel for organic and inorganic phosphate. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 690*(2), 282-289.

Koskella, B., & Brockhurst, M. A. (2014). Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS microbiology reviews, 38*(5), 916-931.

Kropinski, A. M. (2009). Measurement of the Rate of Attachment of Bacteriophage to Cells. In *Bacteriophages* (pp. 151-155): Springer. Berlin, Germany.

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. In *Bacteriophages* (pp. 69-76): Springer. Berlin, Germany.

Krueger, A. P. (1931). The Sorption of Bacteriophage by Living and Dead Susceptible Bacteria. *J Gen Physiol, 14*(4), 493-516. doi:10.1085/jgp.14.4.493

Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., & Hovde, C. J. (1999). Biocontrol of Escherichia coli O157 with O157-specific bacteriophages. *Applied and environmental microbiology, 65*(9), 3767-3773.

Kumar, A., Taneja, N., Kumar, Y., & Sharma, M. (2012). Detection of S higa toxin variants among S higa toxin–forming *E scherichia coli* isolates from animal stool, meat and human stool samples in I ndia. *Journal of applied microbiology, 113*(5), 1208-1216.

Kutateladze, M. (2015). Experience of the Eliava Institute in bacteriophage therapy. *Virol Sin, 30*(1), 80-81. doi:10.1007/s12250-014-3557-0

Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat Rev Microbiol, 8*(5), 317-327. doi:10.1038/nrmicro2315

Lacqua, A., Wanner, O., Colangelo, T., Martinotti, M. G., & Landini, P. (2006). Emergence of biofilm-forming subpopulations upon exposure of Escherichia coli to environmental bacteriophages. *Applied and environmental microbiology, 72*(1), 956-959.

Lairson, L. L., Henrissat, B., Davies, G. J., & Withers, S. G. (2008). Glycosyltransferases: Structures, Functions, and Mechanisms. *Annu Rev Biochem, 77*, 521-555. doi:10.1146/annurev.biochem.76.061005.092322

Lander, G. C., Khayat, R., Li, R., Prevelige, P. E., Potter, C. S., Carragher, B., & Johnson, J. E. (2009). The P22 tail machine at subnanometer resolution reveals the architecture of an infection conduit. *Structure, 17*(6), 789-799.

Lathem, W. W., Grys, T. E., Witowski, S. E., Torres, A. G., Kaper, J. B., Tarr, P. I., & Welch, R. A. (2002). StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Molecular microbiology, 45*(2), 277-288.

Le, S., Yao, X., Lu, S., Tan, Y., Rao, X., Li, M., Jin, X., Wang, J., Zhao, Y., Wu, N. C., Lux, R., He, X., Shi, W., & Hu, F. (2014). Chromosomal DNA deletion confers phage resistance to Pseudomonas aeruginosa. *Sci Rep, 4*, 4738. doi:10.1038/srep04738

Leiman, P. G., Arisaka, F., Van Raaij, M. J., Kostyuchenko, V. A., Aksyuk, A. A., Kanamaru, S., & Rossmann, M. G. (2010). Morphogenesis of the T4 tail and tail fibers. *Virology journal, 7*(1), 1-28.

Leon, M., & Bastias, R. (2015). Virulence reduction in bacteriophage resistant bacteria. *Front Microbiol, 6*, 343. doi:10.3389/fmicb.2015.00343

Letarov, A. V., & Kulikov, E. E. (2017). Adsorption of Bacteriophages on Bacterial Cells. *Biochemistry (Mosc), 82*(13), 1632-1658. doi:10.1134/S0006297917130053

Levine, M. M., Xu, J.-g., Kaper, J. B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H., & Wachsmuth, K. (1987). A DNA probe to identify enterohemorrhagic *Escherichia coli* of 0157: H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *Journal of Infectious Diseases, 156*(1), 175-182.

Lewis, R., & Hill, C. (2020). Overcoming barriers to phage application in food and feed. *Curr Opin Biotechnol, 61*, 38-44. doi:10.1016/j.copbio.2019.09.018

Lhuillier, S., Gallopin, M., Gilquin, B., Brasilès, S., Lancelot, N., Letellier, G., Gilles, M., Dethan, G., Orlova, E. V., & Couprie, J. (2009). Structure of bacteriophage SPP1 head-to-tail connection reveals mechanism for viral DNA gating. *Proceedings of the National Academy of Sciences, 106*(21), 8507-8512.

Liao, W. C., Ng, W. V., Lin, I. H., Syu, W. J., Liu, T. T., & Chang, C. H. (2011). T4-Like genome organization of the Escherichia coli O157:H7 lytic phage AR1. *J Virol, 85*(13), 6567-6578. doi:10.1128/JVI.02378-10

Liebau, J., Pettersson, P., Szpryngiel, S., & Maler, L. (2015). Membrane Interaction of the Glycosyltransferase WaaG. *Biophys J, 109*(3), 552-563. doi:10.1016/j.bpj.2015.06.036

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of microbiology and biotechnology, 20*(1), 5.

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage Therapy: An Alternative to Antibiotics in the Age of Multi-drug Resistance. *World J Gastrointest Pharmacol Ther, 8*(3), 162-173. doi:10.4292/wjgpt.v8.i3.162

Liu, C. G., Green, S. I., Min, L., Clark, J. R., Salazar, K. C., Terwilliger, A. L., Kaplan, H., Trautner, B., Ramig, R. F., & Maresso, A. W. (2020). Phage-Antibiotic Synergy Is Driven By A Unique Combination Of Antibacterial Mechanism Of Action And Stoichiometry. *bioRxiv*.

Liu, X., Turchi, B., Mok, K. C., Taga, M. E., & Miller, M. J. (2017). HM2-phage resistant solventogenic Clostridium saccharoperbutylacetonicum N1-4 shows increased exopolysaccharide production. *FEMS microbiology letters, 364*(19).

Lu, Z., & Breidt, F. (2015). Escherichia coli O157: H7 bacteriophage Φ241 isolated from an industrial cucumber fermentation at high acidity and salinity. *Frontiers in microbiology, 6*, 67.

Luck, S. N., Bennett-Wood, V., Poon, R., Robins-Browne, R. M., & Hartland, E. L. (2005). Invasion of epithelial cells by locus of enterocyte effacement-negative enterohemorrhagic *Escherichia coli*. *Infection and immunity, 73*(5), 3063-3071.

Luck, S. N., Badea, L., Bennett-Wood, V., Robins-Browne, R., & Hartland, E. L. (2006). Contribution of FliC to epithelial cell invasion by enterohemorrhagic *Escherichia coli* O113:H21. *Infection and immunity, 74*(12), 6999-7004.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. *Nucleic Acids Res, 47*(W1), W636-W641. doi:10.1093/nar/gkz268

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., Yeung, D. H., & Kirk, M. D. (2014). Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog Dis, 11*(6), 447-455. doi:10.1089/fpd.2013.1704

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules, 23*(4), 795.

Mathews, C. K. (1983). *Bacteriophage T4*: Wiley Online Library.

Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., Wakiguchi, H., & Imai, S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *J Infect Chemother, 11*(5), 211-219. doi:10.1007/s10156-005-0408-9

McArthur, F., Andersson, C. E., Loutet, S., Mowbray, S. L., & Valvano, M. A. (2005). Functional analysis of the glycero-manno-heptose 7-phosphate kinase domain from the bifunctional HldE protein, which is involved in ADP-L-glycero-D-manno-heptose biosynthesis. *Journal of bacteriology, 187*(15), 5292-5300.

McDanel, J., Schweizer, M., Crabb, V., Nelson, R., Samore, M., Khader, K., Blevins, A. E., Diekema, D., Chiang, H. Y., Nair, R., & Perencevich, E. (2017). Incidence of Extended-Spectrum beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella* Infections in the United States: A Systematic Literature Review. *Infect Control Hosp Epidemiol, 38*(10), 1209-1215. doi:10.1017/ice.2017.156

Melton-Celsa, A. R. (2015). Shiga toxin (Stx) classification, structure, and function. *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli*, 37-53.

Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2006). Growing and analyzing static biofilms. *Current protocols in microbiology*, 1B. 1.1-1B. 1.17.

Meza-Segura, M., Zaidi, M. B., Maldonado-Puga, S., Huerta-Cantillo, J., Chavez-Dueñas, L., Navarro-Garcia, F., & Estrada-Garcia, T. (2017). Cytolethal Distending Toxin-producing *Escherichia coli* Strains Causing Severe Diarrhoea in Young Mexican Children. *JMM Case Reports, 4*(2).

Miller, E. S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T., & Rüger, W. (2003). Bacteriophage T4 genome. *Microbiology and Molecular Biology Reviews, 67*(1), 86-156.

Mills, S., Griffin, C., Coffey, A., Meijer, W., Hafkamp, B., & Ross, R. (2010). CRISPR analysis of bacteriophage-insensitive mutants (BIMs) of industrial Streptococcus thermophilus–implications for starter design. *Journal of applied microbiology, 108*(3), 945-955.

Miyamoto, Y., Iimura, M., Kaper, J. B., Torres, A. G., & Kagnoff, M. F. (2006). Role of Shiga toxin versus H7 flagellin in enterohaemorrhagic Escherichia coli signalling of human colon epithelium in vivo. *Cellular microbiology, 8*(5), 869-879.

Mizoguchi, K., Morita, M., Fischer, C. R., Yoichi, M., Tanji, Y., & Unno, H. (2003). Coevolution of bacteriophage PP01 and Escherichia coli O157:H7 in continuous culture. *Appl Environ Microbiol, 69*(1), 170-176. doi:10.1128/aem.69.1.170-176.2003

Morona, R., Klose, M., & Henning, U. (1984). Escherichia coli K-12 outer membrane protein (OmpA) as a bacteriophage receptor: analysis of mutant genes expressing altered proteins. *Journal of bacteriology, 159*(2), 570-578.

Motto, D. G., Chauhan, A. K., Zhu, G., Homeister, J., Lamb, C. B., Desch, K. C., Zhang, W., Tsai, H.-M., Wagner, D. D., & Ginsburg, D. (2005). Shiga toxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *The Journal of clinical investigation, 115*(10), 2752-2761.

Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. *Viruses, 10*(4), 205. doi:10.3390/v10040205

Muszyński, A., Laus, M., Kijne, J. W., & Carlson, R. W. (2011). Structures of the lipopolysaccharides from Rhizobium leguminosarum RBL5523 and its UDP-glucose dehydrogenase mutant (exo5). *Glycobiology, 21*(1), 55-68.

Nakao, R., Ramstedt, M., Wai, S. N., & Uhlin, B. E. (2012). Enhanced biofilm formation by Escherichia coli LPS mutants defective in Hep biosynthesis. *PLoS One, 7*(12), e51241. doi:10.1371/journal.pone.0051241

Nesper, J., Lauriano, C. M., Klose, K. E., Kapfhammer, D., Kraiß, A., & Reidl, J. (2001). Characterization of Vibrio cholerae O1 El TorgalU and galE Mutants: Influence on lipopolysaccharide structure, colonization, and biofilm formation. *Infection and immunity, 69*(1), 435-445.

Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol Mol Biol Rev, 67*(4), 593-656. doi:10.1128/mmbr.67.4.593-656.2003

Nikaido, H. (2005). Restoring permeability barrier function to outer membrane. *Chem Biol, 12*(5), 507-509. doi:10.1016/j.chembiol.2005.05.001

Nordstrom, K., & Forsgren, A. (1974). Effect of protein A on adsorption of bacteriophages to Staphylococcus aureus. *J Virol, 14*(2), 198-202.

Nyachuba, D. G. (2010). Foodborne illness: is it on the rise? *Nutrition reviews, 68*(5), 257-269.

O'Brien, A. D., Newland, J. W., Miller, S. F., Holmes, R. K., Smith, H. W., & Formal, S. B. (1984). Shiga-like toxin-converting phages from Escherichia coli strains that cause hemorrhagic colitis or infantile diarrhea. *Science, 226*(4675), 694-696.

O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of Escherichia coli O157:H7. *Appl Environ Microbiol, 70*(6), 3417-3424. doi:10.1128/AEM.70.6.3417-3424.2004

O'Neill, J. (2016). Tackling Drug-resistant Infections Globally: Final Report and Recommendations. *UK Government and Wellcome Trust*.

Oechslin, F. (2018). Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. *Viruses, 10*(7), 351. doi:10.3390/v10070351

Ogura, Y., Ooka, T., Iguchi, A., Toh, H., Asadulghani, M., Oshima, K., Kodama, T., Abe, H., Nakayama, K., & Kurokawa, K. (2009). Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Proceedings of the National Academy of Sciences, 106*(42), 17939-17944.

Orth, D., Ehrlenbach, S., Brockmeyer, J., Khan, A. B., Huber, G., Karch, H., Sarg, B., Lindner, H., & Würzner, R. (2010). EspP, a serine protease of enterohemorrhagic *Escherichia coli*,

impairs complement activation by cleaving complement factors C3/C3b and C5. *Infection and immunity, 78*(10), 4294-4301.
Otsuji, N., Sekiguchi, M., Iijima, T., & Takagi, Y. (1959). Induction of phage formation in the lysogenic Escherichia coli K-12 by mitomycin C. *Nature, 184(Suppl 14)*(4692), 1079-1080. doi:10.1038/1841079b0
Pagnout, C., Sohm, B., Razafitianamaharavo, A., Caillet, C., Offroy, M., Leduc, M., Gendre, H., Jomini, S., Beaussart, A., & Bauda, P. (2019). Pleiotropic effects of rfa-gene mutations on Escherichia coli envelope properties. *Scientific reports, 9*(1), 1-16.
Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., Morabito, S., & Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive E. coli (EIEC) toward pathogenicity. *Frontiers in microbiology, 8*, 2390.
Paton, A. W., Ratcliff, R. M., Doyle, R. M., Seymour-Murray, J., Davos, D., Lanser, J. A., & Paton, J. C. (1996). Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *Journal of Clinical Microbiology, 34*(7), 1622-1627.
Paton, A. W., Srimanote, P., Woodrow, M. C., & Paton, J. C. (2001). Characterization of Saa, a Novel Autoagglutinating Adhesin Produced by Locus of Enterocyte Effacement-Negative Shiga-Toxigenic *Escherichia coli* Strains That Are Virulent for Humans. *Infection and immunity, 69*(11), 6999-7009.
Pedruzzi, I., Rosenbusch, J. P., & Locher, K. P. (1998). Inactivation in vitro of the Escherichia coli outer membrane protein FhuA by a phage T5-encoded lipoprotein. *FEMS Microbiol Lett, 168*(1), 119-125. doi:10.1111/j.1574-6968.1998.tb13264.x
Perera, M. N., Abuladze, T., Li, M., Woolston, J., & Sulakvelidze, A. (2015). Bacteriophage cocktail significantly reduces or eliminates Listeria monocytogenes contamination on lettuce, apples, cheese, smoked salmon and frozen foods. *Food microbiology, 52*, 42-48.
PHAC. (2017). Causes of E. coli (Escherichia coli) infection. Retrieved from https://www.canada.ca/en/public-health/services/diseases/e-coli/causes-e-coli.html. Accesed date:[September 4th, 2020]
Picken, R., & Beacham, I. (1977). Bacteriophage-resistant mutants of Escherichia coliK12. Location of receptors within the lipopolysaccharide. *Microbiology, 102*(2), 305-318.
Pitout, J. D. (2012). Extraintestinal Pathogenic *Escherichia coli*: A Combination of Virulence with Antibiotic Resistance. *Front Microbiol, 3*, 9. doi:10.3389/fmicb.2012.00009
Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial Resistance in *Escherichia coli*. *Microbiol Spectr, 6*(4), 289-316. doi:10.1128/microbiolspec.ARBA-0026-2017
Pollari, F., Christidis, T., Pintar, K. D., Nesbitt, A., Farber, J., Lavoie, M.-C., Gill, A., Kirsch, P., & Johnson, R. P. (2017). Evidence for the benefits of food chain interventions on *E. coli* O157:H7/NM prevalence in retail ground beef and human disease incidence: A success story. *Canadian Journal of Public Health, 108*(1), e71-e78.
Poolman, B., Spitzer, J. J., & Wood, J. M. (2004). Bacterial osmosensing: roles of membrane structure and electrostatics in lipid–protein and protein–protein interactions. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1666*(1-2), 88-104.
Prehm, P., Jann, B., Jann, K., Schmidt, G., & Stirm, S. (1976). On a Bacteriophage T3 and T4 Receptor Region within the Cell Wall Lipopolysaccharide of *Escherichia coli* B. *Journal of molecular biology, 101*(2), 277-281.

Public Health Agency of Canada. (2012). National Enteric Surveillance Program (NESP). In: Winnipeg Manitoba, Canada.
Qadri, F., Svennerholm, A.-M., Faruque, A., & Sack, R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical microbiology reviews, 18*(3), 465-483.
Qin, J., Cui, Y., Zhao, X., Rohde, H., Liang, T., Wolters, M., Li, D., Campos, C. B., Christner, M., & Song, Y. (2011). Identification of the Shiga toxin-producing Escherichia coli O104: H4 strain responsible for a food poisoning outbreak in Germany by PCR. *Journal of Clinical Microbiology, 49*(9), 3439-3440.
Rakhuba, D., Kolomiets, E., Dey, E. S., & Novik, G. (2010). Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol. J. Microbiol, 59*(3), 145-155.
Randall-Hazelbauer, L., & Schwartz, M. (1973). Isolation of the bacteriophage lambda receptor from Escherichia coli. *J Bacteriol, 116*(3), 1436-1446.
Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerging infectious diseases, 11*(4), 603.
Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial Drug Resistance in Strains of *Escherichia coli* Isolated from Food Sources. *Rev Inst Med Trop Sao Paulo, 56*(4), 341-346. doi:10.1590/s0036-46652014000400012
Ratnam, S., March, S., Ahmed, R., Bezanson, G., & Kasatiya, S. (1988). Characterization of *Escherichia coli* serotype O157:H7. *Journal of Clinical Microbiology, 26*(10), 2006-2012.
Riede, I. (1987). Receptor specificity of the short tail fibres (gp12) of T-even type Escherichia coli phages. *Mol Gen Genet, 206*(1), 110-115. doi:10.1007/BF00326544
Ripp, S., & Miller, R. V. (1997). The role of pseudolysogeny in bacteriophage-host interactions in a natural freshwater environment. *Microbiology, 143*(6), 2065-2070.
Rodionova, I. A., Zhang, Z., Mehla, J., Goodacre, N., Babu, M., Emili, A., Uetz, P., & Saier, M. H. (2017). The phosphocarrier protein HPr of the bacterial phosphotransferase system globally regulates energy metabolism by directly interacting with multiple enzymes in Escherichia coli. *Journal of Biological Chemistry, 292*(34), 14250-14257.
Rodionova, I. A., Goodacre, N., Babu, M., Emili, A., Uetz, P., & Saier, M. H. (2018). The nitrogen regulatory PII protein (GlnB) and N-acetylglucosamine 6-phosphate epimerase (NanE) allosterically activate glucosamine 6-phosphate deaminase (NagB) in Escherichia coli. *Journal of bacteriology, 200*(5).
Rohwer, F., & Edwards, R. (2002). The Phage Proteomic Tree: a genome-based taxonomy for phage. *J Bacteriol, 184*(16), 4529-4535. doi:10.1128/jb.184.16.4529-4535.2002
Ronner, A. B., & Cliver, D. O. (1990). Isolation and Characterization of a Coliphage Specific for Escherichia coli 0157: H7. *Journal of Food Protection, 53*(11), 944-947.
Rossi, E., Cimdins, A., Lüthje, P., Brauner, A., Sjöling, Å., Landini, P., & Römling, U. (2018). "It'sa gut feeling"–Escherichia coli biofilm formation in the gastrointestinal tract environment. *Critical reviews in microbiology, 44*(1), 1-30.
Saeed, F., Afzaal, M., Tufail, T., & Ahmad, A. (2019). Use of Natural Antimicrobial Agents: A Safe Preservation Approach. *Act. Antimicrob. Food Packag*.
Salmond, G. P., & Fineran, P. C. (2015). A century of the phage: past, present and future. *Nat Rev Microbiol, 13*(12), 777-786. doi:10.1038/nrmicro3564

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*: Cold spring harbor laboratory press. Cold Spring Harbor, USA.
Samson, J. E., Magadán, A. H., Sabri, M., & Moineau, S. (2013). Revenge of the phages: defeating bacterial defences. *Nature Reviews Microbiology, 11*(10), 675-687.
Santander, J., Martin, T., Loh, A., Pohlenz, C., Gatlin III, D. M., & Curtiss III, R. (2013). Mechanisms of intrinsic resistance to antimicrobial peptides of Edwardsiella ictaluri and its influence on fish gut inflammation and virulence. *Microbiology, 159*(Pt 7), 1471.
Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging infectious diseases, 17*(1), 7.
Scanlan, P. D., Bischofberger, A. M., & Hall, A. R. (2017). Modification of *Escherichia coli*-Bacteriophage Interactions by Surfactants and Antibiotics *in vitro*. *FEMS Microbiol Ecol, 93*(1). doi:10.1093/femsec/fiw211
Schmidt, H., Beutin, L., & Karch, H. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. *Infection and immunity, 63*(3), 1055-1061.
Schmidt, H., Henkel, B., & Karch, H. (1997). A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. *FEMS microbiology letters, 148*(2), 265-272.
Schnaitman, C. A., & Klena, J. D. (1993). Genetics of lipopolysaccharide biosynthesis in enteric bacteria. *Microbiology and Molecular Biology Reviews, 57*(3), 655-682.
Scholl, D., Adhya, S., & Merril, C. (2005). Escherichia coli K1's capsule is a barrier to bacteriophage T7. *Appl. Environ. Microbiol., 71*(8), 4872-4874.
Sciara, G., Bebeacua, C., Bron, P., Tremblay, D., Ortiz-Lombardia, M., Lichière, J., van Heel, M., Campanacci, V., Moineau, S., & Cambillau, C. (2010). Structure of lactococcal phage p2 baseplate and its mechanism of activation. *Proceedings of the National Academy of Sciences, 107*(15), 6852-6857.
Scuron, M. D., Boesze-Battaglia, K., Dlakić, M., & Shenker, B. J. (2016). The Cytolethal Distending Toxin Contributes to Microbial Virulence and Disease Pathogenesis by Acting as A Tri-perditious Toxin. *Frontiers in cellular and infection microbiology, 6*, 168.
Sharma, M., Patel, J. R., Conway, W. S., Ferguson, S., & Sulakvelidze, A. (2009). Effectiveness of bacteriophages in reducing Escherichia coli O157: H7 on fresh-cut cantaloupes and lettuce. *Journal of Food Protection, 72*(7), 1481-1485.
Shepherd, A. K., & Pottinger, P. S. (2013). Management of Urinary Tract Infections in the Era of Increasing Antimicrobial Resistance. *Med Clin North Am, 97*(4), 737-757, xii. doi:10.1016/j.mcna.2013.03.006
Sillankorva, S. M., Oliveira, H., & Azeredo, J. (2012). Bacteriophages and their role in food safety. *International journal of microbiology, 2012*.
Smith, H. W., & Huggins, M. B. (1982). Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotics. *Microbiology, 128*(2), 307-318.
Smith, H. W., & Huggins, M. B. (1983). Effectiveness of phages in treating experimental Escherichia coli diarrhoea in calves, piglets and lambs. *J Gen Microbiol, 129*(8), 2659-2675. doi:10.1099/00221287-129-8-2659

Stevens, M. P., Roe, A. J., Vlisidou, I., Van Diemen, P. M., La Ragione, R. M., Best, A., Woodward, M. J., Gally, D. L., & Wallis, T. S. (2004). Mutation of toxB and a truncated version of the efa-1 gene in *Escherichia coli* O157:H7 influences the expression and secretion of locus of enterocyte effacement-encoded proteins but not intestinal colonization in calves or sheep. *Infection and immunity, 72*(9), 5402-5411.

Stirm, S. (1968). Escherichia coli K bacteriophages. I. Isolation and introductory characterization of five Escherichia coli K bacteriophages. *J Virol, 2*(10), 1107-1114.

Strockbine, N. A., Marques, L. R., Newland, J. W., Smith, H. W., Holmes, R. K., & O'Brien, A. D. (1986). Two toxin-converting phages from Escherichia coli O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect Immun, 53*(1), 135-140.

Sulakvelidze, A., Alavidze, Z., & Morris, J. G., Jr. (2001). Bacteriophage therapy. *Antimicrob Agents Chemother, 45*(3), 649-659. doi:10.1128/AAC.45.3.649-659.2001

Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V., & Force, C. P. T. (2001). PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerging infectious diseases, 7*(3), 382.

Tamaki, S., Sato, T., & Matsuhashi, M. (1971). Role of Lipopolysaccharides in Antibiotic Resistance and Bacteriophage Adsorption of *Escherichia coli* K-12. *J Bacteriol, 105*(3), 968-975.

Tan, L., & Darby, C. (2005). Yersinia pestis is viable with endotoxin composed of only lipid A. *Journal of bacteriology, 187*(18), 6599-6600.

Tatsuno, I., Horie, M., Abe, H., Miki, T., Makino, K., Shinagawa, H., Taguchi, H., Kamiya, S., Hayashi, T., & Sasakawa, C. (2001). toxB gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. *Infection and immunity, 69*(11), 6660-6669.

Tchieu, J. H., Norris, V., Edwards, J. S., & Saier Jr, M. H. (2001). The complete phosphotransferase system in Escherichia coli. *Journal of molecular microbiology and biotechnology, 3*(3), 329-346.

Thomas, M. K., Vriezen, R., Farber, J. M., Currie, A., Schlech, W., & Fazil, A. (2015). Economic Cost of a Listeria monocytogenes Outbreak in Canada, 2008. *Foodborne Pathog Dis, 12*(12), 966-971. doi:10.1089/fpd.2015.1965

Tilden Jr, J., Young, W., McNamara, A.-M., Custer, C., Boesel, B., Lambert-Fair, M. A., Majkowski, J., Vugia, D., Werner, S., & Hollingsworth, J. (1996). A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American journal of public health, 86*(8_Pt_1), 1142-1145.

Todd, E. (2014). Foodborne diseases: Overview of biological hazards and foodborne diseases. *Encyclopedia of Food Safety*, 221.

Toivonen, P. M., & Lu, C. (2013). Differential quenching of free chlorine by organic compounds potentially exuded from injured plant tissues. *Postharvest biology and technology, 86*, 192-194.

Totten, S., Medaglia, A., & McDermott, S. (2019). Updates to Canadian Notifiable Disease Surveillance System.

Tritt, A., Eisen, J. A., Facciotti, M. T., & Darling, A. E. (2012). An Integrated Pipeline for *de novo* Assembly of Microbial Genomes. *PLoS One, 7*(9), e42304. doi:10.1371/journal.pone.0042304

Trofa, A. F., Ueno-Olsen, H., Oiwa, R., & Yoshikawa, M. (1999). Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. *Clinical Infectious Diseases, 29*(5), 1303-1306.
Tzipori, S., Chow, C., & Powell, H. (1988). Cerebral infection with *Escherichia coli* O157:H7 in humans and gnotobiotic piglets. *Journal of clinical pathology, 41*(10), 1099-1103.
Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R. P., & Penades, J. R. (2005). Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol Microbiol, 56*(3), 836-844. doi:10.1111/j.1365-2958.2005.04584.x
Uhlich, G. A. (2009). KatP contributes to OxyR-regulated hydrogen peroxide resistance in *Escherichia coli* serotype O157:H7. *Microbiology, 155*(11), 3589-3598.
USDA. USDA Targeting Six Additional Strains of E.coli in Raw Beef Trim Starting Monday [Press release]. Retrieved from https://www.usda.gov/media/press-releases/2012/05/31/usda-targeting-six-additional-strains-ecoli-raw-beef-trim-starting. Accesed date:[September 4th, 2020]
Vaara, M. (1992). Agents that increase the permeability of the outer membrane. *Microbiological reviews, 56*(3), 395-411.
Valério, N., Oliveira, C., Jesus, V., Branco, T., Pereira, C., Moreirinha, C., & Almeida, A. (2017). Effects of Single and Combined Use of Bacteriophages and Antibiotics to Inactivate *Escherichia coli*. *Virus research, 240*, 8-17.
Van der Ley, P., De Graaff, P., & Tommassen, J. (1986). Shielding of Escherichia coli outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. *Journal of bacteriology, 168*(1), 449-451.
van Deurs, B., & Sandvig, K. (1995). Furin-induced cleavage and activation of Shiga toxin. *Journal of Biological Chemistry, 270*(18), 10817-10821.
Viazis, S., Akhtar, M., Feirtag, J., & Diez-Gonzalez, F. (2011). Reduction of Escherichia coli O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. *Food Microbiol, 28*(1), 149-157. doi:10.1016/j.fm.2010.09.009
Vigil, K. J., Jiang, Z.-D., Chen, J. J., Palumbo, K. L., Galbadage, T., Brown, E. L., Yiang, J., Koo, H., DuPont, M. W., & Ericsson, C. (2009). Coliform and Escherichia coli contamination of desserts served in public restaurants from Guadalajara, Mexico, and Houston, Texas. *The American journal of tropical medicine and hygiene, 80*(4), 606-608.
Wang, Z., Wang, J., Ren, G., Li, Y., & Wang, X. (2015). Influence of Core Oligosaccharide of Lipopolysaccharide to Outer Membrane Behavior of Escherichia coli. *Mar Drugs, 13*(6), 3325-3339. doi:10.3390/md13063325
Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the Interactions between *Escherichia coli* Receptors, LPS and OmpC, and Bacteriophage T4 Long Tail Fibers. *Microbiologyopen, 5*(6), 1003-1015. doi:10.1002/mbo3.384
Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2—A Multiple Sequence Alignment Editor and Analysis Workbench. *Bioinformatics, 25*(9), 1189-1191. doi:10.1093/bioinformatics/btp033
Weber-Dąbrowska, B., Mulczyk, M., & Górski, A. (2001). Bacteriophage therapy of bacterial infections: an update of our institute's experience. In *Inflammation* (pp. 201-209): Springer.

Weidel, W. (1953, June 5th, 1953). *Phage Receptor Systems of Escherichia coli B.* Paper presented at the Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor, USA.
Westphal, O., & Jann, K. (1965). Bacterial Lipopolysaccharides. Methods in Carbohydrate Chemistry. In: Academic Press, Cambridge, USA.
Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A, 95*(12), 6578-6583. doi:10.1073/pnas.95.12.6578
WHO. (2014). *Antimicrobial Resistance: Global Report on Surveillance*: World Health Organization. Geneva, Switzerland.
WHO. (2015a). Global Action Plan on Antimicrobial Resistance. In: World Health Organization. Geneva, Switzerland.
WHO. (2015b). *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015*. Retrieved from
WHO. (2016). *National Antimicrobial Resistance Surveillance Systems and Participation in the Global Antimicrobial Resistance Surveillance System (GLASS): A Guide to Planning, Implementation, and Monitoring and Evaluation*. Retrieved from
WHO. (2019). *Shiga Toxin-producing Escherichia Coli (STEC) and Food: Attribution Characterization and Monitoring* (Vol. 19): World Health Organization.
Wilkinson, R., Jun, P. G., & Stocker, B. (1972). Non-smooth mutants of Salmonella typhimurium: differentiation by phage sensitivity and genetic mapping. *Microbiology, 70*(3), 527-554.
Wirth, C., Condemine, G., Boiteux, C., Bernèche, S., Schirmer, T., & Peneff, C. M. (2009). NanC crystal structure, a model for outer-membrane channels of the acidic sugar-specific KdgM porin family. *Journal of molecular biology, 394*(4), 718-731.
Wolfe, M. M., & Soll, A. H. (1988). The physiology of gastric acid secretion. *New England Journal of Medicine, 319*(26), 1707-1715.
Wolschendorf, F., Mahfoud, M., & Niederweis, M. (2007). Porins are required for uptake of phosphates by Mycobacterium smegmatis. *Journal of bacteriology, 189*(6), 2435-2442.
Xicohtencatl-Cortes, J., Monteiro-Neto, V., Ledesma, M. A., Jordan, D. M., Francetic, O., Kaper, J. B., Puente, J. L., & Girón, J. A. (2007). Intestinal adherence associated with type IV pili of enterohemorrhagic *Escherichia coli* O157:H7. *The Journal of clinical investigation, 117*(11), 3519-3529.
Yethon, J. A., Heinrichs, D. E., Monteiro, M. A., Perry, M. B., & Whitfield, C. (1998). Involvement of waaY, waaQ, andwaaP in the Modification of Escherichia coliLipopolysaccharide and Their Role in the Formation of a Stable Outer Membrane. *Journal of Biological Chemistry, 273*(41), 26310-26316.
Yethon, J. A., Vinogradov, E., Perry, M. B., & Whitfield, C. (2000). Mutation of the Lipopolysaccharide Core Glycosyltransferase Encoded by waaG Destabilizes the Outer Membrane of *Escherichia coli* by Interfering with Core Phosphorylation. *J Bacteriol, 182*(19), 5620-5623. doi:10.1128/jb.182.19.5620-5623.2000
Yi, W., Liu, X., Li, Y., Li, J., Xia, C., Zhou, G., Zhang, W., Zhao, W., Chen, X., & Wang, P. G. (2009). Remodeling Bacterial Polysaccharides by Metabolic Pathway Engineering. *Proc Natl Acad Sci U S A, 106*(11), 4207-4212. doi:10.1073/pnas.0812432106
Yoon, S. H., Han, M. J., Jeong, H., Lee, C. H., Xia, X. X., Lee, D. H., Shim, J. H., Lee, S. Y., Oh, T. K., & Kim, J. F. (2012). Comparative Multi-omics Systems Analysis of

Escherichia coli strains B and K-12. *Genome Biol, 13*(5), R37. doi:10.1186/gb-2012-13-5-r37

York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., & Albersheim, P. (1986). Isolation and Characterization of Plant Cell Walls and Cell Wall Components. In *Methods in enzymology* (Vol. 118, pp. 3-40): Elsevier. Amsterdam, Netherlands.

Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein OmpC of Escherichia coli K-12 in the receptor function for bacteriophage T4. *J Bacteriol, 151*(2), 718-722.

Yu, S., Ko, K., Chen, C., Chang, Y., & Syu, W. (2000). Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1, Which Specifically InfectsEscherichia coli O157: H7. *Journal of bacteriology, 182*(21), 5962-5968.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F. M., & Larsen, M. V. (2012). Identification of Acquired Antimicrobial Resistance Genes. *J Antimicrob Chemother, 67*(11), 2640-2644. doi:10.1093/jac/dks261

Zhong, Z., Emond-Rheault, J.-G., Bhandare, S., Lévesque, R., & Goodridge, L. (2020). Bacteriophage-Induced Lipopolysaccharide Mutations in Escherichia coli Lead to Hypersensitivity to Food Grade Surfactant Sodium Dodecyl Sulfate. *Antibiotics, 9*(9), 552.

Zhong, Z., & Goodridge, L. (2020). *Characterization of Phage AR1 Resistant Escherichia coli O157:H7 Mutants*. Manuscript in preparation.

Zumbrun, S. D., Hanson, L., Sinclair, J. F., Freedy, J., Melton-Celsa, A. R., Rodriguez-Canales, J., Hanson, J. C., & O'Brien, A. D. (2010). Human intestinal tissue and cultured colonic cells contain globotriaosylceramide synthase mRNA and the alternate Shiga toxin receptor globotetraosylceramide. *Infection and immunity, 78*(11), 4488-4499.

Chapter VI

General Conclusions, Contribution to Knowledge and Future Work

6.1 General conclusion

This study was conducted to characterize T4-like bacteriophage insensitive *E. coli* mutants and delineate their subsequent fitness changes, as a cost of phage resistance, by means of genomic and phenotypic approaches. Through the characterizations of mutants developed from a non-pathogenic *E. coli* B strain and a pathogenic *E. coli* O157:H7 isolate, we discovered several key findings, which are reported in this Thesis.

Our results showed that all *E. coli* BIMs conferred resistance to T4 and T4-like phages by blocking the initial attachment step. By mapping the sequences of the parental strains to the derived BIMs, various genes involved in the biosynthesis of phage-specific receptors in the LPS or OmpC were found to contain mutations. These putative mutations were confirmed by phenotypic methods, including phage adsorption assays, LPS glycosyl composition analysis by GC-MS and silver stain, collectively supporting the idea that upon T4-like phage infections, *E. coli* mutants developed phage infection by altering their outer membrane components to which phages attach, and thus aborting phage infection.

Provided that OmpC and LPS are important for maintaining outer membrane integrity and mediating transmembrane diffusion, phage-induced alterations in these structures subsequently led to destabilization and increased permeabilities to substrates, including antibiotics, membrane permeabilizers and surfactants. One of the most striking differences is that BIMs of either *E. coli* B or *E. coli* O157:H7 with truncated LPSs showed hypersensitivity to a food-grade surfactant SDS. This feature incentivized the development of a phage-surfactant synergic treatment, which successfully suppressed the emergence of the phage resistant *E. coli* B

mutant and achieved a 5-log bacterial reduction. In addition, the *E. coli* O157:H7 deep rough mutant ZZb2 exhibits increased sensitivity to the bile salt sodium cholate, which might affect the bacterial survivability in the human intestinal environment. On the other hand, the biofilm formation assays showed that this mutant has the tendency to produce significantly more biofilm at 37°C than the wildtype and another BIM ZZb4, possibly representing an increase of tolerance in diverse environments.

Lastly, the metabolic profiles of wildtypes and BIMs with outer membrane alterations were revealed, showing a variety of compounds which BIMs used differently from its parental strain. The decreased tolerance under osmotic and pH pressure were observed in BIMs ZZa3 and ZZb4, and possibly caused by their disrupted membrane structure. In contrast, BIM ZZb2 with enhanced biofilm production showed elevated activities in metabolizing amino acids under acidic pressure and increased tolerance to two food preservatives.

This study is the first attempt to understand the microbial interaction between bacteriophages and foodborne bacterial pathogens in foods by integrating genomic and phenotypic characterizations. The findings of this study reveal the complex physiological modifications of phage resistant *E. coli* mutants, providing insights of their potential impacts on bacterial survival and virulence, as well as advancing the understanding of the consequence of using phage-based antimicrobials as a food safety mitigation.

6.2 Contribution to knowledge

This work presented here provide evidence for the first time that:

1. Whole genome sequencing and bioinformatic analysis of BIMs identified the specific mutations of genes *ompC*, *waaG*, and *hldE* that lead to resistance to T4-like phages. The putative LPS modifications caused by the genetic changes in bacterial genomes

were also confirmed by phenotypic analysis. These findings correlate the resistance-related mutations in receptors to the altered LPSs on the surface of BIMs, accounting for the lack of phage adsorption.

2. The LPS truncation of *E. coli* BIMs sensitized the cells to various substrates and conferred hypersensitivity to the food-grade surfactant SDS. This finding incentivized the design of a synergic phage-SDS combination which was able to suppress the emergence of *E. coli* B BIMs. This result is indicative of a novel strategy to optimize the antimicrobial effectiveness of phage-based antimicrobials by combining phage application with a sub-optimal amount of SDS.
3. *E. coli* mutants with induced LPS modifications showed increased tendency to produce more biofilm. BIM ZZb2, which contains a HldE mutation, produced significantly more biofilm than the wildtype and another mutant. These findings illustrate that this BIM might have increased colonization ability and tolerance to extracellular stress as a result of elevated biofilm production.
4. The overall metabolism of BIMs were detailed using a high throughput phenotyping method. These results delineate the complex metabolic changes which may define bacterial survivability in dynamic environments like foods.

6.3 Future work

In order to further explore the physiological changes of the isolated BIMs, additional studies should be considered. For example, based on the genomic analysis, three *E. coli* O157:H7 BIMs developed resistance to phage AR1 by modifying OmpC. Further protein analysis should be conducted to investigate if OmpC in BIMs are completely eliminated or structurally altered.

This would provide more information to explain the varied membrane permeabilities and metabolic changes of BIMs as a result of phage interaction with this proteinaceous receptor.

The virulence of isolated *E. coli* O157:H7 BIMs in animal hosts should be investigated, provided that previous studies have shown that virulence attenuation is one of the most common fitness changes of BIMs. Also, studying the colonization of these BIMs in animal models would elucidate the impact of the excessive biofilm production

Finally, the results of this study showed that a variety of substrates were metabolized differently between the wildtypes and BIMs. The relevance of these different phenotypes with phage-induced modifications would be better defined by conducting a transcriptomic analysis, which allows the identification of genes that are differentially expressed in the wildtypes and mutants.

References

Abedon, S. T. (2019). Phage-antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? Antibiotics, 8(4), 182.

Abedon, S. T., Kuhl, S. J., Blasdel, B. G., & Kutter, E. M. (2011). Phage treatment of human infections. Bacteriophage, 1(2), 66-85. doi:10.4161/bact.1.2.15845

Abouhamad, W. N., & Manson, M. D. (1994). The dipeptide permease of *Escherichia coli* closely resembles other bacterial transport systems and shows growth-phase-dependent expression. Molecular microbiology, 14(5), 1077-1092.

Ackermann, H. W. (2003). Bacteriophage observations and evolution. Res Microbiol, 154(4), 245-251. doi:10.1016/S0923-2508(03)00067-6

Albesa-Jove, D., Giganti, D., Jackson, M., Alzari, P. M., & Guerin, M. E. (2014). Structure-Function Relationships of Membrane-associated GT-B Glycosyltransferases. Glycobiology, 24(2), 108-124. doi:10.1093/glycob/cwt101

Aldick, T., Bielaszewska, M., Uhlin, B. E., Humpf, H. U., Wai, S. N., & Karch, H. (2009). Vesicular stabilization and activity augmentation of enterohaemorrhagic *Escherichia coli* haemolysin. Molecular microbiology, 71(6), 1496-1508.

Aldick, T., Bielaszewska, M., Zhang, W., Brockmeyer, J., Schmidt, H., Friedrich, A. W., Kim, K. S., Schmidt, M. A., & Karch, H. (2007). Hemolysin from Shiga toxin-negative *Escherichia coli* O26 strains injures microvascular endothelium. Microbes and infection, 9(3), 282-290.

Alisky, J., Iczkowski, K., Rapoport, A., & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. Journal of Infection, 36(1), 5-15.

Allos, B. M., Moore, M. R., Griffin, P. M., & Tauxe, R. V. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. In: The University of Chicago Press.

Altamirano, M. M., Mulliert, G., & Calcagno, M. (1987). Sulfhydryl groups of glucosamine-6-phosphate isomerase deaminase from *Escherichia coli*. Archives of biochemistry and biophysics, 258(1), 95-100.

Álvarez-Añorve, L. I., Bustos-Jaimes, I., Calcagno, M. L., & Plumbridge, J. (2009). Allosteric regulation of glucosamine-6-phosphate deaminase (NagB) and growth of *Escherichia coli* on glucosamine. Journal of bacteriology, 191(20), 6401-6407.

Alvarez-Uria, G., Gandra, S., Mandal, S., & Laxminarayan, R. (2018). Global Forecast of Antimicrobial Resistance in Invasive Isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Int J Infect Dis, 68, 50-53. doi:10.1016/j.ijid.2018.01.011

Amor, K., Heinrichs, D. E., Frirdich, E., Ziebell, K., Johnson, R. P., & Whitfield, C. (2000). Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*. Infect Immun, 68(3), 1116-1124. doi:10.1128/iai.68.3.1116-1124.2000

Andrews, J. C., & Short, S. A. (1985). Genetic analysis of *Escherichia coli* oligopeptide transport mutants. Journal of bacteriology, 161(2), 484-492.

Aoki-Kinoshita, K. F., & Kanehisa, M. (2007). Gene annotation and pathway mapping in KEGG. In Comparative Genomics (pp. 71-91): Springer.

Arunmanee, W., Pathania, M., Solovyova, A. S., Le Brun, A. P., Ridley, H., Baslé, A., van den Berg, B., & Lakey, J. H. (2016). Gram-negative trimeric porins have specific LPS binding sites that are essential for porin biogenesis. Proceedings of the National Academy of Sciences, 113(34), E5034-E5043.

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., Desvaux, M., Di Bonaventura, G., Hébraud, M., & Jaglic, Z. (2017). Critical review on biofilm methods. Critical reviews in microbiology, 43(3), 313-351.

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., & Kubal, M. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. BMC genomics, 9(1), 75.

Bach, S., McAllister, T., Veira, D., Gannon, V., & Holley, R. (2002). Transmission and control of *Escherichia coli* O157:H7—a review. Canadian journal of animal science, 82(4), 475-490.

Bai, X., Hu, B., Xu, Y., Sun, H., Zhao, A., Ba, P., Fu, S., Fan, R., Jin, Y., & Wang, H. (2016). Molecular and phylogenetic characterization of non-O157 Shiga toxin-producing *Escherichia coli* strains in China. Frontiers in cellular and infection microbiology, 6, 143.

Bartual, S. G., Otero, J. M., Garcia-Doval, C., Llamas-Saiz, A. L., Kahn, R., Fox, G. C., & van Raaij, M. J. (2010). Structure of the bacteriophage T4 long tail fiber receptor-binding tip. Proc Natl Acad Sci U S A, 107(47), 20287-20292. doi:10.1073/pnas.1011218107

Baslé, A., Rummel, G., Storici, P., Rosenbusch, J. P., & Schirmer, T. (2006). Crystal structure of osmoporin OmpC from *E. coli* at 2.0 Å. Journal of molecular biology, 362(5), 933-942.

Beef Cattle Research Council. (2014). In-plant Mitigation of Pathogens. Retrieved from http://www.beefresearch.ca/research-topic.cfm/in-plant-mitigation-of-pathogens-12?language=. Accesed date:[Septermber 4th, 2020]

Beloin, C., Michaelis, K., Lindner, K., Landini, P., Hacker, J., Ghigo, J.-M., & Dobrindt, U. (2006). The transcriptional antiterminator RfaH represses biofilm formation in *Escherichia coli*. Journal of bacteriology, 188(4), 1316-1331.

Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In Bacterial Biofilms (pp. 249-289): Springer-Verlag Berlin Heidelberg. Berlin, Germany.

Benz, R., Francis, G., Nakae, T., & Ferenci, T. (1992). Investigation of the selectivity of maltoporin channels using mutant LamB proteins: mutations changing the maltodextrin binding site. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1104(2), 299-307.

Bergh, O., BØrsheim, K. Y., Bratbak, G., & Heldal, M. (1989). High Abundance of Viruses Found in Aquatic Environments. Nature, 340(6233), 467.

Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host Receptors for Bacteriophage Adsorption. FEMS Microbiol Lett, 363(4). doi:10.1093/femsle/fnw002

Bhat, U. R., Forsberg, L. S., & Carlson, R. W. (1994). Structure of Lipid A Component of Rhizobium leguminosarum bv. phaseoli Lipopolysaccharide. . J Biol Chem, 269(20), 14402-14410.

Bielaszewska, M., Fell, M., Greune, L., Prager, R., Fruth, A., Tschäpe, H., Schmidt, M. A., & Karch, H. (2004). Characterization of cytolethal distending toxin genes and expression in Shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups. Infection and immunity, 72(3), 1812-1816.

Bielaszewska, M., Köck, R., Friedrich, A. W., Von Eiff, C., Zimmerhackl, L. B., Karch, H., & Mellmann, A. (2007). Shiga Toxin-mediated Hemolytic Uremic Syndrome: Time to Change the Diagnostic Paradigm? PLoS One, 2(10), e1024.

Bielaszewska, M., Rüter, C., Kunsmann, L., Greune, L., Bauwens, A., Zhang, W., Kuczius, T., Kim, K. S., Mellmann, A., & Schmidt, M. A. (2013). Enterohemorrhagic *Escherichia coli* Hemolysin Employs Outer Membrane Vesicles to Target Mitochondria and Cause Endothelial and Epithelial Apoptosis. PLoS Pathog, 9(12), e1003797.

Blanco, M., Blanco, J., Mora, A., Dahbi, G., Alonso, M., González, E., Bernárdez, M., & Blanco, J. (2004). Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). Journal of Clinical Microbiology, 42(2), 645-651.

Bloom, F. R., & McFALL, E. (1975). Isolation and characterization of D-serine deaminase constitutive mutants by utilization of D-serine as sole carbon or nitrogen source. Journal of bacteriology, 121(3), 1078-1084.

Boerlin, P., McEwen, S. A., Boerlin-Petzold, F., Wilson, J. B., Johnson, R. P., & Gyles, C. L. (1999). Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. Journal of Clinical Microbiology, 37(3), 497-503.

Bokranz, W., Wang, X., Tschäpe, H., & Römling, U. (2005). Expression of cellulose and curli fimbriae by *Escherichia coli* isolated from the gastrointestinal tract. Journal of Medical Microbiology, 54(12), 1171-1182.

Bondy-Denomy, J., & Davidson, A. R. (2014). When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. Journal of Microbiology, 52(3), 235-242.

Bonyadian, M., Moshtaghi, H., & Mohamadtaghipour, L. (2017). Antibiotic Resistance of Verotoxigenic *Escherichia coli* Isolated from Vegetables. Biological Journal of Microorganism, 5(20), 9-16.

Brenner, D. J., Fanning, G., Steigerwalt, A., Ørskov, I., & Ørskov, F. (1972). Polynucleotide sequence relatedness among three groups of pathogenic *Escherichia coli* strains. Infection and immunity, 6(3), 308-315.

Brenner, S. (1955). The Adsorption of Bacteriophages by Sensitive and Resistant Cells of *Escherichia coli* strain B. Proc R Soc Lond B Biol Sci, 144(914), 93-99. doi:10.1098/rspb.1955.0036

Brockmeyer, J., Aldick, T., Soltwisch, J., Zhang, W., Tarr, P. I., Weiss, A., Dreisewerd, K., Müthing, J., Bielaszewska, M., & Karch, H. (2011). Enterohaemorrhagic *Escherichia coli* haemolysin is cleaved and inactivated by serine protease EspPα. Environmental Microbiology, 13(5), 1327-1341.

Brunder, W., Schmidt, H., & Karch, H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology, 142(11), 3305-3315.

Brunder, W., Schmidt, H., & Karch, H. (1997). EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. Molecular microbiology, 24(4), 767-778.

Burland, V., Shao, Y., Perna, N. T., Plunkett, G., Blattner, F. R., & Sofia, H. J. (1998). The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. Nucleic acids research, 26(18), 4196-4204.

Caliezi, C., Wuillemin, W., Zeerleder, S., Redondo, M., Eisele, B., & Hack, C. (2000). C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. Pharmacological reviews, 52(1), 91-112.

Canadian Food Inspection Agency. Permitted Synonyms for Food Additives Table. Retrieved from https://www.inspection.gc.ca/food-label-requirements/labelling/industry/food-additives/permitted-synonyms/eng/1369857665232/1369857767799. Accesed date:[April 16, 2020]

Capparelli, R., Nocerino, N., Iannaccone, M., Ercolini, D., Parlato, M., Chiara, M., & Iannelli, D. (2010). Bacteriophage therapy of *Salmonella enterica*: a fresh appraisal of bacteriophage therapy. J Infect Dis, 201(1), 52-61. doi:10.1086/648478

Carlton, R. M. (1999). Phage therapy: past history and future prospects. Arch Immunol Ther Exp (Warsz), 47(5), 267-274.

Carter, C. D., Parks, A., Abuladze, T., Li, M., Woolston, J., Magnone, J., Senecal, A., Kropinski, A. M., & Sulakvelidze, A. J. B. (2012). Bacteriophage cocktail significantly reduces *Escherichia coli* O157: H7 contamination of lettuce and beef, but does not protect against recontamination. 2(3), 178-185.

Casjens, S. (2003). Prophages and bacterial genomics: what have we learned so far? Molecular microbiology, 49(2), 277-300.

CDC. (2020a). *Salmonella* Hompage. Retrieved from https://www.cdc.gov/*Salmonella*/index.html#:~:text=with%20backyard%20poultry-,Get%20tips%20to%20help%20protect%20you,family%20from%20a%20*Salmonella*%20infection.&text=CDC%20estimates%20*Salmonella*%20bacteria%20cause,the%20United%20States%20every%20year. Accesed date:[September 4th, 2020]

CDC. (2020b). Shigell-Shigellosis Fast Facts. Retrieved from https://www.cdc.gov/shigella/fast-facts.html. Accesed date:[September 4th, 2020]

CDC. (2020c). *E. coli* homepage. Retrieved from https://www.cdc.gov/ecoli/index.html. Accesed date:[September 4th, 2020]

Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage Selection Restores Antibiotic Sensitivity in MDR *Pseudomonas aeruginosa*. Sci Rep, 6, 26717. doi:10.1038/srep26717

Chang, V., Chen, L. Y., Wang, A., & Yuan, X. (2010). The Effect of Lipopolysaccharide Core Structure Defects on Transformation Efficiency in Isogenic *Escherichia coli* BW25113 rfaG, rfaP, and rfaC Mutants. J ExpMicrobiol Immunol (JEMI), 14, 101-107.

Charbit, A., Gehring, K., Nikaido, H., Ferenci, T., & Hofnung, M. (1988). Maltose transport and starch binding in phage-resistant point mutants of maltoporin. Functional and topological implications. J Mol Biol, 201(3), 487-496. doi:10.1016/0022-2836(88)90630-4

Chart, H., Row, B., Threlfall, E. J., & Ward, L. R. (1989). Conversion of *Salmonella* enteritidis phage type 4 to phage type 7 involves loss of lipopolysaccharide with concomitant loss of virulence. FEMS Microbiol Lett, 51(1), 37-40. doi:10.1016/0378-1097(89)90073-6

Chatterjee, S., & Rothenberg, E. (2012). Interaction of bacteriophage l with its *E. coli* receptor, LamB. Viruses, 4(11), 3162-3178. doi:10.3390/v4113162

Clarke, S. C. (2001). Diarrhoeagenic *Escherichia coli*—an emerging problem? Diagnostic microbiology and infectious disease, 41(3), 93-98.

Clokie, M. R., Kropinski, A. M., & Lavigne, R. (2009). Bacteriophages: Springer. Berlin, Germany.

Comeau, A. M., Tetart, F., Trojet, S. N., Prere, M. F., & Krisch, H. M. (2007). Phage-Antibiotic Synergy (PAS): Beta-lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. PLoS One, 2(8), e799. doi:10.1371/journal.pone.0000799

Condemine, G., Berrier, C., Plumbridge, J., & Ghazi, A. (2005). Function and expression of an N-acetylneuraminic acid-inducible outer membrane channel in *Escherichia coli*. Journal of bacteriology, 187(6), 1959-1965.

Confer, A. W., & Ayalew, S. (2013). The OmpA family of proteins: roles in bacterial pathogenesis and immunity. Vet Microbiol, 163(3-4), 207-222. doi:10.1016/j.vetmic.2012.08.019

Coombes, B. K., Wickham, M. E., Mascarenhas, M., Gruenheid, S., Finlay, B. B., & Karmali, M. A. (2008). Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. Applied and environmental microbiology, 74(7), 2153-2160.

Council of Canadian Academies. (2019). When Antibiotics Fail. The Expert Panel on the Potential Socio-Economic Impacts of Antimicrobial Resistance in Canada. Retrieved from

Coutinho, P. M., Deleury, E., Davies, G. J., & Henrissat, B. (2003). An Evolving Hierarchical Family Classification for Glycosyltransferases. J Mol Biol, 328(2), 307-317. doi:10.1016/s0022-2836(03)00307-3

Crawford, R. W., Keestra, A. M., Winter, S. E., Xavier, M. N., Tsolis, R. M., Tolstikov, V., & Bäumler, A. J. (2012). Very long O-antigen chains enhance fitness during *Salmonella*-induced colitis by increasing bile resistance. PLoS Pathog, 8(9), e1002918.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev, 26(4), 822-880. doi:10.1128/CMR.00022-13

Currie, C. G., & Poxton, I. R. (1999). The lipopolysaccharide core type of *Escherichia coli* O157:H7 and other non-O157 verotoxin-producing *E. coli*. FEMS Immunol Med Microbiol, 24(1), 57-62. doi:10.1111/j.1574-695X.1999.tb01265.x

d'Herelle, F. (1918). Sur le rôle du microbe filtrant bactériophage dans la dysentérie bacillaire. CR Acad Sci, 167, 970.

Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta, 1794(5), 808-816. doi:10.1016/j.bbapap.2008.11.005

Deresinski, S. (2009). Bacteriophage therapy: exploiting smaller fleas. Clinical Infectious Diseases, 48(8), 1096-1101.

Destoumieux-Garzón, D., Duquesne, S., Peduzzi, J., Goulard, C., Desmadril, M., Letellier, L., Rebuffat, S., & Boulanger, P. (2005). The iron–siderophore transporter FhuA is the receptor for the antimicrobial peptide microcin J25: role of the microcin Val11–Pro16 β-hairpin region in the recognition mechanism. Biochemical Journal, 389(3), 869-876.

Doughty, S., Sloan, J., Bennett-Wood, V., Robertson, M., Robins-Browne, R. M., & Hartland, E. L. (2002). Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. Infection and immunity, 70(12), 6761-6769.

Duckworth, D. H. (1976). " Who discovered bacteriophage?". Bacteriological reviews, 40(4), 793.

Dunne, M., Hupfeld, M., Klumpp, J., & Loessner, M. J. (2018). Molecular Basis of Bacterial Host Interactions by Gram-Positive Targeting Bacteriophages. Viruses, 10(8), 397. doi:10.3390/v10080397

Dupree, D. E., Price, R. E., Burgess, B. A., Andress, E. L., & Breidt, F. (2019). Effects of sodium chloride or calcium chloride concentration on the growth and survival of *Escherichia coli* O157: H7 in model vegetable fermentations. Journal of Food Protection, 82(4), 570-578.

EFSA, & ECDC. (2019). The European Union one health 2018 zoonoses report. EFSA Journal, 17(12), e05926.
Endriss, F., & Braun, V. (2004). Loop deletions indicate regions important for FhuA transport and receptor functions in *Escherichia coli*. J Bacteriol, 186(14), 4818-4823. doi:10.1128/JB.186.14.4818-4823.2004
Farfan, M. J., Cantero, L., Vidal, R., Botkin, D. J., & Torres, A. G. (2011). Long polar fimbriae of enterohemorrhagic *Escherichia coli* O157:H7 bind to extracellular matrix proteins. Infection and immunity, 79(9), 3744-3750.
Faruque, S. M., & Mekalanos, J. J. (2012). Phage-bacterial interactions in the evolution of toxigenic *Vibrio cholerae*. Virulence, 3(7), 556-565.
FDA. (2017). CFR-code of federal regulations title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=186.1797&SearchTerm=sodium%20sulfate#:~:text=(a)%20sodium%20sulfate%20(Na2SO4,No.&text=It%20is%20prepared%20by%20the,used%20for%20dry%20food%20packaging. Accesed date:[December 4th, 2020]
FDA. (2017). Retail food protection: Employee health and personal hygiene handbook. Retrieved from https://www.fda.gov/media/77065/download. Accesed date:[Septerber 3rd, 2020]
Feige, U., & Stirm, S. (1976). On the structure of the *Escherichia coli* C cell wall lipopolysaccharide core and on its øX174 receptor region. Biochemical and biophysical research communications, 71(2), 566-573.
Filippov, A. A., Sergueev, K. V., He, Y., Huang, X. Z., Gnade, B. T., Mueller, A. J., Fernandez-Prada, C. M., & Nikolich, M. P. (2011). Bacteriophage-resistant mutants in *Yersinia pestis*: identification of phage receptors and attenuation for mice. PLoS One, 6(9), e25486. doi:10.1371/journal.pone.0025486
Fong, K., Mu, K., Rheault, J.-G., Levesque, R. C., Kitts, D. D., Delaquis, P., Goodridge, L., & Wang, S. (2020). Bacteriophage-Insensitive Mutants of Antimicrobial-Resistant *Salmonella Enterica* are Altered in their Tetracycline Resistance and Virulence in Caco-2 Intestinal Cells. International journal of molecular sciences, 21(5), 1883.
Food and Drug Administration. Code of Federal Regulations Title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.822. Accesed date:[Apr 16, 2020]
Food Standard Agency. (2020). Approved Additives and their E Numbers. Food Standard Agency, London. Retrieved from https://www.food.gov.uk/business-guidance/approved-additives-and-e-numbers. Accesed date:[December 4th, 2020]
Forst, S., Delgado, J., Ramakrishnan, G., & Inouye, M. (1988). Regulation of ompC and ompF expression in *Escherichia coli* in the absence of envZ. J Bacteriol, 170(11), 5080-5085. doi:10.1128/jb.170.11.5080-5085.1988
Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., & Spode, A. (2011). Epidemic profile of Shiga-toxin–producing *Escherichia coli* O104:H4 outbreak in Germany. New England Journal of Medicine, 365(19), 1771-1780.
Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B., & Knutton, S. (1998). Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. Molecular microbiology, 30(5), 911-921.

Fuller, C. A., Pellino, C. A., Flagler, M. J., Strasser, J. E., & Weiss, A. A. (2011). Shiga toxin subtypes display dramatic differences in potency. Infection and immunity, 79(3), 1329-1337.

Gabig, M., Herman-Antosiewicz, A., Kwiatkowska, M., Los, M., Thomas, M. S., & Wegrzyn, G. (2002). The Cell Surface Protein Ag43 Facilitates Phage Infection of *Escherichia coli* in the Presence of Bile Salts and Carbohydrates. Microbiology, 148(Pt 5), 1533-1542. doi:10.1099/00221287-148-5-1533

Galardini, M., Mengoni, A., Biondi, E. G., Semeraro, R., Florio, A., Bazzicalupo, M., Benedetti, A., & Mocali, S. (2014). DuctApe: a suite for the analysis and correlation of genomic and OmniLog Phenotype Microarray data. Genomics, 103(1), 1-10. doi:10.1016/j.ygeno.2013.11.005

Gao, L., Hu, J., Zhang, X., Wei, L., Li, S., Miao, Z., & Chai, T. (2015). Application of Swine Manure on Agricultural Ffields Contributes to Extended-spectrum Beta-lactamase-producing *Escherichia coli* Spread in Tai'an, China. Front Microbiol, 6, 313. doi:10.3389/fmicb.2015.00313

Ghosh, C., Sarkar, P., Issa, R., & Haldar, J. (2019). Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance. Trends Microbiol, 27(4), 323-338. doi:10.1016/j.tim.2018.12.010

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. Int J Food Microbiol, 134(1-2), 37-45. doi:10.1016/j.ijfoodmicro.2009.05.021

Gill, J., & Abedon, S. T. (2003). Bacteriophage ecology and plants. APSnet Feature, 1-17.

Gilmore, B. F. (2012). Bacteriophages as anti-infective agents: recent developments and regulatory challenges. Expert review of anti-infective therapy, 10(5), 533-535.

Girgis, H. S., Liu, Y., Ryu, W. S., & Tavazoie, S. (2007). A comprehensive genetic characterization of bacterial motility. PLoS Genet, 3(9), 1644-1660. doi:10.1371/journal.pgen.0030154

Goerke, C., Koller, J., & Wolz, C. (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. Antimicrob Agents Chemother, 50(1), 171-177. doi:10.1128/AAC.50.1.171-177.2006

Goodridge, L., & Abedon, S. T. (2003). Bacteriophage biocontrol and bioprocessing: application of phage therapy to industry. SIM news, 53(6), 254-262.

Goodridge, L., Gallaccio, A., & Griffiths, M. W. (2003). Morphological, host range, and genetic characterization of two coliphages. Appl Environ Microbiol, 69(9), 5364-5371. doi:10.1128/aem.69.9.5364-5371.2003

Greer, G. G. (2005). Bacteriophage Control of Foodborne Bacteria. Journal of Food Protection, 68(5), 1102-1111.

Grys, T. E., Siegel, M. B., Lathem, W. W., & Welch, R. A. (2005). The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells. Infection and immunity, 73(3), 1295-1303.

Gunn, J. S. (2000). Mechanisms of bacterial resistance and response to bile. Microbes and infection, 2(8), 907-913.

Hammad, A. (1998). Evaluation of alginate-encapsulated *Azotobacter chroococcum* as a phage-resistant and an effective inoculum. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 38(1), 9-16.

Hanlon, G. W., Denyer, S. P., Olliff, C. J., & Ibrahim, L. J. (2001). Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol., 67(6), 2746-2753.

Hatfull, G. F., & Hendrix, R. W. (2011). Bacteriophages and their genomes. Current opinion in virology, 1(4), 298-303.

Heiman, K. E., Mody, R. K., Johnson, S. D., Griffin, P. M., & Gould, L. H. (2015). *Escherichia coli* O157 outbreaks in the United States, 2003–2012. Emerging infectious diseases, 21(8), 1293.

Heller, K., & Braun, V. (1982). Polymannose O-antigens of *Escherichia coli*, the binding sites for the reversible adsorption of bacteriophage T5+ via the L-shaped tail fibers. J Virol, 41(1), 222-227.

Ho, T. D., Davis, B. M., Ritchie, J. M., & Waldor, M. K. (2008). Type 2 secretion promotes enterohemorrhagic *Escherichia coli* adherence and intestinal colonization. Infection and immunity, 76(5), 1858-1865.

Hobbs, Z., & Abedon, S. T. (2016). Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. FEMS microbiology letters, 363(7).

Hosseinidoust, Z., Tufenkji, N., & van de Ven, T. G. (2013). Formation of biofilms under phage predation: considerations concerning a biofilm increase. Biofouling, 29(4), 457-468.

Hu, B., Margolin, W., Molineux, I. J., & Liu, J. (2015). Structural remodeling of bacteriophage T4 and host membranes during infection initiation. Proceedings of the National Academy of Sciences, 112(35), E4919-E4928.

Hua, Y., An, X., Pei, G., Li, S., Wang, W., Xu, X., Fan, H., Huang, Y., Zhang, Z., & Mi, Z. (2014). Characterization of the morphology and genome of an *Escherichia coli* podovirus. Archives of virology, 159(12), 3249-3256.

Hussein, H. (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. Journal of animal science, 85(suppl_13), E63-E72.

Hwang, C.-A., & Juneja, V. (2011). Effects of salt, sodium pyrophosphate, and sodium lactate on the probability of growth of *Escherichia coli* O157: H7 in ground beef. Journal of Food Protection, 74(4), 622-626.

International Agency for Research on Cancer. (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. In Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances.

Janka, A., Bielaszewska, M., Dobrindt, U., & Karch, H. (2002). Identification and distribution of the enterohemorrhagic *Escherichia coli* factor for adherence (efa1) gene in sorbitol-fermenting *Escherichia coli* O157:H−. International Journal of Medical Microbiology, 292(3-4), 207-214.

Jeong, H., Barbe, V., Lee, C. H., Vallenet, D., Yu, D. S., Choi, S. H., Couloux, A., Lee, S. W., Yoon, S. H., Cattolico, L., Hur, C. G., Park, H. S., Segurens, B., Kim, S. C., Oh, T. K., Lenski, R. E., Studier, F. W., Daegelen, P., & Kim, J. F. (2009). Genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3). J Mol Biol, 394(4), 644-652. doi:10.1016/j.jmb.2009.09.052

Kadner, R. J. (1977). Transport and utilization of D-methionine and other methionine sources in *Escherichia coli*. Journal of bacteriology, 129(1), 207-216.

Kadner, R. J., & Watson, W. J. (1974). Methionine transport in *Escherichia coli*: physiological and genetic evidence for two uptake systems. Journal of bacteriology, 119(2), 401-409.

Karam, J. D., & Drake, J. W. (1994). Molecular Biology of Bacteriophage (Vol. 4): American Society for Microbiology. Washington DC, USA.
Karmali, M. A., Gannon, V., & Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol, 140(3-4), 360-370. doi:10.1016/j.vetmic.2009.04.011
Karmali, M. A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., Isaac-Renton, J., Clark, C., Rahn, K., & Kaper, J. B. (2003). Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. Journal of Clinical Microbiology, 41(11), 4930-4940.
Kasman, L. M., Kasman, A., Westwater, C., Dolan, J., Schmidt, M. G., & Norris, J. S. (2002). Overcoming the Phage Replication Threshold: A Mathematical Model with Implications for Phage Therapy. J Virol, 76(11), 5557-5564. doi:10.1128/jvi.76.11.5557-5564.2002
Kaufmann, A., Stierhof, Y. D., & Henning, U. (1994). New outer membrane-associated protease of *Escherichia coli* K-12. J Bacteriol, 176(2), 359-367. doi:10.1128/jb.176.2.359-367.1994
Killmann, H., Videnov, G., Jung, G., Schwarz, H., & Braun, V. (1995). Identification of receptor binding sites by competitive peptide mapping: phages T1, T5, and phi 80 and colicin M bind to the gating loop of FhuA. J Bacteriol, 177(3), 694-698. doi:10.1128/jb.177.3.694-698.1995
Kim, M., & Ryu, S. (2012). Spontaneous and transient defence against bacteriophage by phase-variable glucosylation of O-antigen in *Salmonella enterica* serovar *Typhimurium*. Molecular microbiology, 86(2), 411-425.
Kirk, M. D., Angulo, F. J., Havelaar, A. H., & Black, R. E. (2017). Diarrhoeal disease in children due to contaminated food. Bulletin of the World Health Organization, 95(3), 233.
Kita, E., Yunou, Y., Kurioka, T., Harada, H., Yoshikawa, S., Mikasa, K., & Higashi, N. (2000). Pathogenic Mechanism of Mouse Brain Damage Caused by Oral Infection with Shiga Toxin-Producing *Escherichia coli* O157:H7. Infection and immunity, 68(3), 1207-1214.
Koebnik, R., Locher, K. P., & Van Gelder, P. (2000). Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol Microbiol, 37(2), 239-253. doi:10.1046/j.1365-2958.2000.01983.x
Kondou, Y., Kitazawa, D., Takeda, S., Tsuchiya, Y., Yamashita, E., Mizuguchi, M., Kawano, K., & Tsukihara, T. (2005). Structure of the central hub of bacteriophage Mu baseplate determined by X-ray crystallography of gp44. Journal of molecular biology, 352(4), 976-985.
Konowalchuk, J., Speirs, J., & Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. Infection and immunity, 18(3), 775-779.
Koo, H. L., Jiang, Z.-D., Brown, E., Garcia, C., Qi, H., & DuPont, H. L. (2008). Coliform contamination of vegetables obtained from popular restaurants in Guadalajara, Mexico, and Houston, Texas. Clinical Infectious Diseases, 47(2), 221-221.
Korteland, J., Tommassen, J., & Lugtenberg, B. (1982). PhoE protein pore of the outer membrane of *Escherichia coli* K12 is a particularly efficient channel for organic and inorganic phosphate. Biochimica et Biophysica Acta (BBA)-Biomembranes, 690(2), 282-289.
Koskella, B., & Brockhurst, M. A. (2014). Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. FEMS microbiology reviews, 38(5), 916-931.

Kropinski, A. M. (2009). Measurement of the Rate of Attachment of Bacteriophage to Cells. In Bacteriophages (pp. 151-155): Springer. Berlin, Germany.
Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay. In Bacteriophages (pp. 69-76): Springer. Berlin, Germany.
Krueger, A. P. (1931). The Sorption of Bacteriophage by Living and Dead Susceptible Bacteria. J Gen Physiol, 14(4), 493-516. doi:10.1085/jgp.14.4.493
Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., & Hovde, C. J. (1999). Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Applied and environmental microbiology, 65(9), 3767-3773.
Kumar, A., Taneja, N., Kumar, Y., & Sharma, M. (2012). Detection of S higa toxin variants among S higa toxin–forming E scherichia coli isolates from animal stool, meat and human stool samples in I ndia. Journal of applied microbiology, 113(5), 1208-1216.

Kutateladze, M. (2015). Experience of the Eliava Institute in bacteriophage therapy. Virol Sin, 30(1), 80-81. doi:10.1007/s12250-014-3557-0
Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. Nat Rev Microbiol, 8(5), 317-327. doi:10.1038/nrmicro2315
Lacqua, A., Wanner, O., Colangelo, T., Martinotti, M. G., & Landini, P. (2006). Emergence of biofilm-forming subpopulations upon exposure of *Escherichia coli* to environmental bacteriophages. Applied and environmental microbiology, 72(1), 956-959.
Lairson, L. L., Henrissat, B., Davies, G. J., & Withers, S. G. (2008). Glycosyltransferases: Structures, Functions, and Mechanisms. Annu Rev Biochem, 77, 521-555. doi:10.1146/annurev.biochem.76.061005.092322
Lander, G. C., Khayat, R., Li, R., Prevelige, P. E., Potter, C. S., Carragher, B., & Johnson, J. E. (2009). The P22 tail machine at subnanometer resolution reveals the architecture of an infection conduit. Structure, 17(6), 789-799.
Lathem, W. W., Grys, T. E., Witowski, S. E., Torres, A. G., Kaper, J. B., Tarr, P. I., & Welch, R. A. (2002). StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. Molecular microbiology, 45(2), 277-288.
Le, S., Yao, X., Lu, S., Tan, Y., Rao, X., Li, M., Jin, X., Wang, J., Zhao, Y., Wu, N. C., Lux, R., He, X., Shi, W., & Hu, F. (2014). Chromosomal DNA deletion confers phage resistance to *Pseudomonas aeruginosa*. Sci Rep, 4, 4738. doi:10.1038/srep04738
Leiman, P. G., Arisaka, F., Van Raaij, M. J., Kostyuchenko, V. A., Aksyuk, A. A., Kanamaru, S., & Rossmann, M. G. (2010). Morphogenesis of the T4 tail and tail fibers. Virology journal, 7(1), 1-28.
Leon, M., & Bastias, R. (2015). Virulence reduction in bacteriophage resistant bacteria. Front Microbiol, 6, 343. doi:10.3389/fmicb.2015.00343
Letarov, A. V., & Kulikov, E. E. (2017). Adsorption of Bacteriophages on Bacterial Cells. Biochemistry (Mosc), 82(13), 1632-1658. doi:10.1134/S0006297917130053
Levine, M. M., Xu, J.-g., Kaper, J. B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H., & Wachsmuth, K. (1987). A DNA probe to identify enterohemorrhagic *Escherichia coli* of 0157: H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. Journal of Infectious Diseases, 156(1), 175-182.
Lewis, R., & Hill, C. (2020). Overcoming barriers to phage application in food and feed. Current Opinion in Biotechnology, 61, 38-44.

Lhuillier, S., Gallopin, M., Gilquin, B., Brasilès, S., Lancelot, N., Letellier, G., Gilles, M., Dethan, G., Orlova, E. V., & Couprie, J. (2009). Structure of bacteriophage SPP1 head-to-tail connection reveals mechanism for viral DNA gating. Proceedings of the National Academy of Sciences, 106(21), 8507-8512.

Liao, W. C., Ng, W. V., Lin, I. H., Syu, W. J., Liu, T. T., & Chang, C. H. (2011). T4-Like genome organization of the *Escherichia coli* O157:H7 lytic phage AR1. J Virol, 85(13), 6567-6578. doi:10.1128/JVI.02378-10

Liebau, J., Pettersson, P., Szpryngiel, S., & Maler, L. (2015). Membrane Interaction of the Glycosyltransferase WaaG. Biophys J, 109(3), 552-563. doi:10.1016/j.bpj.2015.06.036

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. Journal of microbiology and biotechnology, 20(1), 5.

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage Therapy: An Alternative to Antibiotics in the Age of Multi-drug Resistance. World J Gastrointest Pharmacol Ther, 8(3), 162-173. doi:10.4292/wjgpt.v8.i3.162

Liu, C. G., Green, S. I., Min, L., Clark, J. R., Salazar, K. C., Terwilliger, A. L., Kaplan, H., Trautner, B., Ramig, R. F., & Maresso, A. W. (2020). Phage-Antibiotic Synergy Is Driven By A Unique Combination Of Antibacterial Mechanism Of Action And Stoichiometry. bioRxiv.

Liu, X., Turchi, B., Mok, K. C., Taga, M. E., & Miller, M. J. (2017). HM2-phage resistant solventogenic *Clostridium saccharoperbutylacetonicum* N1-4 shows increased exopolysaccharide production. FEMS microbiology letters, 364(19).

Lu, Z., & Breidt, F. (2015). *Escherichia coli* O157: H7 bacteriophage Φ241 isolated from an industrial cucumber fermentation at high acidity and salinity. Frontiers in microbiology, 6, **67.**

Luck, S. N., Badea, L., Bennett-Wood, V., Robins-Browne, R., & Hartland, E. L. (2006). Contribution of FliC to epithelial cell invasion by enterohemorrhagic *Escherichia coli* O113:H21. Infection and immunity, 74(12), 6999-7004.

Luck, S. N., Bennett-Wood, V., Poon, R., Robins-Browne, R. M., & Hartland, E. L. (2005). Invasion of epithelial cells by locus of enterocyte effacement-negative enterohemorrhagic *Escherichia coli*. Infection and immunity, 73(5), 3063-3071.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI Search and Sequence Analysis Tools APIs in 2019. Nucleic Acids Res, 47(W1), W636-W641. doi:10.1093/nar/gkz268

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., Yeung, D. H., & Kirk, M. D. (2014). Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis, 11(6), 447-455. doi:10.1089/fpd.2013.1704

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and its Consequential Resistance in Environmental Sources: Potential Public Health Implications. Molecules, 23(4), 795.

Mathews, C. K. (1983). Bacteriophage T4: Wiley Online Library.

Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., Wakiguchi, H., & Imai, S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother, 11(5), 211-219. doi:10.1007/s10156-005-0408-9

McArthur, F., Andersson, C. E., Loutet, S., Mowbray, S. L., & Valvano, M. A. (2005). Functional analysis of the glycero-manno-heptose 7-phosphate kinase domain from the bifunctional HldE protein, which is involved in ADP-L-glycero-D-manno-heptose biosynthesis. Journal of bacteriology, 187(15), 5292-5300.

McDanel, J., Schweizer, M., Crabb, V., Nelson, R., Samore, M., Khader, K., Blevins, A. E., Diekema, D., Chiang, H. Y., Nair, R., & Perencevich, E. (2017). Incidence of Extended-Spectrum beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella* Infections in the United States: A Systematic Literature Review. Infect Control Hosp Epidemiol, 38(10), 1209-1215. doi:10.1017/ice.2017.156

Melton-Celsa, A. R. (2015). Shiga toxin (Stx) classification, structure, and function. Enterohemorrhagic *Escherichia coli* and Other Shiga Toxin-Producing *E. coli*, 37-53.

Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2006). Growing and analyzing static biofilms. Current protocols in microbiology, 1B. 1.1-1B. 1.17.

Meza-Segura, M., Zaidi, M. B., Maldonado-Puga, S., Huerta-Cantillo, J., Chavez-Dueñas, L., Navarro-Garcia, F., & Estrada-Garcia, T. (2017). Cytolethal Distending Toxin-producing *Escherichia coli* Strains Causing Severe Diarrhoea in Young Mexican Children. JMM Case Reports, 4(2).

Miller, E. S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T., & Rüger, W. (2003). Bacteriophage T4 genome. Microbiology and Molecular Biology Reviews, 67(1), 86-156.

Mills, S., Griffin, C., Coffey, A., Meijer, W., Hafkamp, B., & Ross, R. (2010). CRISPR analysis of bacteriophage-insensitive mutants (BIMs) of industrial *Streptococcus thermophilus*–implications for starter design. Journal of applied microbiology, 108(3), 945-955.

Miyamoto, Y., Iimura, M., Kaper, J. B., Torres, A. G., & Kagnoff, M. F. (2006). Role of Shiga toxin versus H7 flagellin in enterohaemorrhagic *Escherichia coli* signalling of human colon epithelium in vivo. Cellular microbiology, 8(5), 869-879.

Mizoguchi, K., Morita, M., Fischer, C. R., Yoichi, M., Tanji, Y., & Unno, H. (2003). Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. Appl Environ Microbiol, 69(1), 170-176. doi:10.1128/aem.69.1.170-176.2003

Morona, R., Klose, M., & Henning, U. (1984). *Escherichia coli* K-12 outer membrane protein (OmpA) as a bacteriophage receptor: analysis of mutant genes expressing altered proteins. Journal of bacteriology, 159(2), 570-578.

Motto, D. G., Chauhan, A. K., Zhu, G., Homeister, J., Lamb, C. B., Desch, K. C., Zhang, W., Tsai, H.-M., Wagner, D. D., & Ginsburg, D. (2005). Shiga toxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. The Journal of clinical investigation, 115(10), 2752-2761.

Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. Viruses, 10(4), 205. doi:10.3390/v10040205

Muszyński, A., Laus, M., Kijne, J. W., & Carlson, R. W. (2011). Structures of the lipopolysaccharides from Rhizobium leguminosarum RBL5523 and its UDP-glucose dehydrogenase mutant (exo5). Glycobiology, 21(1), 55-68.

Nakao, R., Ramstedt, M., Wai, S. N., & Uhlin, B. E. (2012). Enhanced biofilm formation by *Escherichia coli* LPS mutants defective in Hep biosynthesis. PLoS One, 7(12), e51241. doi:10.1371/journal.pone.0051241

Nesper, J., Lauriano, C. M., Klose, K. E., Kapfhammer, D., Kraiß, A., & Reidl, J. (2001). Characterization of *Vibrio cholerae* O1 El TorgalU and galE Mutants: Influence on

lipopolysaccharide structure, colonization, and biofilm formation. Infection and immunity, 69(1), 435-445.
Nikaido, H. (2003). Molecular Basis of Bacterial Outer Membrane Permeability Revisited. Microbiol Mol Biol Rev, 67(4), 593-656. doi:10.1128/mmbr.67.4.593-656.2003
Nikaido, H. (2005). Restoring permeability barrier function to outer membrane. Chem Biol, 12(5), 507-509. doi:10.1016/j.chembiol.2005.05.001
Nordstrom, K., & Forsgren, A. (1974). Effect of protein A on adsorption of bacteriophages to Staphylococcus aureus. J Virol, 14(2), 198-202.
O'Brien, A. D., Newland, J. W., Miller, S. F., Holmes, R. K., Smith, H. W., & Formal, S. B. (1984). Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science, 226(4675), 694-696.
O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. Appl Environ Microbiol, 70(6), 3417-3424. doi:10.1128/AEM.70.6.3417-3424.2004
O'Neill, J. (2016). Tackling Drug-resistant Infections Globally: Final Report and Recommendations. UK Government and Wellcome Trust.
Oechslin, F. (2018). Resistance Development to Bacteriophages Occurring during Bacteriophage Therapy. Viruses, 10(7), 351. doi:10.3390/v10070351
Ogura, Y., Ooka, T., Iguchi, A., Toh, H., Asadulghani, M., Oshima, K., Kodama, T., Abe, H., Nakayama, K., & Kurokawa, K. (2009). Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. Proceedings of the National Academy of Sciences, 106(42), 17939-17944.
Orth, D., Ehrlenbach, S., Brockmeyer, J., Khan, A. B., Huber, G., Karch, H., Sarg, B., Lindner, H., & Würzner, R. (2010). EspP, a serine protease of enterohemorrhagic *Escherichia coli*, impairs complement activation by cleaving complement factors C3/C3b and C5. Infection and immunity, 78(10), 4294-4301.
Otsuji, N., Sekiguchi, M., Iijima, T., & Takagi, Y. (1959). Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin C. Nature, 184(Suppl 14)(4692), 1079-1080. doi:10.1038/1841079b0
Pagnout, C., Sohm, B., Razafitianamaharavo, A., Caillet, C., Offroy, M., Leduc, M., Gendre, H., Jomini, S., Beaussart, A., & Bauda, P. (2019). Pleiotropic effects of rfa-gene mutations on *Escherichia coli* envelope properties. Scientific reports, 9(1), 1-16.
Pasqua, M., Michelacci, V., Di Martino, M. L., Tozzoli, R., Grossi, M., Colonna, B., Morabito, S., & Prosseda, G. (2017). The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. Frontiers in microbiology, 8, 2390.
Paton, A. W., Ratcliff, R. M., Doyle, R. M., Seymour-Murray, J., Davos, D., Lanser, J. A., & Paton, J. C. (1996). Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. Journal of Clinical Microbiology, 34(7), 1622-1627.
Paton, A. W., Srimanote, P., Woodrow, M. C., & Paton, J. C. (2001). Characterization of Saa, a Novel Autoagglutinating Adhesin Produced by Locus of Enterocyte Effacement-Negative Shiga-Toxigenic *Escherichia coli* Strains That Are Virulent for Humans. Infection and immunity, 69(11), 6999-7009.
Pedruzzi, I., Rosenbusch, J. P., & Locher, K. P. (1998). Inactivation in vitro of the *Escherichia coli* outer membrane protein FhuA by a phage T5-encoded lipoprotein. FEMS Microbiol Lett, 168(1), 119-125. doi:10.1111/j.1574-6968.1998.tb13264.x

Perera, M. N., Abuladze, T., Li, M., Woolston, J., & Sulakvelidze, A. (2015). Bacteriophage cocktail significantly reduces or eliminates Listeria monocytogenes contamination on lettuce, apples, cheese, smoked salmon and frozen foods. Food microbiology, 52, 42-48.
PHAC. (2017). Causes of *E. coli* (*Escherichia coli*) infection. Retrieved from https://www.canada.ca/en/public-health/services/diseases/e-coli/causes-e-coli.html. Accesed date:[September 4th, 2020]
Picken, R., & Beacham, I. (1977). Bacteriophage-resistant mutants of *Escherichia coli* K12. Location of receptors within the lipopolysaccharide. Microbiology, 102(2), 305-318.
Pitout, J. D. (2012). Extraintestinal Pathogenic *Escherichia coli*: A Combination of Virulence with Antibiotic Resistance. Front Microbiol, 3, 9. doi:10.3389/fmicb.2012.00009
Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial Resistance in *Escherichia coli*. Microbiol Spectr, 6(4), 289-316. doi:10.1128/microbiolspec.ARBA-0026-2017
Pollari, F., Christidis, T., Pintar, K. D., Nesbitt, A., Farber, J., Lavoie, M.-C., Gill, A., Kirsch, P., & Johnson, R. P. (2017). Evidence for the benefits of food chain interventions on *E. coli* O157:H7/NM prevalence in retail ground beef and human disease incidence: A success story. Canadian Journal of Public Health, 108(1), e71-e78.
Poolman, B., Spitzer, J. J., & Wood, J. M. (2004). Bacterial osmosensing: roles of membrane structure and electrostatics in lipid–protein and protein–protein interactions. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1666(1-2), 88-104.
Prehm, P., Jann, B., Jann, K., Schmidt, G., & Stirm, S. (1976). On a Bacteriophage T3 and T4 Receptor Region within the Cell Wall Lipopolysaccharide of *Escherichia coli* B. Journal of molecular biology, 101(2), 277-281.
Public Health Agency of Canada. (2012). National Enteric Surveillance Program (NESP). In: Winnipeg Manitoba, Canada.
Qadri, F., Svennerholm, A.-M., Faruque, A., & Sack, R. B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clinical microbiology reviews, 18(3), 465-483.
Qin, J., Cui, Y., Zhao, X., Rohde, H., Liang, T., Wolters, M., Li, D., Campos, C. B., Christner, M., & Song, Y. (2011). Identification of the Shiga toxin-producing *Escherichia coli* O104: H4 strain responsible for a food poisoning outbreak in Germany by PCR. Journal of Clinical Microbiology, 49(9), 3439-3440.
Rakonjac, J., Bennett, N. J., Spagnuolo, J., Gagic, D., & Russel, M. (2011). Filamentous bacteriophage: biology, phage display and nanotechnology applications. Current issues in molecular biology, 13(2), 51.
Randall-Hazelbauer, L., & Schwartz, M. (1973). Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J Bacteriol, 116(3), 1436-1446.
Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerging infectious diseases, 11(4), 603.
Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial Drug Resistance in Strains of *Escherichia coli* Isolated from Food Sources. Rev Inst Med Trop Sao Paulo, 56(4), 341-346. doi:10.1590/s0036-46652014000400012
Ratnam, S., March, S., Ahmed, R., Bezanson, G., & Kasatiya, S. (1988). Characterization of *Escherichia coli* serotype O157:H7. Journal of Clinical Microbiology, 26(10), 2006-2012.

Riede, I. (1987). Receptor specificity of the short tail fibres (gp12) of T-even type *Escherichia coli* phages. Mol Gen Genet, 206(1), 110-115. doi:10.1007/BF00326544
Ripp, S., & Miller, R. V. (1997). The role of pseudolysogeny in bacteriophage-host interactions in a natural freshwater environment. Microbiology, 143(6), 2065-2070.
Rodionova, I. A., Goodacre, N., Babu, M., Emili, A., Uetz, P., & Saier, M. H. (2018). The nitrogen regulatory PII protein (GlnB) and N-acetylglucosamine 6-phosphate epimerase (NanE) allosterically activate glucosamine 6-phosphate deaminase (NagB) in *Escherichia coli*. Journal of bacteriology, 200(5).
Rodionova, I. A., Zhang, Z., Mehla, J., Goodacre, N., Babu, M., Emili, A., Uetz, P., & Saier, M. H. (2017). The phosphocarrier protein HPr of the bacterial phosphotransferase system globally regulates energy metabolism by directly interacting with multiple enzymes in *Escherichia coli*. Journal of Biological Chemistry, 292(34), 14250-14257.
Rohwer, F., & Edwards, R. (2002). The Phage Proteomic Tree: a genome-based taxonomy for phage. J Bacteriol, 184(16), 4529-4535. doi:10.1128/jb.184.16.4529-4535.2002
Ronner, A. B., & Cliver, D. O. (1990). Isolation and Characterization of a Coliphage Specific for *Escherichia coli* 0157: H7. Journal of Food Protection, 53(11), 944-947.
Rossi, E., Cimdins, A., Lüthje, P., Brauner, A., Sjöling, Å., Landini, P., & Römling, U. (2018). "It's a gut feeling"–*Escherichia coli* biofilm formation in the gastrointestinal tract environment. Critical reviews in microbiology, 44(1), 1-30.
Salmond, G. P., & Fineran, P. C. (2015). A century of the phage: past, present and future. Nat Rev Microbiol, 13(12), 777-786. doi:10.1038/nrmicro3564
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual: Cold spring harbor laboratory press. Cold Spring Harbor, USA.
Samson, J. E., Magadán, A. H., Sabri, M., & Moineau, S. (2013). Revenge of the phages: defeating bacterial defences. Nature Reviews Microbiology, 11(10), 675-687.
Santander, J., Martin, T., Loh, A., Pohlenz, C., Gatlin III, D. M., & Curtiss III, R. (2013). Mechanisms of intrinsic resistance to antimicrobial peptides of *Edwardsiella ictaluri* and its influence on fish gut inflammation and virulence. Microbiology, 159(Pt 7), 1471.
Scaletsky, I., Silva, M., & Trabulsi, L. R. (1984). Distinctive patterns of adherence of enteropathogenic Escherichia coli to HeLa cells. *Infection and immunity, 45*(2), 534-536.
Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. Emerging infectious diseases, 17(1), 7.
Scanlan, P. D., Bischofberger, A. M., & Hall, A. R. (2017). Modification of *Escherichia coli*-Bacteriophage Interactions by Surfactants and Antibiotics in vitro. FEMS Microbiol Ecol, 93(1). doi:10.1093/femsec/fiw211
Schmidt, H., Beutin, L., & Karch, H. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infection and immunity, 63(3), 1055-1061.
Schmidt, H., Henkel, B., & Karch, H. (1997). A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. FEMS microbiology letters, 148(2), 265-272.
Schnaitman, C. A., & Klena, J. D. (1993). Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiology and Molecular Biology Reviews, 57(3), 655-682.

Scholl, D., Adhya, S., & Merril, C. (2005). *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. Appl. Environ. Microbiol., 71(8), 4872-4874.
Sciara, G., Bebeacua, C., Bron, P., Tremblay, D., Ortiz-Lombardia, M., Lichière, J., van Heel, M., Campanacci, V., Moineau, S., & Cambillau, C. (2010). Structure of lactococcal phage p2 baseplate and its mechanism of activation. Proceedings of the National Academy of Sciences, 107(15), 6852-6857.
Scuron, M. D., Boesze-Battaglia, K., Dlakić, M., & Shenker, B. J. (2016). The Cytolethal Distending Toxin Contributes to Microbial Virulence and Disease Pathogenesis by Acting as A Tri-perditious Toxin. Frontiers in cellular and infection microbiology, 6, 168.
Sharma, M., Patel, J. R., Conway, W. S., Ferguson, S., & Sulakvelidze, A. (2009). Effectiveness of bacteriophages in reducing *Escherichia coli* O157: H7 on fresh-cut cantaloupes and lettuce. Journal of Food Protection, 72(7), 1481-1485.
Shepherd, A. K., & Pottinger, P. S. (2013). Management of Urinary Tract Infections in the Era of Increasing Antimicrobial Resistance. Med Clin North Am, 97(4), 737-757, xii. doi:10.1016/j.mcna.2013.03.006
Siringan, P., Connerton, P. L., Cummings, N. J., & Connerton, I. F. (2014). Alternative bacteriophage life cycles: the carrier state of *Campylobacter jejuni*. Open biology, 4(3), 130200.
Smith, H. W., & Huggins, M. B. (1982). Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. Microbiology, 128(2), 307-318.
Smith, H. W., & Huggins, M. B. (1983). Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J Gen Microbiol, 129(8), 2659-2675. doi:10.1099/00221287-129-8-2659
Stevens, M. P., Roe, A. J., Vlisidou, I., Van Diemen, P. M., La Ragione, R. M., Best, A., Woodward, M. J., Gally, D. L., & Wallis, T. S. (2004). Mutation of toxB and a truncated version of the efa-1 gene in *Escherichia coli* O157:H7 influences the expression and secretion of locus of enterocyte effacement-encoded proteins but not intestinal colonization in calves or sheep. Infection and immunity, 72(9), 5402-5411.
Stirm, S. (1968). *Escherichia coli* K bacteriophages. I. Isolation and introductory characterization of five *Escherichia coli* K bacteriophages. J Virol, 2(10), 1107-1114.
Strockbine, N. A., Marques, L. R., Newland, J. W., Smith, H. W., Holmes, R. K., & O'Brien, A. D. (1986). Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun, 53(1), 135-140.
Sulakvelidze, A., Alavidze, Z., & Morris, J. G., Jr. (2001). Bacteriophage therapy. Antimicrob Agents Chemother, 45(3), 649-659. doi:10.1128/AAC.45.3.649-659.2001
Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V., & Force, C. P. T. (2001). PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerging infectious diseases, 7(3), 382.
Tamaki, S., Sato, T., & Matsuhashi, M. (1971). Role of Lipopolysaccharides in Antibiotic Resistance and Bacteriophage Adsorption of *Escherichia coli* K-12. J Bacteriol, 105(3), 968-975.
Tan, L., & Darby, C. (2005). *Yersinia pestis* is viable with endotoxin composed of only lipid A. Journal of bacteriology, 187(18), 6599-6600.

Tatsuno, I., Horie, M., Abe, H., Miki, T., Makino, K., Shinagawa, H., Taguchi, H., Kamiya, S., Hayashi, T., & Sasakawa, C. (2001). toxB gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. Infection and immunity, 69(11), 6660-6669.

Tchieu, J. H., Norris, V., Edwards, J. S., & Saier Jr, M. H. (2001). The complete phosphotransferase system in *Escherichia coli*. Journal of molecular microbiology and biotechnology, 3(3), 329-346.

Tilden Jr, J., Young, W., McNamara, A.-M., Custer, C., Boesel, B., Lambert-Fair, M. A., Majkowski, J., Vugia, D., Werner, S., & Hollingsworth, J. (1996). A new route of transmission for *Escherichia coli*: infection from dry fermented salami. American journal of public health, 86(8_Pt_1), 1142-1145.

Todd, E. (2014). Foodborne diseases: Overview of biological hazards and foodborne diseases. Encyclopedia of Food Safety, 221.

Toivonen, P. M., & Lu, C. (2013). Differential quenching of free chlorine by organic compounds potentially exuded from injured plant tissues. Postharvest biology and technology, 86, 192-194.

Totten, S., Medaglia, A., & McDermott, S. (2019). Updates to Canadian Notifiable Disease Surveillance System.

Tritt, A., Eisen, J. A., Facciotti, M. T., & Darling, A. E. (2012). An Integrated Pipeline for de novo Assembly of Microbial Genomes. PLoS One, 7(9), e42304. doi:10.1371/journal.pone.0042304

Trofa, A. F., Ueno-Olsen, H., Oiwa, R., & Yoshikawa, M. (1999). Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. Clinical Infectious Diseases, 29(5), 1303-1306.

Tzipori, S., Chow, C., & Powell, H. (1988). Cerebral infection with *Escherichia coli* O157:H7 in humans and gnotobiotic piglets. Journal of clinical pathology, 41(10), 1099-1103.

Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R. P., & Penades, J. R. (2005). Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol Microbiol, 56(3), 836-844. doi:10.1111/j.1365-2958.2005.04584.x

Uhlich, G. A. (2009). KatP contributes to OxyR-regulated hydrogen peroxide resistance in *Escherichia coli* serotype O157:H7. Microbiology, 155(11), 3589-3598.

USDA. USDA Targeting Six Additional Strains of *E. coli* in Raw Beef Trim Starting Monday [Press release]. Retrieved from https://www.usda.gov/media/press-releases/2012/05/31/usda-targeting-six-additional-strains-ecoli-raw-beef-trim-starting. Accessed date:[September 4th, 2020]

Vaara, M. (1992). Agents that increase the permeability of the outer membrane. Microbiological reviews, 56(3), 395-411.

Valério, N., Oliveira, C., Jesus, V., Branco, T., Pereira, C., Moreirinha, C., & Almeida, A. (2017). Effects of Single and Combined Use of Bacteriophages and Antibiotics to Inactivate *Escherichia coli*. Virus research, 240, 8-17.

Van der Ley, P., De Graaff, P., & Tommassen, J. (1986). Shielding of *Escherichia coli* outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. Journal of bacteriology, 168(1), 449-451.

van Deurs, B., & Sandvig, K. (1995). Furin-induced cleavage and activation of Shiga toxin. Journal of Biological Chemistry, 270(18), 10817-10821.

Viator, C., Muth, M., & Brophy, J. (2015). Costs of Food Safety Investments. RTI International. Prepared for USDA, Food Safety and Inspection Service, Office of Policy and Program Development.
Viazis, S., Akhtar, M., Feirtag, J., & Diez-Gonzalez, F. (2011b). Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. Food Microbiol, 28(1), 149-157. doi:10.1016/j.fm.2010.09.009
Viazis, S., Akhtar, M., Feirtag, J., Brabban, A., & Diez-Gonzalez, F. (2011a). Isolation and characterization of lytic bacteriophages against enterohaemorrhagic *Escherichia coli*. Journal of applied microbiology, 110(5), 1323-1331.
Vigil, K. J., Jiang, Z.-D., Chen, J. J., Palumbo, K. L., Galbadage, T., Brown, E. L., Yiang, J., Koo, H., DuPont, M. W., & Ericsson, C. (2009). Coliform and *Escherichia coli* contamination of desserts served in public restaurants from Guadalajara, Mexico, and Houston, Texas. The American journal of tropical medicine and hygiene, 80(4), 606-608.
Wang, Z., Wang, J., Ren, G., Li, Y., & Wang, X. (2015). Influence of Core Oligosaccharide of Lipopolysaccharide to Outer Membrane Behavior of *Escherichia coli*. Mar Drugs, 13(6), 3325-3339. doi:10.3390/md13063325
Washizaki, A., Yonesaki, T., & Otsuka, Y. (2016). Characterization of the Interactions between *Escherichia coli* Receptors, LPS and OmpC, and Bacteriophage T4 Long Tail Fibers. Microbiologyopen, 5(6), 1003-1015. doi:10.1002/mbo3.384
Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2—A Multiple Sequence Alignment Editor and Analysis Workbench. Bioinformatics, 25(9), 1189-1191. doi:10.1093/bioinformatics/btp033
Weber-Dąbrowska, B., Mulczyk, M., & Górski, A. (2001). Bacteriophage therapy of bacterial infections: an update of our institute's experience. In Inflammation (pp. 201-209): Springer.
Weidel, W. (1953, June 5th, 1953). Phage Receptor Systems of *Escherichia coli* B. Paper presented at the Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor, USA.
Westphal, O., & Jann, K. (1965). Bacterial Lipopolysaccharides. Methods in Carbohydrate Chemistry. In: Academic Press, Cambridge, USA.
WHO. (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. Retrieved from
WHO. (2019). Shiga Toxin-producing *Escherichia coli* (STEC) and Food: Attribution Characterization and Monitoring (Vol. 19): World Health Organization.
Wilkinson, R., Jun, P. G., & Stocker, B. (1972). Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. Microbiology, 70(3), 527-554.
Wirth, C., Condemine, G., Boiteux, C., Bernèche, S., Schirmer, T., & Peneff, C. M. (2009). NanC crystal structure, a model for outer-membrane channels of the acidic sugar-specific KdgM porin family. Journal of molecular biology, 394(4), 718-731.
Wolfe, M. M., & Soll, A. H. (1988). The physiology of gastric acid secretion. New England Journal of Medicine, 319(26), 1707-1715.
Wolschendorf, F., Mahfoud, M., & Niederweis, M. (2007). Porins are required for uptake of phosphates by Mycobacterium smegmatis. Journal of bacteriology, 189(6), 2435-2442.

World Health Organization. (2014). Antimicrobial Resistance: Global Report on Surveillance: World Health Organization. Geneva, Switzerland.
World Health Organization. (2015). Global Action Plan on Antimicrobial Resistance. In: World Health Organization. Geneva, Switzerland.
World Health Organization. (2016). National Antimicrobial Resistance Surveillance Systems and Participation in the Global Antimicrobial Resistance Surveillance System (GLASS): A Guide to Planning, Implementation, and Monitoring and Evaluation. Retrieved from
Xicohtencatl-Cortes, J., Monteiro-Neto, V., Ledesma, M. A., Jordan, D. M., Francetic, O., Kaper, J. B., Puente, J. L., & Girón, J. A. (2007). Intestinal adherence associated with type IV pili of enterohemorrhagic *Escherichia coli* O157:H7. The Journal of clinical investigation, 117(11), 3519-3529.
Yethon, J. A., Heinrichs, D. E., Monteiro, M. A., Perry, M. B., & Whitfield, C. (1998). Involvement of waaY, waaQ, andwaaP in the Modification of *Escherichia coli*Lipopolysaccharide and Their Role in the Formation of a Stable Outer Membrane. Journal of Biological Chemistry, 273(41), 26310-26316.
Yethon, J. A., Vinogradov, E., Perry, M. B., & Whitfield, C. (2000). Mutation of the Lipopolysaccharide Core Glycosyltransferase Encoded by waaG Destabilizes the Outer Membrane of *Escherichia coli* by Interfering with Core Phosphorylation. J Bacteriol, 182(19), 5620-5623. doi:10.1128/jb.182.19.5620-5623.2000
Yi, W., Liu, X., Li, Y., Li, J., Xia, C., Zhou, G., Zhang, W., Zhao, W., Chen, X., & Wang, P. G. (2009). Remodeling Bacterial Polysaccharides by Metabolic Pathway Engineering. Proc Natl Acad Sci U S A, 106(11), 4207-4212. doi:10.1073/pnas.0812432106
Yoon, S. H., Han, M. J., Jeong, H., Lee, C. H., Xia, X. X., Lee, D. H., Shim, J. H., Lee, S. Y., Oh, T. K., & Kim, J. F. (2012). Comparative Multi-omics Systems Analysis of *Escherichia coli* strains B and K-12. Genome Biol, 13(5), R37. doi:10.1186/gb-2012-13-5-r37
York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., & Albersheim, P. (1986). Isolation and Characterization of Plant Cell Walls and Cell Wall Components. In Methods in enzymology (Vol. 118, pp. 3-40): Elsevier. Amsterdam, Netherlands.
Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. J Bacteriol, 151(2), 718-722.
Yu, S., Ko, K., Chen, C., Chang, Y., & Syu, W. (2000). Characterization of the Distal Tail Fiber Locus and Determination of the Receptor for Phage AR1, Which Specifically Infects*Escherichia coli* O157: H7. Journal of bacteriology, 182(21), 5962-5968.
Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F. M., & Larsen, M. V. (2012). Identification of Acquired Antimicrobial Resistance Genes. J Antimicrob Chemother, 67(11), 2640-2644. doi:10.1093/jac/dks261
Zhong, Z., & Goodridge, L. (2020). Characterization of Phage AR1 Resistant *Escherichia coli* O157:H7 Mutants. Manuscript in preparation.
Zhong, Z., Emond-Rheault, J.-G., Bhandare, S., Lévesque, R., & Goodridge, L. (2020). Bacteriophage-Induced Lipopolysaccharide Mutations in *Escherichia coli* Lead to Hypersensitivity to Food Grade Surfactant Sodium Dodecyl Sulfate. Antibiotics, 9(9), 552.
Zumbrun, S. D., Hanson, L., Sinclair, J. F., Freedy, J., Melton-Celsa, A. R., Rodriguez-Canales, J., Hanson, J. C., & O'Brien, A. D. (2010). Human intestinal tissue and cultured colonic

cells contain globotriaosylceramide synthase mRNA and the alternate Shiga toxin receptor globotetraosylceramide. Infection and immunity, 78(11), 4488-4499.

Appendix

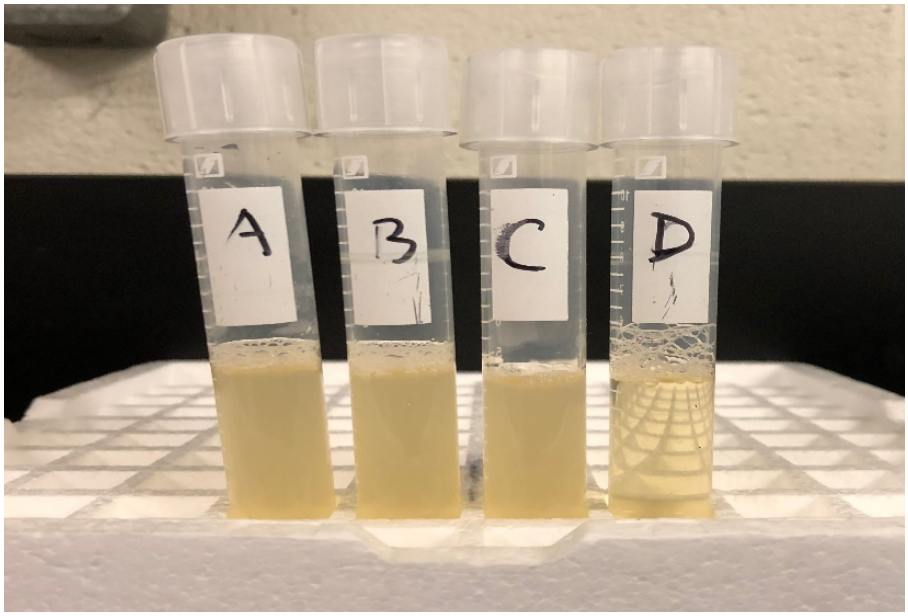

Figure S3.1. Visual appearances of four settings in phage resistance inhibition tests

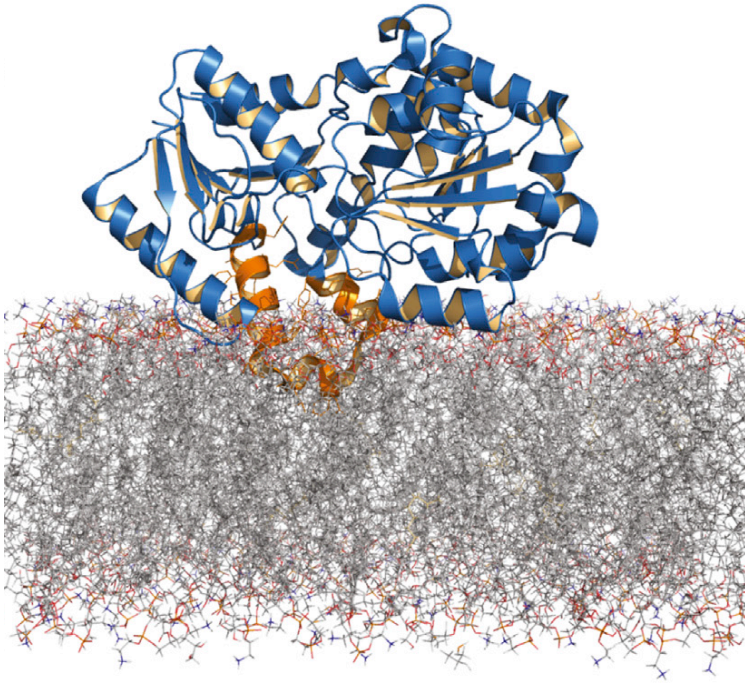

Figure S3.2. Model of WaaG from *Escherichia coli* K-12 anchoring to simulated membrane (grey). Crystal structure of glycosyltransferase waaG (PDB accession number 2IW1) is in blue and MIR-waaG is in orange. This figure was adapted from Liebau et al (2015).

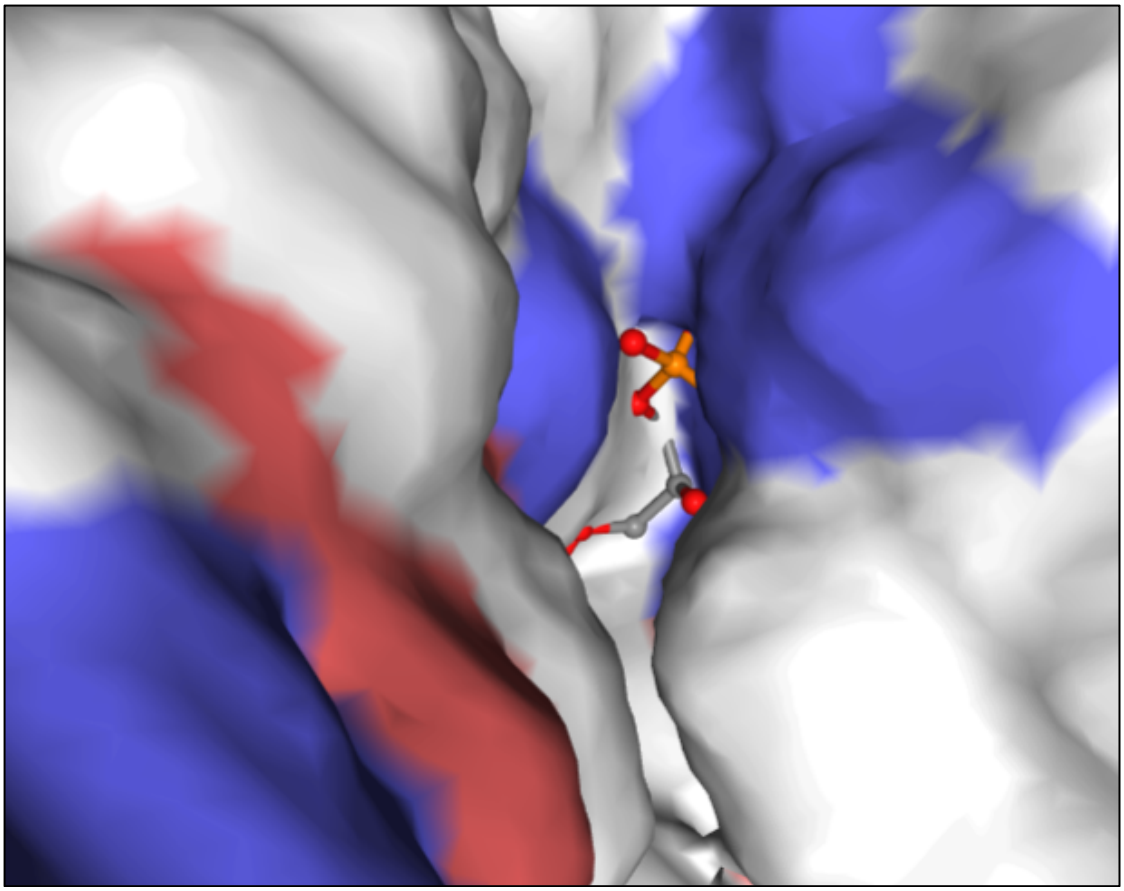

Figure S4.1. Homology modelling of BIM ZZb2 HldE protein. The substituted tyrosine (in balls and sticks) at position 18 overlaps with the solvent-accessible surface of the protein.

Table S5.1: Overall metabolism profiles of WT-B and ZZa3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM01	A01	Negative Control	C-Source, negative control		0	0
PM01	A02	L-Arabinose	C-Source, carbohydrate	C00259	3	4
PM01	A03	N-Acetyl-D-Glucosamine	C-Source, carbohydrate	C00140	4	4
PM01	A04	D-Saccharic acid	C-Source, carboxylic acid	C00818	3	3
PM01	A05	Succinic acid	C-Source, carboxylic acid	C00042	3	4
PM01	A06	D-Galactose	C-Source, carbohydrate	C00124	4	4
PM01	A07	L-Aspartic acid	C-Source, amino acid	C00049	3	4
PM01	A08	L-Proline	C-Source, amino acid	C00148	3	3
PM01	A09	D-Alanine	C-Source, amino acid	C00133	4	4
PM01	A10	D-Trehalose	C-Source, carbohydrate	C01083	4	4
PM01	A11	D-Mannose	C-Source, carbohydrate	C00159	4	4
PM01	A12	Dulcitol	C-Source, carbohydrate	C01697	3	2.5
PM01	B01	D-Serine	C-Source, amino acid	C00740	4	4
PM01	B02	D-Sorbitol	C-Source, carbohydrate	C00794	4	4
PM01	B03	Glycerol	C-Source, carbohydrate	C00116	4	4
PM01	B04	L-Fucose	C-Source, carbohydrate	C01019	0	0
PM01	B05	D-Glucuronic acid	C-Source, carboxylic acid	C00191	4	3.5
PM01	B06	D-Gluconic acid	C-Source, carboxylic acid	C00257	4	4
PM01	B07	DL-a-Glycerol Phosphate	C-Source, carbohydrate	C00093	3	3
PM01	B08	D-Xylose	C-Source, carbohydrate	C00181	3	4
PM01	B09	L-Lactic acid	C-Source, carboxylic acid	C01432	3	3
PM01	B10	Formic acid	C-Source, carboxylic acid	C00058	0	0
PM01	B11	D-Mannitol	C-Source, carbohydrate	C00392	4	4
PM01	B12	L-Glutamic acid	C-Source, amino acid	C00025	3.5	3
PM01	C01	D-Glucose-6-Phosphate	C-Source, carbohydrate	C00092	4	4
PM01	C02	D-Galactonic acid-g-Lactone	C-Source, carboxylic acid	C03383	3.5	4
PM01	C03	DL-Malic acid	C-Source, carboxylic acid	C00497	2	3
PM01	C04	D-Ribose	C-Source, carbohydrate	C00121	3	3
PM01	C05	Tween 20	C-Source, fatty acid	C11624	3	4
PM01	C06	L-Rhamnose	C-Source, carbohydrate	C00507	3	3
PM01	C07	D-Fructose	C-Source, carbohydrate	C00095	4	4
PM01	C08	Acetic acid	C-Source, carboxylic acid	C00033	2.5	3
PM01	C09	a-D-Glucose	C-Source, carbohydrate	C00031	4	4
PM01	C10	Maltose	C-Source, carbohydrate	C00208	3	3
PM01	C11	D-Melibiose	C-Source, carbohydrate	C05402	4	4

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM01	C12	Thymidine	C-Source, carbohydrate	C00214	4	4
PM01	D01	L-Asparagine	C-Source, amino acid	C00152	3	4
PM01	D02	D-Aspartic acid	C-Source, amino acid	C00402	0.5	0
PM01	D03	D-Glucosaminic acid	C-Source, carboxylic acid	C03752	1.5	1
PM01	D04	1,2-Propanediol	C-Source, alcohol	C00583	0.5	0
PM01	D05	Tween 40	C-Source, fatty acid		2	3.5
PM01	D06	a-Ketoglutaric acid	C-Source, carboxylic acid	C00026	2	1
PM01	D07	a-Ketobutyric acid	C-Source, carboxylic acid	C00109	2	3
PM01	D08	a-Methyl-D-Galactoside	C-Source, carbohydrate	C03619	4	4
PM01	D09	a-D-Lactose	C-Source, carbohydrate	C00243	3.5	4
PM01	D10	Lactulose	C-Source, carbohydrate	C07064	2.5	3
PM01	D11	Sucrose	C-Source, carbohydrate	C00089	1	0.5
PM01	D12	Uridine	C-Source, carbohydrate	C00299	4	4
PM01	E01	L-Glutamine	C-Source, amino acid	C00064	3	3
PM01	E02	m-Tartaric acid	C-Source, carboxylic acid	C00552	1.5	3
PM01	E03	D-Glucose-1-Phosphate	C-Source, carbohydrate	C00103	4	4
PM01	E04	D-Fructose-6-Phosphate	C-Source, carbohydrate	C00085	4	4
PM01	E05	Tween 80	C-Source, fatty acid	C11625	1.5	1.5
PM01	E06	a-Hydroxyglutaric acid-g-Lactone	C-Source, carboxylic acid		0	0.5
PM01	E07	a-Hydroxybutyric acid	C-Source, carboxylic acid	C05984	2	2
PM01	E08	b-Methyl-D-Glucoside	C-Source, carbohydrate		3	3
PM01	E09	Adonitol	C-Source, carbohydrate	C00474	0.5	0.5
PM01	E10	Maltotriose	C-Source, carbohydrate	C01835	3	3
PM01	E11	2`-Deoxyadenosine	C-Source, carbohydrate	C00559	4	4
PM01	E12	Adenosine	C-Source, carbohydrate	C00212	4	4
PM01	F01	Gly-Asp	C-Source, amino acid	C02871	3.5	3
PM01	F02	Citric acid	C-Source, carboxylic acid	C00158	1	0.5
PM01	F03	m-Inositol	C-Source, carbohydrate	C00137	0.5	0.5
PM01	F04	D-Threonine	C-Source, amino acid	C00820	2	0
PM01	F05	Fumaric acid	C-Source, carboxylic acid	C00122	2	3.5
PM01	F06	Bromosuccinic acid	C-Source, carboxylic acid		2	3
PM01	F07	Propionic acid	C-Source, carboxylic acid	C00163	3	3
PM01	F08	Mucic acid	C-Source, carboxylic acid	C01807	4	3
PM01	F09	Glycolic acid	C-Source, carboxylic acid	C00160	2	2
PM01	F10	Glyoxylic acid	C-Source, carboxylic acid	C00048	3	3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM01	F11	D-Cellobiose	C-Source, carbohydrate	C00185	0.5	0
PM01	F12	Inosine	C-Source, carbohydrate	C00294	4	4
PM01	G01	Gly-Glu	C-Source, amino acid		3	2.5
PM01	G02	Tricarballylic acid	C-Source, carboxylic acid		0.5	0.5
PM01	G03	L-Serine	C-Source, amino acid	C00065	4	3.5
PM01	G04	L-Threonine	C-Source, amino acid	C00188	3	3
PM01	G05	L-Alanine	C-Source, amino acid	C00041	3	3.5
PM01	G06	Ala-Gly	C-Source, amino acid		3	3.5
PM01	G07	Acetoacetic acid	C-Source, carboxylic acid	C00164	1	0
PM01	G08	N-Acetyl-D-Mannosamine	C-Source, carbohydrate	C00645	3	3
PM01	G09	Mono-Methylsuccinate	C-Source, carboxylic acid		0	0
PM01	G10	Methylpyruvate	C-Source, ester		3.5	3.5
PM01	G11	D-Malic acid	C-Source, carboxylic acid	C00497	2	3
PM01	G12	L-Malic acid	C-Source, carboxylic acid	C00149	2.5	4
PM01	H01	Gly-Pro	C-Source, amino acid		3.5	4
PM01	H02	p-Hydroxyphenyl Acetic acid	C-Source, carboxylic acid	C00642	4	4
PM01	H03	m-Hydroxyphenyl Acetic acid	C-Source, carboxylic acid	C05593	4	4
PM01	H04	Tyramine	C-Source, amine	C00483	0.5	0
PM01	H05	D-Psicose	C-Source, carbohydrate	C06468	2	3
PM01	H06	L-Lyxose	C-Source, carbohydrate	C01508	3	3
PM01	H07	Glucuronamide	C-Source, amide	D01791	2	2
PM01	H08	Pyruvic acid	C-Source, carboxylic acid	C00022	4	4
PM01	H09	L-Galactonic acid-g-Lactone	C-Source, carboxylic acid	C01115	3	3
PM01	H10	D-Galacturonic acid	C-Source, carboxylic acid	C00333	4	4
PM01	H11	Phenylethylamine	C-Source, amine	C05332	1	0.5
PM01	H12	2-Aminoethanol	C-Source, alcohol	C00189	0	0.5
PM02	A01	Negative Control	C-Source, negative control		0	0
PM02	A02	Chondroitin Sulfate C	C-Source, polymer	C00635	0	0
PM02	A03	a-Cyclodextrin	C-Source, polymer		0.5	0
PM02	A04	b-Cyclodextrin	C-Source, polymer		0	0
PM02	A05	g-Cyclodextrin	C-Source, polymer		0.5	0
PM02	A06	Dextrin	C-Source, polymer	C00721	0.5	0
PM02	A07	Gelatin	C-Source, polymer	C01498	0	0
PM02	A08	Glycogen	C-Source, polymer	C00182	0	0
PM02	A09	Inulin	C-Source, polymer	C00368	0	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM02	A10	Laminarin	C-Source, polymer	C00771	0.5	0
PM02	A11	Mannan	C-Source, polymer	C00464	0	0
PM02	A12	Pectin	C-Source, polymer	C00714	2	2
PM02	B01	N-Acetyl-D-Galactosamine	C-Source, carbohydrate	C01074	3.5	3.5
PM02	B02	N-Acetyl-Neuraminic acid	C-Source, carboxylic acid	C00270	2.5	3
PM02	B03	b-D-Allose	C-Source, carbohydrate	C01487	3	3
PM02	B04	Amygdalin	C-Source, carbohydrate	C08325	0.5	0
PM02	B05	D-Arabinose	C-Source, carbohydrate	C00216	3	3
PM02	B06	D-Arabitol	C-Source, carbohydrate	C01904	0	0
PM02	B07	L-Arabitol	C-Source, carbohydrate	C00532	0	0
PM02	B08	Arbutin	C-Source, carbohydrate	C06186	0.5	0
PM02	B09	2-Deoxy-D-Ribose	C-Source, carbohydrate	C01801	0.5	0
PM02	B10	i-Erythritol	C-Source, carbohydrate	C00503	0	0
PM02	B11	D-Fucose	C-Source, carbohydrate	C01018	0	0
PM02	B12	3-O-b-D-Galactopyranosyl-D-Arabinose	C-Source, carbohydrate		3	3
PM02	C01	Gentiobiose	C-Source, carbohydrate	C08240	0	0
PM02	C02	L-Glucose	C-Source, carbohydrate		0	0
PM02	C03	D-Lactitol	C-Source, carbohydrate		1	1
PM02	C04	D-Melezitose	C-Source, carbohydrate	C08243	0.5	0
PM02	C05	Maltitol	C-Source, carbohydrate	G00275	0.5	0
PM02	C06	a-Methyl-D-Glucoside	C-Source, carbohydrate		0	0
PM02	C07	b-Methyl-D-Galactoside	C-Source, carbohydrate	C03619	3	3
PM02	C08	3-Methylglucose	C-Source, carbohydrate		0	0
PM02	C09	b-Methyl-D-Glucuronic acid	C-Source, carboxylic acid	C08350	3	3
PM02	C10	a-Methyl-D-Mannoside	C-Source, carbohydrate		0	0
PM02	C11	b-Methyl-D-Xyloside	C-Source, carbohydrate		0.5	0
PM02	C12	Palatinose	C-Source, carbohydrate	C01742	0	0
PM02	D01	D-Raffinose	C-Source, carbohydrate	C00492	0	0
PM02	D02	Salicin	C-Source, carbohydrate	C01451	0.5	1.5
PM02	D03	Sedoheptulosan	C-Source, carbohydrate		0	0
PM02	D04	L-Sorbose	C-Source, carbohydrate	C00247	0	0
PM02	D05	Stachyose	C-Source, carbohydrate	C01613	1	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM02	D06	D-Tagatose	C-Source, carbohydrate	C00795	2	1
PM02	D07	Turanose	C-Source, carbohydrate	G03588	0	0
PM02	D08	Xylitol	C-Source, carbohydrate	C00379	0	0
PM02	D09	N-Acetyl-D-Glucosaminitol	C-Source, carbohydrate		0	0
PM02	D10	g-Amino-N-Butyric acid	C-Source, carboxylic acid	C00334	0.5	0
PM02	D11	d-Amino Valeric acid	C-Source, carboxylic acid	C00431	0	0
PM02	D12	Butyric acid	C-Source, carboxylic acid	C00246	0	0
PM02	E01	Capric acid	C-Source, carboxylic acid	C01571	0	0
PM02	E02	Caproic acid	C-Source, carboxylic acid	C01585	0	0
PM02	E03	Citraconic acid	C-Source, carboxylic acid	C02226	0	0
PM02	E04	Citramalic acid	C-Source, carboxylic acid	C00815	0	0
PM02	E05	D-Glucosamine	C-Source, carbohydrate	C00329	3.5	3
PM02	E06	2-Hydroxybenzoic acid	C-Source, carboxylic acid	C00805	0	0
PM02	E07	4-Hydroxybenzoic acid	C-Source, carboxylic acid	C00156	0	0
PM02	E08	b-Hydroxybutyric acid	C-Source, carboxylic acid	C01089	1	0
PM02	E09	g-Hydroxybutyric acid	C-Source, carboxylic acid	C00989	0	0
PM02	E10	a-Keto-Valeric acid	C-Source, carboxylic acid	C00567	0	0
PM02	E11	Itaconic acid	C-Source, carboxylic acid	C00490	0	0
PM02	E12	5-Keto-D-Gluconic acid	C-Source, carboxylic acid	C01062	2.5	2
PM02	F01	D-Lactic acid Methyl Ester	C-Source, ester		0	0
PM02	F02	Malonic acid	C-Source, carboxylic acid	C00383	0	0
PM02	F03	Melibionic acid	C-Source, carbohydrate		3	2.5
PM02	F04	Oxalic acid	C-Source, carboxylic acid	C00209	0	0
PM02	F05	Oxalomalic acid	C-Source, carboxylic acid	C01990	0	0
PM02	F06	Quinic acid	C-Source, carboxylic acid	C00296	0	0
PM02	F07	D-Ribono-1,4-Lactone	C-Source, carboxylic acid		0	0
PM02	F08	Sebacic acid	C-Source, carboxylic acid	C08277	0	0
PM02	F09	Sorbic acid	C-Source, carboxylic acid		0	0
PM02	F10	Succinamic acid	C-Source, carboxylic acid		0	0
PM02	F11	D-Tartaric acid	C-Source, carboxylic acid	C02107	0.5	0
PM02	F12	L-Tartaric acid	C-Source, carboxylic acid	C00898	0	0
PM02	G01	Acetamide	C-Source, amide	C06244	0	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM02	G02	L-Alaninamide	C-Source, amide		2	2
PM02	G03	N-Acetyl-L-Glutamic acid	C-Source, amino acid	C00624	2	1
PM02	G04	L-Arginine	C-Source, amino acid	C00062	2	1.5
PM02	G05	Glycine	C-Source, amino acid	C00037	2.5	2
PM02	G06	L-Histidine	C-Source, amino acid	C00135	0	0
PM02	G07	L-Homoserine	C-Source, amino acid	C00263	0	0
PM02	G08	Hydroxy-L-Proline	C-Source, amino acid	C01015	0	0
PM02	G09	L-Isoleucine	C-Source, amino acid	C00407	0	0
PM02	G10	L-Leucine	C-Source, amino acid	C00123	0	0
PM02	G11	L-Lysine	C-Source, amino acid	C00047	0	0
PM02	G12	L-Methionine	C-Source, amino acid	C00073	0	0
PM02	H01	L-Ornithine	C-Source, amino acid	C00077	2	1
PM02	H02	L-Phenylalanine	C-Source, amino acid	C00079	0.5	0
PM02	H03	L-Pyroglutamic acid	C-Source, amino acid	C02238	0	0
PM02	H04	L-Valine	C-Source, amino acid	C00183	0	0
PM02	H05	D,L-Carnitine	C-Source, carboxylic acid	C00487	0	0
PM02	H06	sec-Butylamine	C-Source, amine		0	0
PM02	H07	D,L-Octopamine	C-Source, amine	C04227	0	0
PM02	H08	Putrescine	C-Source, amine	C00134	0	0
PM02	H09	Dihydroxyacetone	C-Source, alcohol	C00184	2.5	3
PM02	H10	2,3-Butanediol	C-Source, alcohol	C03044	0	0
PM02	H11	2,3-Butanedione	C-Source, alcohol	C00741	0	0
PM02	H12	3-Hydroxy-2-buta	C-Source, alcohol	C00466	0	0
PM03	A01	Negative Control	N-Source, Negative control		0	0
PM03	A02	Ammonia	N-Source, inorganic	C00014	4	3
PM03	A03	Nitrite	N-Source, inorganic	C00088	2	2
PM03	A04	Nitrate	N-Source, inorganic	C00244	2	1
PM03	A05	Urea	N-Source, other	C00086	0	0
PM03	A06	Biuret	N-Source, other	C06555	2	1
PM03	A07	L-Alanine	N-Source, amino acid	C00041	4	3
PM03	A08	L-Arginine	N-Source, amino acid	C00062	4	3
PM03	A09	L-Asparagine	N-Source, amino acid	C00152	4	4
PM03	A10	L-Aspartic acid	N-Source, amino acid	C00049	4	4
PM03	A11	L-Cysteine	N-Source, amino acid	C00097	4	4
PM03	A12	L-Glutamic acid	N-Source, amino acid	C00025	4	4
PM03	B01	L-Glutamine	N-Source, amino acid	C00064	4	4
PM03	B02	Glycine	N-Source, amino acid	C00037	4	3
PM03	B03	L-Histidine	N-Source, amino acid	C00135	2	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM03	B04	L-Isoleucine	N-Source, amino acid	C00407	2	1.5
PM03	B05	L-Leucine	N-Source, amino acid	C00123	3	2
PM03	B06	L-Lysine	N-Source, amino acid	C00047	3	2
PM03	B07	L-Methionine	N-Source, amino acid	C00073	3	2
PM03	B08	L-Phenylalanine	N-Source, amino acid	C00079	3	2
PM03	B09	L-Proline	N-Source, amino acid	C00148	3.5	3
PM03	B10	L-Serine	N-Source, amino acid	C00065	4	4
PM03	B11	L-Threonine	N-Source, amino acid	C00188	3	2
PM03	B12	L-Tryptophan	N-Source, amino acid	C00078	3	2.5
PM03	C01	L-Tyrosine	N-Source, amino acid	C00082	2	2
PM03	C02	L-Valine	N-Source, amino acid	C00183	3	2
PM03	C03	D-Alanine	N-Source, amino acid	C00133	4	4
PM03	C04	D-Asparagine	N-Source, amino acid	C01905	3.5	3
PM03	C05	D-Aspartic acid	N-Source, amino acid	C00402	0	0
PM03	C06	D-Glutamic acid	N-Source, amino acid	C00217	0	0
PM03	C07	D-Lysine	N-Source, amino acid	C00739	1	0
PM03	C08	D-Serine	N-Source, amino acid	C00740	4	3
PM03	C09	D-Valine	N-Source, amino acid	C06417	1.5	1
PM03	C10	L-Citrulline	N-Source, amino acid	C00327	2	2
PM03	C11	L-Homoserine	N-Source, amino acid	C00263	2	2
PM03	C12	L-Ornithine	N-Source, amino acid	C00077	3.5	3
PM03	D01	N-Acetyl-L-Glutamic acid	N-Source, amino acid	C00624	3	2
PM03	D02	N-Phthaloyl-L-Glutamic acid	N-Source, amino acid		0	0
PM03	D03	L-Pyroglutamic acid	N-Source, amino acid	C02238	1	0.5
PM03	D04	Hydroxylamine	N-Source, other	C00192	0	0
PM03	D05	Methylamine	N-Source, other	C00218	0	0
PM03	D06	N-Amylamine	N-Source, other		0	0
PM03	D07	N-Butylamine	N-Source, other		0	0
PM03	D08	Ethylamine	N-Source, other	C00797	1	0
PM03	D09	Ethanolamine	N-Source, other	C00189	0.5	0
PM03	D10	Ethylenediamine	N-Source, other	C12511	0	0
PM03	D11	Putrescine	N-Source, other	C00134	1	1
PM03	D12	Agmatine	N-Source, other	C00179	0	0
PM03	E01	Histamine	N-Source, other	C00388	0	0
PM03	E02	b-Phenylethylamine	N-Source, other	C05332	0	0
PM03	E03	Tyramine	N-Source, other	C00483	0.5	0
PM03	E04	Acetamide	N-Source, other	C06244	0.5	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM03	E05	Formamide	N-Source, other	C00488	0.5	0.5
PM03	E06	Glucuronamide	N-Source, other	D01791	1.5	2
PM03	E07	DL-Lactamide	N-Source, other		0	0
PM03	E08	D-Glucosamine	N-Source, other	C00329	3	3
PM03	E09	D-Galactosamine	N-Source, other	C02262	2	2
PM03	E10	D-Mannosamine	N-Source, other	C03570	2	2
PM03	E11	N-Acetyl-D-Glucosamine	N-Source, other	C00140	2.5	2
PM03	E12	N-Acetyl-D-Galactosamine	N-Source, other	C01074	3.5	3
PM03	F01	N-Acetyl-D-Mannosamine	N-Source, other	C00645	2	1
PM03	F02	Adenine	N-Source, other	C00147	3	3
PM03	F03	Adenosine	N-Source, other	C00212	4	4
PM03	F04	Cytidine	N-Source, other	C00475	4	4
PM03	F05	Cytosine	N-Source, other	C00380	4	4
PM03	F06	Guanine	N-Source, other	C00242	0	0
PM03	F07	Guanosine	N-Source, other	C00387	1.5	1.5
PM03	F08	Thymine	N-Source, other	C00178	3	2
PM03	F09	Thymidine	N-Source, other	C00214	2	2
PM03	F10	Uracil	N-Source, other	C00106	3	2
PM03	F11	Uridine	N-Source, other	C00299	2.5	2
PM03	F12	Inosine	N-Source, other	C00294	0	0
PM03	G01	Xanthine	N-Source, other	C00385	2	2
PM03	G02	Xanthosine	N-Source, other	C01762	2	2
PM03	G03	Uric acid	N-Source, other	C00366	1	1
PM03	G04	Alloxan	N-Source, other	C07599	1.5	1.5
PM03	G05	Allantoin	N-Source, other	C01551	1.5	1
PM03	G06	Parabanic acid	N-Source, other		1.5	1
PM03	G07	DL-a-Amino-N-Butyric acid	N-Source, other	C02261	4	3
PM03	G08	g-Amino-N-Butyric acid	N-Source, other	C00334	3	2
PM03	G09	e-Amino-N-Caproic acid	N-Source, other	C02378	2	2
PM03	G10	DL-a-Amino-Caprylic acid	N-Source, other		3	2
PM03	G11	d-Amino-N-Valeric acid	N-Source, other	C00431	3	2
PM03	G12	a-Amino-N-Valeric acid	N-Source, other	C01826	3.5	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM03	H01	Ala-Asp	N-Source, peptide	C02871	4	4
PM03	H02	Ala-Gln	N-Source, peptide		4	4
PM03	H03	Ala-Glu	N-Source, peptide		4	4
PM03	H04	Ala-Gly	N-Source, peptide		4	3.5
PM03	H05	Ala-His	N-Source, peptide		4	3
PM03	H06	Ala-Leu	N-Source, peptide	C05035	4	3
PM03	H07	Ala-Thr	N-Source, peptide		4	3
PM03	H08	Gly-Asn	N-Source, peptide		4	4
PM03	H09	Gly-Gln	N-Source, peptide		4	4
PM03	H10	Gly-Glu	N-Source, peptide		4	3.5
PM03	H11	Gly-Met	N-Source, peptide		4	3
PM03	H12	Met-Ala	N-Source, peptide		4	3
PM04	A01	Negative Control	P-Source, Negative control		0	0
PM04	A02	Phosphate	P-Source, inorganic	C00009	4	4
PM04	A03	Pyrophosphate	P-Source, inorganic	C00013	4	4
PM04	A04	Trimetaphosphate	P-Source, inorganic	C02466	4	3.5
PM04	A05	Tripolyphosphate	P-Source, inorganic	C00536	4	3.5
PM04	A06	Triethyl Phosphate	P-Source, organic		0.5	0
PM04	A07	Hypophosphite	P-Source, inorganic		2	0
PM04	A08	Adenosine 2`-Monophosphate	P-Source, organic	C00946	4	3
PM04	A09	Adenosine 3`-Monophosphate	P-Source, organic	C01367	4	4
PM04	A10	Adenosine 5`-Monophosphate	P-Source, organic	C00020	4	3.5
PM04	A11	Adenosine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	4
PM04	A12	Adenosine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00575	3.5	3
PM04	B01	Thiophosphate	P-Source, inorganic		4	3
PM04	B02	Dithiophosphate	P-Source, inorganic		4	3
PM04	B03	DL-a-Glycerol Phosphate	P-Source, organic	C00093	4	3
PM04	B04	b-Glycerol Phosphate	P-Source, organic	C02979	4	3
PM04	B05	Carbamyl Phosphate	P-Source, organic	C00169	4	3.5
PM04	B06	D-2-Phospho-Glyceric acid	P-Source, organic	C00631	4	4
PM04	B07	D-3-Phospho-Glyceric acid	P-Source, organic	C00197	4	3.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM04	B08	Guanosine 2`-Monophosphate	P-Source, organic		4	3.5
PM04	B09	Guanosine 3`-Monophosphate	P-Source, organic	C06193	4	4
PM04	B10	Guanosine 5`-Monophosphate	P-Source, organic	C00144	4	4
PM04	B11	Guanosine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	3.5
PM04	B12	Guanosine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00942	1.5	1.5
PM04	C01	Phosphoenol Pyruvate	P-Source, organic	C00074	4	3
PM04	C02	Phospho-Glycolic acid	P-Source, organic	C00988	4	3.5
PM04	C03	D-Glucose-1-Phosphate	P-Source, organic	C00103	4	3
PM04	C04	D-Glucose-6-Phosphate	P-Source, organic	C00092	3.5	3
PM04	C05	2-Deoxy-D-Glucose 6-Phosphate	P-Source, organic	C06369	0.5	0.5
PM04	C06	D-Glucosamine-6-Phosphate	P-Source, organic	C00352	4	4
PM04	C07	6-Phospho-Gluconic acid	P-Source, organic		4	3.5
PM04	C08	Cytidine 2`-Monophosphate	P-Source, organic	C03104	4	3
PM04	C09	Cytidine 3`-Monophosphate	P-Source, organic	C05822	0.5	0
PM04	C10	Cytidine 5`-Monophosphate	P-Source, organic	C00055	4	4
PM04	C11	Cytidine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	3
PM04	C12	Cytidine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00941	2	2
PM04	D01	D-Mannose-1-Phosphate	P-Source, organic	C00636	4	3.5
PM04	D02	D-Mannose-6-Phosphate	P-Source, organic	C00275	3.5	3
PM04	D03	Cysteamine-S-Phosphate	P-Source, organic		4	4
PM04	D04	Phospho-L-Arginine	P-Source, organic	C05945	4	3.5
PM04	D05	O-Phospho-D-Serine	P-Source, organic	C02532	4	3
PM04	D06	O-Phospho-L-Serine	P-Source, organic	C01005	4	4

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM04	D07	O-Phospho-L-Threonine	P-Source, organic		4	3.5
PM04	D08	Uridine 2`-Monophosphate	P-Source, organic	C03031	4	3
PM04	D09	Uridine 3`-Monophosphate	P-Source, organic	C01368	4	4
PM04	D10	Uridine 5`-Monophosphate	P-Source, organic	C00105	4	4
PM04	D11	Uridine 2`,3`-Cyclic Monophosphate	P-Source, organic	C02355	4	3
PM04	D12	Uridine 3`,5`-Cyclic Monophosphate	P-Source, organic		1.5	2
PM04	E01	O-Phospho-D-Tyrosine	P-Source, organic	C06501	4	4
PM04	E02	O-Phospho-L-Tyrosine	P-Source, organic	C06501	4	3
PM04	E03	Phosphocreatine	P-Source, organic	C02305	4	4
PM04	E04	Phosphoryl Choline	P-Source, organic	C00588	4	3
PM04	E05	O-Phosphoryl-Ethanolamine	P-Source, organic	C00346	4	3
PM04	E06	Phosphono Acetic acid	P-Source, organic	C05682	2	1
PM04	E07	2-Aminoethyl Phosphonic acid	P-Source, organic	C03557	4	3.5
PM04	E08	Methylene Diphosphonic acid	P-Source, organic		0	0
PM04	E09	Thymidine 3`-Monophosphate	P-Source, organic		4	4
PM04	E10	Thymidine 5`-Monophosphate	P-Source, organic	C00364	4	4
PM04	E11	Inositol Hexaphosphate	P-Source, organic	C01204	4	4
PM04	E12	Thymidine 3`,5`-Cyclic Monophosphate	P-Source, organic		1.5	1.5
PM04	F01	Negative Control	S-Source, Negative control		0	0
PM04	F02	Sulfate	S-Source, inorganic	C00059	3	2.5
PM04	F03	Thiosulfate	S-Source, inorganic	C00320	3	2.5
PM04	F04	Tetrathionate	S-Source, inorganic	C02084	3.5	2.5
PM04	F05	Thiophosphate	S-Source, inorganic		3	2.5
PM04	F06	Dithiophosphate	S-Source, inorganic		3	3
PM04	F07	L-Cysteine	S-Source, organic	C00097	3	2.5
PM04	F08	D-Cysteine	S-Source, organic	C00793	2	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM04	F09	Cys-Gly	S-Source, organic		3	3
PM04	F10	L-Cysteic acid	S-Source, organic	C00506	3	2
PM04	F11	Cysteamine	S-Source, organic	C01678	1	0
PM04	F12	L-Cysteine Sulfinic acid	S-Source, organic	C00606	3	2.5
PM04	G01	N-Acetyl-L-Cysteine	S-Source, organic	C06809	0	0
PM04	G02	S-Methyl-L-Cysteine	S-Source, organic	C03540	0	0
PM04	G03	Cystathionine	S-Source, organic	C00542	2	1
PM04	G04	Lanthionine	S-Source, organic		1	0
PM04	G05	Glutathione	S-Source, organic	C00051	3	2.5
PM04	G06	DL-Ethionine	S-Source, organic	C11227	0	0
PM04	G07	L-Methionine	S-Source, organic	C00073	3	2
PM04	G08	D-Methionine	S-Source, organic	C00855	2	0
PM04	G09	Gly-Met	S-Source, organic		3	2
PM04	G10	N-Acetyl-D,L-Methionine	S-Source, organic	C02712	2.5	2
PM04	G11	L-Methionine Sulfoxide	S-Source, organic	C02989	3	2
PM04	G12	L-Methionine Sulfone	S-Source, organic		0	0
PM04	H01	L-Djenkolic acid	S-Source, organic	C08275	1	1
PM04	H02	Thiourea	S-Source, organic	C14415	1	0
PM04	H03	1-Thio-b-D-Glucose	S-Source, organic		2	0
PM04	H04	DL-Lipoamide	S-Source, organic	C00248	0	0
PM04	H05	Taurocholic acid	S-Source, organic	C05122	2	1
PM04	H06	Taurine	S-Source, organic	C00245	3	3
PM04	H07	Hypotaurine	S-Source, organic	C00519	3	2
PM04	H08	p-Aminobenzene Sulfonic acid	S-Source, organic	C00568	0	0
PM04	H09	Butane Sulfonic acid	S-Source, organic		4	3
PM04	H10	2-Hydroxyethane Sulfonic acid	S-Source, organic	C05123	3.5	3
PM04	H11	Methane Sulfonic acid	S-Source, organic	C11145	3	3
PM04	H12	Tetramethylene Sulfone	S-Source, organic		0	0
PM05	A01	Negative Control	Nutritional supplement, Negative control		0	0
PM05	A02	Positive Control	Nutritional supplement, Positive control		1	0
PM05	A03	L-Alanine	Nutritional supplement	C00041	0	1
PM05	A04	L-Arginine	Nutritional supplement	C00062	0	0.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM05	A05	L-Asparagine	Nutritional supplement	C00152	0	0
PM05	A06	L-Aspartic acid	Nutritional supplement	C00049	0.5	0.5
PM05	A07	L-Cysteine	Nutritional supplement	C00097	0	0.5
PM05	A08	L-Glutamic acid	Nutritional supplement	C00025	0.5	0
PM05	A09	Adenosine 3`,5`-Cyclic Monophosphate	Nutritional supplement	C00575	0	0
PM05	A10	Adenine	Nutritional supplement	C00147	0	0
PM05	A11	Adenosine	Nutritional supplement	C00212	0	0
PM05	A12	2`-Deoxyadenosine	Nutritional supplement	C00559	0	0
PM05	B01	L-Glutamine	Nutritional supplement	C00064	0.5	0
PM05	B02	Glycine	Nutritional supplement	C00037	0	0
PM05	B03	L-Histidine	Nutritional supplement	C00135	0	0.5
PM05	B04	L-Isoleucine	Nutritional supplement	C00407	0	0
PM05	B05	L-Leucine	Nutritional supplement	C00123	0	0
PM05	B06	L-Lysine	Nutritional supplement	C00047	0	0
PM05	B07	L-Methionine	Nutritional supplement	C00073	0	0
PM05	B08	L-Phenylalanine	Nutritional supplement	C00079	0	0
PM05	B09	Guanosine 3`,5`-Cyclic Monophosphate	Nutritional supplement	C00942	0	0.5
PM05	B10	Guanine	Nutritional supplement	C00242	0	0
PM05	B11	Guanosine	Nutritional supplement	C00387	0	0
PM05	B12	2`-Deoxyguanosine	Nutritional supplement	C00330	0	0.5
PM05	C01	L-Proline	Nutritional supplement	C00148	0.5	0.5
PM05	C02	L-Serine	Nutritional supplement	C00065	0	0
PM05	C03	L-Threonine	Nutritional supplement	C00188	0	0
PM05	C04	L-Tryptophan	Nutritional supplement	C00078	0	0.5
PM05	C05	L-Tyrosine	Nutritional supplement	C00082	0	0
PM05	C06	L-Valine	Nutritional supplement	C00183	0	0
PM05	C07	L-Isoleucine + L-Valine	Nutritional supplement		0.5	0
PM05	C08	Hydroxy-L-Proline	Nutritional supplement	C01015	0	0
PM05	C09	(5) 4-Amino-Imidazole-4(5)-Carboxamide	Nutritional supplement	C04051	0	0
PM05	C10	Hypoxanthine	Nutritional supplement	C00262	0	0
PM05	C11	Inosine	Nutritional supplement	C00294	0	0
PM05	C12	2`-Deoxyinosine	Nutritional supplement	C05512	0	0.5
PM05	D01	L-Ornithine	Nutritional supplement	C00077	0.5	0.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM05	D02	L-Citrulline	Nutritional supplement	C00327	0	0
PM05	D03	Chorismic acid	Nutritional supplement	C00251	0	0
PM05	D04	(-)Shikimic acid	Nutritional supplement	C00493	0	0
PM05	D05	L-Homoserine Lactone	Nutritional supplement		0	0
PM05	D06	D-Alanine	Nutritional supplement	C00133	0	0
PM05	D07	D-Aspartic acid	Nutritional supplement	C00402	0	0
PM05	D08	D-Glutamic acid	Nutritional supplement	C00217	0.5	0
PM05	D09	DL-Diamino-a,e-Pimelic acid	Nutritional supplement	C00680	0	0
PM05	D10	Cytosine	Nutritional supplement	C00380	0	0
PM05	D11	Cytidine	Nutritional supplement	C00475	0	0
PM05	D12	2`-Deoxycytidine	Nutritional supplement	C00881	0	0
PM05	E01	Putrescine	Nutritional supplement	C00134	0	0.5
PM05	E02	Spermidine	Nutritional supplement	C00315	0	0
PM05	E03	Spermine	Nutritional supplement	C00750	0	0
PM05	E04	Pyridoxine	Nutritional supplement	C00314	0	0
PM05	E05	Pyridoxal	Nutritional supplement	C00250	0.5	0
PM05	E06	Pyridoxamine	Nutritional supplement	C00534	0.5	0
PM05	E07	b-Alanine	Nutritional supplement	C00099	0.5	0.5
PM05	E08	D-Pantothenic acid	Nutritional supplement	C00864	0	0
PM05	E09	Orotic acid	Nutritional supplement	C00295	0.5	0.5
PM05	E10	Uracil	Nutritional supplement	C00106	0	0
PM05	E11	Uridine	Nutritional supplement	C00299	0	0
PM05	E12	2`-Deoxyuridine	Nutritional supplement	C00526	0	0.5
PM05	F01	Quinolinic acid	Nutritional supplement	C03722	0.5	1
PM05	F02	Nicotinic acid	Nutritional supplement	C00253	0	0
PM05	F03	Nicotinamide	Nutritional supplement	C00153	0	0
PM05	F04	b-Nicotinamide Adenine Dinucleotide	Nutritional supplement	C00003	0	0
PM05	F05	d-Amino-levulinic acid	Nutritional supplement	C00431	0	0
PM05	F06	Hematin	Nutritional supplement		0	0
PM05	F07	Deferoxamine	Nutritional supplement	C06940	0	0
PM05	F08	a-D-Glucose	Nutritional supplement	C00031	0.5	0
PM05	F09	N-Acetyl-D-Glucosamine	Nutritional supplement	C00140	0	0
PM05	F10	Thymine	Nutritional supplement	C00178	0	0.5
PM05	F11	Glutathione	Nutritional supplement	C00051	0	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM05	F12	Thymidine	Nutritional supplement	C00214	0	0
PM05	G01	Oxaloacetic acid	Nutritional supplement	C00036	0	0
PM05	G02	D-Biotin	Nutritional supplement	C00120	0.5	0.5
PM05	G03	Cyanocobalamin	Nutritional supplement	C02823	0	0.5
PM05	G04	p-Amino-Benzoic acid	Nutritional supplement	C00568	0	0
PM05	G05	Folic acid	Nutritional supplement	C00504	0	0
PM05	G06	Inosine + Thiamine	Nutritional supplement		0	0
PM05	G07	Thiamine	Nutritional supplement	C00378	0	0
PM05	G08	Thiamine Pyrophosphate	Nutritional supplement	C00068	0	0.5
PM05	G09	Riboflavin	Nutritional supplement	C00255	0	0.5
PM05	G10	Pyrrolo-Quinoline Qui	Nutritional supplement	C00113	0	0
PM05	G11	Menadione	Nutritional supplement	C05377	0	0
PM05	G12	m-Inositol	Nutritional supplement	C00137	0	0
PM05	H01	Butyric acid	Nutritional supplement	C00246	0	0
PM05	H02	a-Hydroxybutyric acid	Nutritional supplement	C05984	0.5	0.5
PM05	H03	a-Ketobutyric acid	Nutritional supplement	C00109	0	0
PM05	H04	Caprylic acid	Nutritional supplement	C06423	0	0.5
PM05	H05	DL-Thioctic acid	Nutritional supplement	C00725	0	0.5
PM05	H06	DL-Mevalonic acid Lactone	Nutritional supplement	C02104	0	0
PM05	H07	DL-Carnitine	Nutritional supplement	C00487	0	0
PM05	H08	Choline	Nutritional supplement	C00114	0	0.5
PM05	H09	Tween 20	Nutritional supplement	C11624	0	0.5
PM05	H10	Tween 40	Nutritional supplement		0	0
PM05	H11	Tween 60	Nutritional supplement		0	0
PM05	H12	Tween 80	Nutritional supplement	C11625	0	0.5
PM06	A01	Negative Control	N-Source, Negative control		0	0
PM06	A02	L-Glutamine	N-Source, amino acid	C00064	4	4
PM06	A03	Ala-Ala	N-Source, peptide		4	3
PM06	A04	Ala-Arg	N-Source, peptide	C05034	4	3
PM06	A05	Ala-Asn	N-Source, peptide		4	4
PM06	A06	Ala-Glu	N-Source, peptide		4	3
PM06	A07	Ala-Gly	N-Source, peptide		4	3.5
PM06	A08	Ala-His	N-Source, peptide		3.5	3
PM06	A09	Ala-Leu	N-Source, peptide	C05035	4	3
PM06	A10	Ala-Lys	N-Source, peptide	C05036	4	3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM06	A11	Ala-Phe	N-Source, peptide		4	3.5
PM06	A12	Ala-Pro	N-Source, peptide		4	3.5
PM06	B01	Ala-Ser	N-Source, peptide		4	3
PM06	B02	Ala-Thr	N-Source, peptide		3.5	3
PM06	B03	Ala-Trp	N-Source, peptide	C02732	3.5	3
PM06	B04	Ala-Tyr	N-Source, peptide	C05039	3.5	2.5
PM06	B05	Arg-Ala	N-Source, peptide		4	4
PM06	B06	Arg-Arg	N-Source, peptide	C05034	3.5	3
PM06	B07	Arg-Asp	N-Source, peptide	C02871	4	4
PM06	B08	Arg-Gln	N-Source, peptide		4	3.5
PM06	B09	Arg-Glu	N-Source, peptide		4	4
PM06	B10	Arg-Ile	N-Source, peptide		4	4
PM06	B11	Arg-Leu	N-Source, peptide	C05035	3.5	3
PM06	B12	Arg-Lys	N-Source, peptide	C05036	4	3
PM06	C01	Arg-Met	N-Source, peptide		4	3
PM06	C02	Arg-Phe	N-Source, peptide		3.5	3
PM06	C03	Arg-Ser	N-Source, peptide		4	4
PM06	C04	Arg-Trp	N-Source, peptide	C02732	4	2.5
PM06	C05	Arg-Tyr	N-Source, peptide	C05039	4	4
PM06	C06	Arg-Val	N-Source, peptide		4	3.5
PM06	C07	Asn-Glu	N-Source, peptide		4	4
PM06	C08	Asn-Val	N-Source, peptide		4	3.5
PM06	C09	Asp-Asp	N-Source, peptide	C02871	4	3
PM06	C10	Asp-Glu	N-Source, peptide		3.5	3
PM06	C11	Asp-Leu	N-Source, peptide	C05035	3.5	3
PM06	C12	Asp-Lys	N-Source, peptide	C05036	4	3
PM06	D01	Asp-Phe	N-Source, peptide		4	3
PM06	D02	Asp-Trp	N-Source, peptide	C02732	2.5	2
PM06	D03	Asp-Val	N-Source, peptide		3.5	3
PM06	D04	Cys-Gly	N-Source, peptide		4	3.5
PM06	D05	Gln-Gln	N-Source, peptide		4	4
PM06	D06	Gln-Gly	N-Source, peptide		4	4
PM06	D07	Glu-Asp	N-Source, peptide	C02871	4	3.5
PM06	D08	Glu-Glu	N-Source, peptide		3.5	2.5
PM06	D09	Glu-Gly	N-Source, peptide		3.5	3
PM06	D10	Glu-Ser	N-Source, peptide		4	4
PM06	D11	Glu-Trp	N-Source, peptide	C02732	3.5	3
PM06	D12	Glu-Tyr	N-Source, peptide	C05039	4	3
PM06	E01	Glu-Val	N-Source, peptide		4	3.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM06	E02	Gly-Ala	N-Source, peptide		4	3
PM06	E03	Gly-Arg	N-Source, peptide	C05034	4	4
PM06	E04	Gly-Cys	N-Source, peptide		3.5	2.5
PM06	E05	Gly-Gly	N-Source, peptide		4	4
PM06	E06	Gly-His	N-Source, peptide		4	3.5
PM06	E07	Gly-Leu	N-Source, peptide	C05035	4	3.5
PM06	E08	Gly-Lys	N-Source, peptide	C05036	3.5	2.5
PM06	E09	Gly-Met	N-Source, peptide		4	3
PM06	E10	Gly-Phe	N-Source, peptide		4	4
PM06	E11	Gly-Pro	N-Source, peptide		3.5	3
PM06	E12	Gly-Ser	N-Source, peptide		4	4
PM06	F01	Gly-Thr	N-Source, peptide		3	2
PM06	F02	Gly-Trp	N-Source, peptide	C02732	4	3
PM06	F03	Gly-Tyr	N-Source, peptide	C05039	4	3
PM06	F04	Gly-Val	N-Source, peptide		4	3.5
PM06	F05	His-Asp	N-Source, peptide	C02871	4	3.5
PM06	F06	His-Gly	N-Source, peptide		3.5	3
PM06	F07	His-Leu	N-Source, peptide	C05035	2.5	2
PM06	F08	His-Lys	N-Source, peptide	C05036	1	1
PM06	F09	His-Met	N-Source, peptide		3.5	2.5
PM06	F10	His-Pro	N-Source, peptide		3	2.5
PM06	F11	His-Ser	N-Source, peptide		4	3
PM06	F12	His-Trp	N-Source, peptide	C02732	3.5	2.5
PM06	G01	His-Tyr	N-Source, peptide	C05039	2	2
PM06	G02	His-Val	N-Source, peptide		3	2
PM06	G03	Ile-Ala	N-Source, peptide		4	4
PM06	G04	Ile-Arg	N-Source, peptide	C05034	4	3.5
PM06	G05	Ile-Gln	N-Source, peptide		4	4
PM06	G06	Ile-Gly	N-Source, peptide		4	3.5
PM06	G07	Ile-His	N-Source, peptide		2.5	2
PM06	G08	Ile-Ile	N-Source, peptide		2	1.5
PM06	G09	Ile-Met	N-Source, peptide		3	2.5
PM06	G10	Ile-Phe	N-Source, peptide		3	2
PM06	G11	Ile-Pro	N-Source, peptide		3	3
PM06	G12	Ile-Ser	N-Source, peptide		4	4
PM06	H01	Ile-Trp	N-Source, peptide	C02732	3.5	3
PM06	H02	Ile-Tyr	N-Source, peptide	C05039	3.5	2
PM06	H03	Ile-Val	N-Source, peptide		2.5	2
PM06	H04	Leu-Ala	N-Source, peptide		4	3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM06	H05	Leu-Arg	N-Source, peptide	C05034	4	3
PM06	H06	Leu-Asp	N-Source, peptide	C02871	4	4
PM06	H07	Leu-Glu	N-Source, peptide		4	3
PM06	H08	Leu-Gly	N-Source, peptide		4	3
PM06	H09	Leu-Ile	N-Source, peptide		2.5	2
PM06	H10	Leu-Leu	N-Source, peptide	C05035	3	3
PM06	H11	Leu-Met	N-Source, peptide		4	3
PM06	H12	Leu-Phe	N-Source, peptide		3.5	3
PM07	A01	Negative Control	N-Source, Negative control		0	0
PM07	A02	L-Glutamine	N-Source, amino acid	C00064	4	4
PM07	A03	Leu-Ser	N-Source, peptide		4	3
PM07	A04	Leu-Trp	N-Source, peptide	C02732	4	3
PM07	A05	Leu-Val	N-Source, peptide		3	2
PM07	A06	Lys-Ala	N-Source, peptide		4	3
PM07	A07	Lys-Arg	N-Source, peptide	C05034	4	3
PM07	A08	Lys-Glu	N-Source, peptide		4	3
PM07	A09	Lys-Ile	N-Source, peptide		3	2
PM07	A10	Lys-Leu	N-Source, peptide	C05035	3	2
PM07	A11	Lys-Lys	N-Source, peptide	C05036	1.5	0.5
PM07	A12	Lys-Phe	N-Source, peptide		3	2
PM07	B01	Lys-Pro	N-Source, peptide		4	3
PM07	B02	Lys-Ser	N-Source, peptide		4	3
PM07	B03	Lys-Thr	N-Source, peptide		3.5	2.5
PM07	B04	Lys-Trp	N-Source, peptide	C02732	3.5	3
PM07	B05	Lys-Tyr	N-Source, peptide	C05039	2.5	2
PM07	B06	Lys-Val	N-Source, peptide		2.5	2
PM07	B07	Met-Arg	N-Source, peptide	C05034	4	3
PM07	B08	Met-Asp	N-Source, peptide	C02871	4	4
PM07	B09	Met-Gln	N-Source, peptide		4	4
PM07	B10	Met-Glu	N-Source, peptide		4	3.5
PM07	B11	Met-Gly	N-Source, peptide		4	3
PM07	B12	Met-His	N-Source, peptide		3	2
PM07	C01	Met-Ile	N-Source, peptide		4	3
PM07	C02	Met-Leu	N-Source, peptide	C05035	3.5	3
PM07	C03	Met-Lys	N-Source, peptide	C05036	3.5	2
PM07	C04	Met-Met	N-Source, peptide		3	2
PM07	C05	Met-Phe	N-Source, peptide		3	2
PM07	C06	Met-Pro	N-Source, peptide		4	3
PM07	C07	Met-Trp	N-Source, peptide	C02732	4	3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM07	C08	Met-Val	N-Source, peptide		4	3
PM07	C09	Phe-Ala	N-Source, peptide		4	4
PM07	C10	Phe-Gly	N-Source, peptide		4	3.5
PM07	C11	Phe-Ile	N-Source, peptide		3	3
PM07	C12	Phe-Phe	N-Source, peptide		3	2
PM07	D01	Phe-Pro	N-Source, peptide		3.5	3
PM07	D02	Phe-Ser	N-Source, peptide		4	4
PM07	D03	Phe-Trp	N-Source, peptide	C02732	4	2
PM07	D04	Pro-Ala	N-Source, peptide		4	4
PM07	D05	Pro-Asp	N-Source, peptide	C02871	4	3.5
PM07	D06	Pro-Gln	N-Source, peptide		4	4
PM07	D07	Pro-Gly	N-Source, peptide		3	3
PM07	D08	Pro-Hyp	N-Source, peptide		1	1
PM07	D09	Pro-Leu	N-Source, peptide	C05035	4	3
PM07	D10	Pro-Phe	N-Source, peptide		4	3
PM07	D11	Pro-Pro	N-Source, peptide		3.5	3
PM07	D12	Pro-Tyr	N-Source, peptide	C05039	4	3
PM07	E01	Ser-Ala	N-Source, peptide		4	3.5
PM07	E02	Ser-Gly	N-Source, peptide		4	4
PM07	E03	Ser-His	N-Source, peptide		4	4
PM07	E04	Ser-Leu	N-Source, peptide	C05035	4	4
PM07	E05	Ser-Met	N-Source, peptide		4	4
PM07	E06	Ser-Phe	N-Source, peptide		4	4
PM07	E07	Ser-Pro	N-Source, peptide		4	4
PM07	E08	Ser-Ser	N-Source, peptide		4	3.5
PM07	E09	Ser-Tyr	N-Source, peptide	C05039	4	3
PM07	E10	Ser-Val	N-Source, peptide		4	4
PM07	E11	Thr-Ala	N-Source, peptide		4	3.5
PM07	E12	Thr-Arg	N-Source, peptide	C05034	4	3
PM07	F01	Thr-Glu	N-Source, peptide		4	3.5
PM07	F02	Thr-Gly	N-Source, peptide		3	2
PM07	F03	Thr-Leu	N-Source, peptide	C05035	4	3
PM07	F04	Thr-Met	N-Source, peptide		4	4
PM07	F05	Thr-Pro	N-Source, peptide		4	3
PM07	F06	Trp-Ala	N-Source, peptide		4	4
PM07	F07	Trp-Arg	N-Source, peptide	C05034	4	3.5
PM07	F08	Trp-Asp	N-Source, peptide	C02871	4	3.5
PM07	F09	Trp-Glu	N-Source, peptide		3.5	3
PM07	F10	Trp-Gly	N-Source, peptide		4	3.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM07	F11	Trp-Leu	N-Source, peptide	C05035	3.5	3
PM07	F12	Trp-Lys	N-Source, peptide	C05036	4	2.5
PM07	G01	Trp-Phe	N-Source, peptide		4	3
PM07	G02	Trp-Ser	N-Source, peptide		4	4
PM07	G03	Trp-Trp	N-Source, peptide	C02732	3.5	3
PM07	G04	Trp-Tyr	N-Source, peptide	C05039	4	3
PM07	G05	Tyr-Ala	N-Source, peptide		4	3
PM07	G06	Tyr-Gln	N-Source, peptide		4	3.5
PM07	G07	Tyr-Glu	N-Source, peptide		4	3
PM07	G08	Tyr-Gly	N-Source, peptide		4	3.5
PM07	G09	Tyr-His	N-Source, peptide		3	2
PM07	G10	Tyr-Leu	N-Source, peptide	C05035	3.5	2.5
PM07	G11	Tyr-Lys	N-Source, peptide	C05036	3.5	2
PM07	G12	Tyr-Phe	N-Source, peptide		3	2
PM07	H01	Tyr-Trp	N-Source, peptide	C02732	4	2.5
PM07	H02	Tyr-Tyr	N-Source, peptide	C05039	4	2
PM07	H03	Val-Arg	N-Source, peptide	C05034	4	3
PM07	H04	Val-Asn	N-Source, peptide		4	4
PM07	H05	Val-Asp	N-Source, peptide	C02871	4	4
PM07	H06	Val-Gly	N-Source, peptide		4	4
PM07	H07	Val-His	N-Source, peptide		3	2
PM07	H08	Val-Ile	N-Source, peptide		3	2
PM07	H09	Val-Leu	N-Source, peptide	C05035	3	3
PM07	H10	Val-Tyr	N-Source, peptide	C05039	4	2.5
PM07	H11	Val-Val	N-Source, peptide		3	2
PM07	H12	g-Glu-Gly	N-Source, peptide		0	0
PM08	A01	Negative Control	N-Source, Negative control		0	0
PM08	A02	L-Glutamine	N-Source, amino acid	C00064	4	4
PM08	A03	Ala-Asp	N-Source, peptide	C02871	4	4
PM08	A04	Ala-Gln	N-Source, peptide		4	4
PM08	A05	Ala-Ile	N-Source, peptide		4	3
PM08	A06	Ala-Met	N-Source, peptide		4	3
PM08	A07	Ala-Val	N-Source, peptide		4	3
PM08	A08	Asp-Ala	N-Source, peptide		4	3
PM08	A09	Asp-Gln	N-Source, peptide		4	4
PM08	A10	Asp-Gly	N-Source, peptide		4	3
PM08	A11	Glu-Ala	N-Source, peptide		4	4
PM08	A12	Gly-Asn	N-Source, peptide		4	4
PM08	B01	Gly-Asp	N-Source, peptide	C02871	4	4

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM08	B02	Gly-Ile	N-Source, peptide		3.5	3
PM08	B03	His-Ala	N-Source, peptide		4	3
PM08	B04	His-Glu	N-Source, peptide		3	2
PM08	B05	His-His	N-Source, peptide		2	2
PM08	B06	Ile-Asn	N-Source, peptide		4	4
PM08	B07	Ile-Leu	N-Source, peptide	C05035	3	3
PM08	B08	Leu-Asn	N-Source, peptide		4	4
PM08	B09	Leu-His	N-Source, peptide		3.5	3
PM08	B10	Leu-Pro	N-Source, peptide		3.5	3
PM08	B11	Leu-Tyr	N-Source, peptide	C05039	3.5	2
PM08	B12	Lys-Asp	N-Source, peptide	C02871	4	3
PM08	C01	Lys-Gly	N-Source, peptide		3.5	3
PM08	C02	Lys-Met	N-Source, peptide		2.5	2
PM08	C03	Met-Thr	N-Source, peptide		4	3
PM08	C04	Met-Tyr	N-Source, peptide	C05039	3.5	2.5
PM08	C05	Phe-Asp	N-Source, peptide	C02871	4	3
PM08	C06	Phe-Glu	N-Source, peptide		3.5	3.5
PM08	C07	Gln-Glu	N-Source, peptide		4	4
PM08	C08	Phe-Met	N-Source, peptide		3.5	3
PM08	C09	Phe-Tyr	N-Source, peptide	C05039	2.5	2
PM08	C10	Phe-Val	N-Source, peptide		3.5	3
PM08	C11	Pro-Arg	N-Source, peptide	C05034	4	3
PM08	C12	Pro-Asn	N-Source, peptide		4	4
PM08	D01	Pro-Glu	N-Source, peptide		3.5	3
PM08	D02	Pro-lle	N-Source, peptide		3.5	2.5
PM08	D03	Pro-Lys	N-Source, peptide	C05036	3.5	3
PM08	D04	Pro-Ser	N-Source, peptide		4	3.5
PM08	D05	Pro-Trp	N-Source, peptide	C02732	3	3
PM08	D06	Pro-Val	N-Source, peptide		4	3.5
PM08	D07	Ser-Asn	N-Source, peptide		4	4
PM08	D08	Ser-Asp	N-Source, peptide	C02871	4	4
PM08	D09	Ser-Gln	N-Source, peptide		4	4
PM08	D10	Ser-Glu	N-Source, peptide		4	3
PM08	D11	Thr-Asp	N-Source, peptide	C02871	3.5	3
PM08	D12	Thr-Gln	N-Source, peptide		4	4
PM08	E01	Thr-Phe	N-Source, peptide		3.5	3
PM08	E02	Thr-Ser	N-Source, peptide		4	4
PM08	E03	Trp-Val	N-Source, peptide		4	3
PM08	E04	Tyr-Ile	N-Source, peptide		3	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM08	E05	Tyr-Val	N-Source, peptide		3.5	3
PM08	E06	Val-Ala	N-Source, peptide		4	4
PM08	E07	Val-Gln	N-Source, peptide		4	4
PM08	E08	Val-Glu	N-Source, peptide		3.5	3
PM08	E09	Val-Lys	N-Source, peptide	C05036	2.5	2
PM08	E10	Val-Met	N-Source, peptide		3.5	3
PM08	E11	Val-Phe	N-Source, peptide		3	3
PM08	E12	Val-Pro	N-Source, peptide		4	3
PM08	F01	Val-Ser	N-Source, peptide		4	3
PM08	F02	b-Ala-Ala	N-Source, peptide		3.5	2
PM08	F03	b-Ala-Gly	N-Source, peptide		0.5	0.5
PM08	F04	b-Ala-His	N-Source, peptide		1	0
PM08	F05	Met-b-Ala	N-Source, peptide		2.5	2
PM08	F06	b-Ala-Phe	N-Source, peptide		1.5	0.5
PM08	F07	D-Ala-D-Ala	N-Source, peptide		1.5	2
PM08	F08	D-Ala-Gly	N-Source, peptide		3.5	3
PM08	F09	D-Ala-Leu	N-Source, peptide	C05035	2	2
PM08	F10	D-Leu-D-Leu	N-Source, peptide		0.5	0.5
PM08	F11	D-Leu-Gly	N-Source, peptide		1.5	1
PM08	F12	D-Leu-Tyr	N-Source, peptide	C05039	0	0
PM08	G01	g-Glu-Gly	N-Source, peptide		1	0.5
PM08	G02	g-D-Glu-Gly	N-Source, peptide		1.5	1
PM08	G03	Gly-D-Ala	N-Source, peptide		2.5	2
PM08	G04	Gly-D-Asp	N-Source, peptide		2	1
PM08	G05	Gly-D-Ser	N-Source, peptide		2.5	2
PM08	G06	Gly-D-Thr	N-Source, peptide		1	1
PM08	G07	Gly-D-Val	N-Source, peptide		0	0
PM08	G08	Leu-b-Ala	N-Source, peptide		2	1.5
PM08	G09	Leu-D-Leu	N-Source, peptide		1	0
PM08	G10	Phe-b-Ala	N-Source, peptide		2	2
PM08	G11	Ala-Ala-Ala	N-Source, peptide		4	3
PM08	G12	D-Ala-Gly-Gly	N-Source, peptide		3	2
PM08	H01	Gly-Gly-Ala	N-Source, peptide		4	3
PM08	H02	Gly-Gly-D-Leu	N-Source, peptide		3.5	2
PM08	H03	Gly-Gly-Gly	N-Source, peptide		3.5	3
PM08	H04	Gly-Gly-Ile	N-Source, peptide		4	3.5
PM08	H05	Gly-Gly-Leu	N-Source, peptide	C05035	4	3
PM08	H06	Gly-Gly-Phe	N-Source, peptide		4	3.5
PM08	H07	Val-Tyr-Val	N-Source, peptide		3.5	2.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM08	H08	Gly-Phe-Phe	N-Source, peptide		4	3.5
PM08	H09	Leu-Gly-Gly	N-Source, peptide		4	3.5
PM08	H10	Leu-Leu-Leu	N-Source, peptide	C05035	3	2
PM08	H11	Phe-Gly-Gly	N-Source, peptide		4	4
PM08	H12	Tyr-Gly-Gly	N-Source, peptide		4	3
PM09	A01	1% NaCl	osmotic sensitivity, NaCl	C13563	4	3
PM09	A02	2% NaCl	osmotic sensitivity, NaCl	C13563	4	3
PM09	A03	3% NaCl	osmotic sensitivity, NaCl	C13563	2	2
PM09	A04	4% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A05	5% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A06	5.5% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A07	6% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A08	6.5% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A09	7% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A10	8% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A11	9% NaCl	osmotic sensitivity, NaCl	C13563	0	0
PM09	A12	10% NaCl	osmotic sensitivity, NaCl	C13563	1	1
PM09	B01	6% NaCl	osmotic sensitivity, NaCl control		0	0
PM09	B02	6% NaCl + Betaine	osmolyte, betaine	C00719	0	0
PM09	B03	6% NaCl + NN Dimethyl Glycine	osmolyte, dimethylglycine	C01026	0	0
PM09	B04	6% NaCl + Sarcosine	osmolyte, sarcosine	C00213	0	0
PM09	B05	6% NaCl + Dimethyl Sulphonyl Propionate	osmolyte, dimethyl sulphonyl propionate		0	0
PM09	B06	6% NaCl + MOPS	osmolyte, MOPS		0	0
PM09	B07	6% NaCl + Ectoine	osmolyte, ectoine	C06231	0	0
PM09	B08	6% NaCl + Choline	osmolyte, choline	C00114	0	0
PM09	B09	6% NaCl + Phosphorylcholine	osmolyte, phosphorylcholine	C00588	0	0
PM09	B10	6% NaCl + Creatine	osmolyte, creatine	C00300	0	0
PM09	B11	6% NaCl + Creatinine	osmolyte, creatinine	C00791	0	0
PM09	B12	6% NaCl + L-Carnitine	osmolyte, carnitine	C00318	0	0
PM09	C01	6% NaCl + KCl	osmolyte, KCl	C13567	0	0
PM09	C02	6% NaCl + L-Proline	osmolyte, proline	C00148	0	0
PM09	C03	6% NaCl + N-Acetyl-L-Glutamine	osmolyte, acetyl glutamine		0	0

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM09	C04	6% NaCl + b-Glutamic acid	osmolyte, b-glutamate		0	0
PM09	C05	6% NaCl + g-Amino-N-Butyric acid	osmolyte, g-amino butyric acid		0	0
PM09	C06	6% NaCl + Glutathione	osmolyte, glutathione	C00051	0	0
PM09	C07	6% NaCl + Glycerol	osmolyte, glycerol	C00116	0	0
PM09	C08	6% NaCl + Trehalose	osmolyte, trehalose	C01083	0	0
PM09	C09	6% NaCl + Trimethylamine-N-Oxide	osmolyte, trimethylamine-N-oxide	C01104	0	0.5
PM09	C10	6% NaCl + Trimethylamine	osmolyte, trimethylamine	C00565	0	0
PM09	C11	6% NaCl + Octopine	osmolyte, octopine	C04137	0	0
PM09	C12	6% NaCl + Trigonelline	osmolyte, trigonelline	C01004	0	0
PM09	D01	3% Potassium Chloride	osmotic sensitivity, KCl	C13567	3	3
PM09	D02	4% Potassium chloride	osmotic sensitivity, KCl	C13567	1	2
PM09	D03	5% Potassium Chloride	osmotic sensitivity, KCl	C13567	0.5	1
PM09	D04	6% Potassium chloride	osmotic sensitivity, KCl	C13567	0	0
PM09	D05	2% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	3	3
PM09	D06	3% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	3	2
PM09	D07	4% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	3	2
PM09	D08	5% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	3	0
PM09	D09	5% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4
PM09	D10	10% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	3
PM09	D11	15% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4
PM09	D12	20% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4
PM09	E01	1% Sodium Formate	osmotic sensitivity, sodium formate		3	3
PM09	E02	2% Sodium Formate	osmotic sensitivity, sodium formate		2	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM09	E03	3% Sodium Formate	osmotic sensitivity, sodium formate		2	2
PM09	E04	4% Sodium Formate	osmotic sensitivity, sodium formate		2	1.5
PM09	E05	5% Sodium Formate	osmotic sensitivity, sodium formate		1.5	0
PM09	E06	6% Sodium Formate	osmotic sensitivity, sodium formate		0	0
PM09	E07	2% Urea	osmotic sensitivity, urea	C00086	4	4
PM09	E08	3% Urea	osmotic sensitivity, urea	C00086	4	4
PM09	E09	4% Urea	osmotic sensitivity, urea	C00086	4	3
PM09	E10	5% Urea	osmotic sensitivity, urea	C00086	1	1.5
PM09	E11	6% Urea	osmotic sensitivity, urea	C00086	0	0
PM09	E12	7% Urea	osmotic sensitivity, urea	C00086	0	0
PM09	F01	1% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	4	3
PM09	F02	2% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	3	3
PM09	F03	3% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	2.5	2
PM09	F04	4% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	2	2
PM09	F05	5% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	2	2
PM09	F06	6% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	1.5	0.5
PM09	F07	7% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	1	1
PM09	F08	8% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	1.5	1.5
PM09	F09	9% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	1	1
PM09	F10	10% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	1	0
PM09	F11	11% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	0
PM09	F12	12% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	0
PM09	G01	20mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	3.5
PM09	G02	50mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	3
PM09	G03	100mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	3
PM09	G04	200mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		3	1.5
PM09	G05	20mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	3	3

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM09	G06	50mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	2	2
PM09	G07	100mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	0	0
PM09	G08	200mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	0	0
PM09	G09	10mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4
PM09	G10	20mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4
PM09	G11	50mM Ammonium Sulfate pH 8	toxicity, ammonia		4	3
PM09	G12	100mM Ammonium Sulfate pH 8	toxicity, ammonia		4	3
PM09	H01	10mM Sodium Nitrate	toxicity, nitrate	C00244	4	4
PM09	H02	20mM Sodium Nitrate	toxicity, nitrate	C00244	4	4
PM09	H03	40mM Sodium Nitrate	toxicity, nitrate	C00244	4	4
PM09	H04	60mM Sodium Nitrate	toxicity, nitrate	C00244	3	3.5
PM09	H05	80mM Sodium Nitrate	toxicity, nitrate	C00244	3	4
PM09	H06	100mM Sodium Nitrate	toxicity, nitrate	C00244	3	3
PM09	H07	10mM Sodium Nitrite	toxicity, nitrite	C00088	4	3.5
PM09	H08	20mM Sodium Nitrite	toxicity, nitrite	C00088	4	3
PM09	H09	40mM Sodium Nitrite	toxicity, nitrite	C00088	3.5	3
PM09	H10	60mM Sodium Nitrite	toxicity, nitrite	C00088	3.5	3
PM09	H11	80mM Sodium Nitrite	toxicity, nitrite	C00088	3	3
PM09	H12	100mM Sodium Nitrite	toxicity, nitrite	C00088	3	3
PM10	A01	pH 3.5	pH, growth at 3.5		0	0
PM10	A02	pH 4	pH, growth at 4		0	0
PM10	A03	pH 4.5	pH, growth at 4.5		0	0
PM10	A04	pH 5	pH, growth at 5		4	4
PM10	A05	pH 5.5	pH, growth at 5.5		4	4
PM10	A06	pH 6	pH, growth at 6		4	4

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM10	A07	pH 7	pH, growth at 7		4	4
PM10	A08	pH 8	pH, growth at 8		4	4
PM10	A09	pH 8.5	pH, growth at 8.5		4	4
PM10	A10	pH 9	pH, growth at 9		4	4
PM10	A11	pH 9.5	pH, growth at 9.5		4	4
PM10	A12	pH 10	pH, growth at 10		4	4
PM10	B01	pH 4.5	pH, decarboxylase control		2	0
PM10	B02	pH 4.5 + L-Alanine	pH, decarboxylase	C00041	3	0.5
PM10	B03	pH 4.5 + L-Arginine	pH, decarboxylase	C00062	1	0
PM10	B04	pH 4.5 + L-Asparagine	pH, decarboxylase	C00152	4	1
PM10	B05	pH 4.5 + L-Aspartic acid	pH, decarboxylase	C00049	1	0
PM10	B06	pH 4.5 + L-Glutamic acid	pH, decarboxylase	C00025	0	0
PM10	B07	pH 4.5 + L-Glutamine	pH, decarboxylase	C00064	3	1.5
PM10	B08	pH 4.5 + Glycine	pH, decarboxylase	C00037	3.5	3.5
PM10	B09	pH 4.5 + L-Histidine	pH, decarboxylase	C00135	1.5	0
PM10	B10	pH 4.5 + L-Isoleucine	pH, decarboxylase	C00407	0	0
PM10	B11	pH 4.5 + L-Leucine	pH, decarboxylase	C00123	0	0
PM10	B12	pH 4.5 + L-Lysine	pH, decarboxylase	C00047	4	3
PM10	C01	pH 4.5 + L-Methionine	pH, decarboxylase	C00073	2	0
PM10	C02	pH 4.5 + L-Phenylalanine	pH, decarboxylase	C00079	1	0
PM10	C03	pH 4.5 + L-Proline	pH, decarboxylase	C00148	3	1
PM10	C04	pH 4.5 + L-Serine	pH, decarboxylase	C00065	3.5	1
PM10	C05	pH 4.5 + L-Threonine	pH, decarboxylase	C00188	3	1
PM10	C06	pH 4.5 + L-Tryptophan	pH, decarboxylase	C00078	0	0
PM10	C07	pH 4.5 + L-Citrulline	pH, decarboxylase	C00082	3	1
PM10	C08	pH 4.5 + L-Valine	pH, decarboxylase	C00183	2.5	3
PM10	C09	pH 4.5 + Hydroxy-L-Proline	pH, decarboxylase	C01015	3	2
PM10	C10	pH 4.5 + L-Ornithine	pH, decarboxylase	C00077	2.5	0.5
PM10	C11	pH 4.5 + L-Homoarginine	pH, decarboxylase		3	1
PM10	C12	pH 4.5 + L-Homoserine	pH, decarboxylase	C00263	2	2

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM10	D01	pH 4.5 + Anthranilic acid	pH, decarboxylase	C00108	1	1.5
PM10	D02	pH 4.5 + L-Norleucine	pH, decarboxylase	C01933	0	0
PM10	D03	pH 4.5 + L-Norvaline	pH, decarboxylase		4	3
PM10	D04	pH 4.5 + a-Amino-N-Butyric acid	pH, decarboxylase		3.5	3
PM10	D05	pH 4.5 + p-Aminobenzoate	pH, decarboxylase		0	0
PM10	D06	pH 4.5 + L-Cysteic acid	pH, decarboxylase	C00506	3	3
PM10	D07	pH 4.5 + D-Lysine	pH, decarboxylase	C00739	4	3
PM10	D08	pH 4.5 + 5-Hydroxy-L-Lysine	pH, decarboxylase	C01211	3.5	3.5
PM10	D09	pH 4.5 + 5-Hydroxy-L-Tryptophan	pH, decarboxylase		2	2
PM10	D10	pH 4.5 + DL-Diamino-a,e-Pimelic acid	pH, decarboxylase	C00680	3	3
PM10	D11	pH 4.5 + Trimethylamine-N-Oxide	pH, decarboxylase	C01104	0	0
PM10	D12	pH 4.5 + Urea	pH, decarboxylase	C00086	4	3
PM10	E01	pH 9.5	pH, deaminase control		4	3.5
PM10	E02	pH 9.5 + L-Alanine	pH, deaminase	C00041	4	3.5
PM10	E03	pH 9.5 + L-Arginine	pH, deaminase	C00062	3.5	3
PM10	E04	pH 9.5 + L-Asparagine	pH, deaminase	C00152	4	3.5
PM10	E05	pH 9.5 + L-Aspartic acid	pH, deaminase	C00049	4	3.5
PM10	E06	pH 9.5 + L-Glutamic acid	pH, deaminase	C00025	4	4
PM10	E07	pH 9.5 + L-Glutamine	pH, deaminase	C00064	4	3.5
PM10	E08	pH 9.5 + Glycine	pH, deaminase	C00037	3.5	3.5
PM10	E09	pH 9.5 + L-Histidine	pH, deaminase	C00135	3	2
PM10	E10	pH 9.5 + L-Isoleucine	pH, deaminase	C00407	3.5	3.5
PM10	E11	pH 9.5 + L-Leucine	pH, deaminase	C00123	4	2.5
PM10	E12	pH 9.5 + L-Lysine	pH, deaminase	C00047	3.5	3
PM10	F01	pH 9.5 + L-Methionine	pH, deaminase	C00073	4	1.5

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM10	F02	pH 9.5 + L-Phenylalanine	pH, deaminase	C00079	3	0
PM10	F03	pH 9.5 + L-Proline	pH, deaminase	C00148	4	4
PM10	F04	pH 9.5 + L-Serine	pH, deaminase	C00065	4	4
PM10	F05	pH 9.5 + L-Threonine	pH, deaminase	C00188	4	3
PM10	F06	pH 9.5 + L-Tryptophan	pH, deaminase	C00078	0	0
PM10	F07	pH 9.5 + L-Tyrosine	pH, deaminase	C00082	3	3
PM10	F08	pH 9.5 + L-Valine	pH, deaminase	C00183	3	0.5
PM10	F09	pH 9.5 + Hydroxy-L-Proline	pH, deaminase	C01015	4	4
PM10	F10	pH 9.5 + L-Ornithine	pH, deaminase	C00077	3.5	3
PM10	F11	pH 9.5 + L-Homoarginine	pH, deaminase		3.5	3.5
PM10	F12	pH 9.5 + L-Homoserine	pH, deaminase	C00263	4	3
PM10	G01	pH 9.5 + Anthranilic acid	pH, deaminase	C00108	4	3.5
PM10	G02	pH 9.5 + L-Norleucine	pH, deaminase	C01933	0	0
PM10	G03	pH 9.5 + L-Norvaline	pH, deaminase		4	3.5
PM10	G04	pH 9.5 + Agmatine	pH, deaminase	C00179	1	0
PM10	G05	pH 9.5 + Cadaverine	pH, deaminase	C01672	1	0
PM10	G06	pH 9.5 + Putrescine	pH, deaminase	C00134	3	2
PM10	G07	pH 9.5 + Histamine	pH, deaminase	C00388	0	0
PM10	G08	pH 9.5 + b-Phenylethylamine	pH, deaminase	C05332	2	2
PM10	G09	pH 9.5 + Tyramine	pH, deaminase	C00483	2	2
PM10	G10	pH 9.5 + Creatine	pH, deaminase	C00300	4	4
PM10	G11	pH 9.5 + Trimethylamine-N-Oxide	pH, deaminase	C01104	4	4
PM10	G12	pH 9.5 + Urea	pH, deaminase	C00086	4	4
PM10	H01	X-Caprylate	caprylate esterase		4	3.5
PM10	H02	X-a-D-Glucoside	a-D-glucosidase		4	3.5
PM10	H03	X-b-D-Glucoside	b-D-glucosidase		4	3.5
PM10	H04	X-a-D-Galactoside	a-D-galactosidase		4	4
PM10	H05	X-b-D-Galactoside	b-D-galactosidase		4	4
PM10	H06	X-a-D-Glucuronide	a-D-glucuronidase		4	3.5
PM10	H07	X-b-D-Glucuronide	b-D-glucuronidase		4	4

Plate	Well	Chemical	Mode of action	CO_ID	WT-B	ZZa3
PM10	H08	X-b-D-Glucosaminide	b-D-glucosaminidase		3.5	4
PM10	H09	X-b-D-Galactosaminide	b-D-galactosaminidase		4	3.5
PM10	H10	X-a-D-Mannoside	a-D-mannosidase		4	3.5
PM10	H11	X-PO4	aryl phosphatase		4	4
PM10	H12	X-SO4	aryl sulfatase		4	4

*. Compound ID in the KEGG database.

Table S5.2: Overall metabolism profiles of WT-O157, ZZb2 and ZZa4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM01	A01	Negative Control	C-Source, negative control		0	0	0
PM01	A02	L-Arabinose	C-Source, carbohydrate	C00259	4	3.5	4
PM01	A03	N-Acetyl-D-Glucosamine	C-Source, carbohydrate	C00140	3	3	4
PM01	A04	D-Saccharic acid	C-Source, carboxylic acid	C00818	0.5	0	0
PM01	A05	Succinic acid	C-Source, carboxylic acid	C00042	4	4	4
PM01	A06	D-Galactose	C-Source, carbohydrate	C00124	4	4	4
PM01	A07	L-Aspartic acid	C-Source, amino acid	C00049	4	4	4
PM01	A08	L-Proline	C-Source, amino acid	C00148	2.5	1	2.5
PM01	A09	D-Alanine	C-Source, amino acid	C00133	4	3	4
PM01	A10	D-Trehalose	C-Source, carbohydrate	C01083	4	4	4
PM01	A11	D-Mannose	C-Source, carbohydrate	C00159	4	3.5	4
PM01	A12	Dulcitol	C-Source, carbohydrate	C01697	4	3	4
PM01	B01	D-Serine	C-Source, amino acid	C00740	0	0	0
PM01	B02	D-Sorbitol	C-Source, carbohydrate	C00794	0	0.5	0
PM01	B03	Glycerol	C-Source, carbohydrate	C00116	4	4	4
PM01	B04	L-Fucose	C-Source, carbohydrate	C01019	4	4	4
PM01	B05	D-Glucuronic acid	C-Source, carboxylic acid	C00191	4	4	4
PM01	B06	D-Gluconic acid	C-Source, carboxylic acid	C00257	4	4	4
PM01	B07	DL-a-Glycerol Phosphate	C-Source, carbohydrate	C00093	4	4	4
PM01	B08	D-Xylose	C-Source, carbohydrate	C00181	4	3.5	4
PM01	B09	L-Lactic acid	C-Source, carboxylic acid	C01432	4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM01	B10	Formic acid	C-Source, carboxylic acid	C00058	0	0	0
PM01	B11	D-Mannitol	C-Source, carbohydrate	C00392	4	3	4
PM01	B12	L-Glutamic acid	C-Source, amino acid	C00025	3	0.5	2
PM01	C01	D-Glucose-6-Phosphate	C-Source, carbohydrate	C00092	4	4	4
PM01	C02	D-Galactonic acid-g-Lactone	C-Source, carboxylic acid	C03383	0	0	0
PM01	C03	DL-Malic acid	C-Source, carboxylic acid	C00497	4	4	4
PM01	C04	D-Ribose	C-Source, carbohydrate	C00121	4	4	4
PM01	C05	Tween 20	C-Source, fatty acid	C11624	0	0	0
PM01	C06	L-Rhamnose	C-Source, carbohydrate	C00507	3	3	3
PM01	C07	D-Fructose	C-Source, carbohydrate	C00095	4	3.5	4
PM01	C08	Acetic acid	C-Source, carboxylic acid	C00033	4	3.5	4
PM01	C09	a-D-Glucose	C-Source, carbohydrate	C00031	3	3	4
PM01	C10	Maltose	C-Source, carbohydrate	C00208	4	3.5	4
PM01	C11	D-Melibiose	C-Source, carbohydrate	C05402	4	3.5	4
PM01	C12	Thymidine	C-Source, carbohydrate	C00214	4	4	4
PM01	D01	L-Asparagine	C-Source, amino acid	C00152	4	3	4
PM01	D02	D-Aspartic acid	C-Source, amino acid	C00402	0	0	0
PM01	D03	D-Glucosaminic acid	C-Source, carboxylic acid	C03752	0	0	0
PM01	D04	1,2-Propanediol	C-Source, alcohol	C00583	0	0	0
PM01	D05	Tween 40	C-Source, fatty acid		0	0.5	0
PM01	D06	a-Ketoglutaric acid	C-Source, carboxylic acid	C00026	0	0	0
PM01	D07	a-Ketobutyric acid	C-Source, carboxylic acid	C00109	3	0.5	4
PM01	D08	a-Methyl-D-Galactoside	C-Source, carbohydrate	C03619	4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM01	D09	a-D-Lactose	C-Source, carbohydrate	C00243	4	4	4
PM01	D10	Lactulose	C-Source, carbohydrate	C07064	3	3	3.5
PM01	D11	Sucrose	C-Source, carbohydrate	C00089	4	4	4
PM01	D12	Uridine	C-Source, carbohydrate	C00299	4	4	4
PM01	E01	L-Glutamine	C-Source, amino acid	C00064	3	3	3
PM01	E02	m-Tartaric acid	C-Source, carboxylic acid	C00552	0.5	0.5	2
PM01	E03	D-Glucose-1-Phosphate	C-Source, carbohydrate	C00103	4	4	4
PM01	E04	D-Fructose-6-Phosphate	C-Source, carbohydrate	C00085	4	4	4
PM01	E05	Tween 80	C-Source, fatty acid	C11625	0	0	0
PM01	E06	a-Hydroxyglutaric acid-g-Lactone	C-Source, carboxylic acid		0	0	0
PM01	E07	a-Hydroxybutyric acid	C-Source, carboxylic acid	C05984	4	1.5	4
PM01	E08	b-Methyl-D-Glucoside	C-Source, carbohydrate		4	3	4
PM01	E09	Adonitol	C-Source, carbohydrate	C00474	0	0	0
PM01	E10	Maltotriose	C-Source, carbohydrate	C01835	4	3.5	4
PM01	E11	2`-Deoxyadenosine	C-Source, carbohydrate	C00559	4	4	4
PM01	E12	Adenosine	C-Source, carbohydrate	C00212	4	4	4
PM01	F01	Gly-Asp	C-Source, amino acid	C02871	4	3	4
PM01	F02	Citric acid	C-Source, carboxylic acid	C00158	0	0	0
PM01	F03	m-Inositol	C-Source, carbohydrate	C00137	0	0	0
PM01	F04	D-Threonine	C-Source, amino acid	C00820	0	0	0
PM01	F05	Fumaric acid	C-Source, carboxylic acid	C00122	4	3	4
PM01	F06	Bromosuccinic acid	C-Source, carboxylic acid		3.5	3	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM01	F07	Propionic acid	C-Source, carboxylic acid	C00163	4	3	4
PM01	F08	Mucic acid	C-Source, carboxylic acid	C01807	3	0	3
PM01	F09	Glycolic acid	C-Source, carboxylic acid	C00160	0	0	0
PM01	F10	Glyoxylic acid	C-Source, carboxylic acid	C00048	1	0	1
PM01	F11	D-Cellobiose	C-Source, carbohydrate	C00185	0	0	0
PM01	F12	Inosine	C-Source, carbohydrate	C00294	4	4	4
PM01	G01	Gly-Glu	C-Source, amino acid		3	0	3
PM01	G02	Tricarballylic acid	C-Source, carboxylic acid		0	0	0
PM01	G03	L-Serine	C-Source, amino acid	C00065	4	4	4
PM01	G04	L-Threonine	C-Source, amino acid	C00188	0	0	0
PM01	G05	L-Alanine	C-Source, amino acid	C00041	4	3	4
PM01	G06	Ala-Gly	C-Source, amino acid		4	4	4
PM01	G07	Acetoacetic acid	C-Source, carboxylic acid	C00164	0	0	0
PM01	G08	N-Acetyl-D-Mannosamine	C-Source, carbohydrate	C00645	4	1.5	3.5
PM01	G09	Mono-Methylsuccinate	C-Source, carboxylic acid		0	0	0
PM01	G10	Methylpyruvate	C-Source, ester		4	4	4
PM01	G11	D-Malic acid	C-Source, carboxylic acid	C00497	4	3	4
PM01	G12	L-Malic acid	C-Source, carboxylic acid	C00149	4	4	4
PM01	H01	Gly-Pro	C-Source, amino acid		4	3	4
PM01	H02	p-Hydroxyphenyl Acetic acid	C-Source, carboxylic acid	C00642	0	0	0
PM01	H03	m-Hydroxyphenyl Acetic acid	C-Source, carboxylic acid	C05593	0	0	0
PM01	H04	Tyramine	C-Source, amine	C00483	0	0	0
PM01	H05	D-Psicose	C-Source, carbohydrate	C06468	3	0	3

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM01	H06	L-Lyxose	C-Source, carbohydrate	C01508	1	0	0
PM01	H07	Glucuronamide	C-Source, amide	D01791	0	0	0
PM01	H08	Pyruvic acid	C-Source, carboxylic acid	C00022	4	4	4
PM01	H09	L-Galactonic acid-g-Lactone	C-Source, carboxylic acid	C01115	4	4	4
PM01	H10	D-Galacturonic acid	C-Source, carboxylic acid	C00333	4	4	4
PM01	H11	Phenylethylamine	C-Source, amine	C05332	0	0	0
PM01	H12	2-Aminoethanol	C-Source, alcohol	C00189	0	0	0
PM02	A01	Negative Control	C-Source, negative control		0	0	0
PM02	A02	Chondroitin Sulfate C	C-Source, polymer	C00635	0	0	0
PM02	A03	a-Cyclodextrin	C-Source, polymer		0	0	0
PM02	A04	b-Cyclodextrin	C-Source, polymer		0	0	0
PM02	A05	g-Cyclodextrin	C-Source, polymer		0	0	0
PM02	A06	Dextrin	C-Source, polymer	C00721	3	2	4
PM02	A07	Gelatin	C-Source, polymer	C01498	0	0	0
PM02	A08	Glycogen	C-Source, polymer	C00182	0.5	0	0
PM02	A09	Inulin	C-Source, polymer	C00368	0	0	0
PM02	A10	Laminarin	C-Source, polymer	C00771	0	2	0
PM02	A11	Mannan	C-Source, polymer	C00464	0	0	0
PM02	A12	Pectin	C-Source, polymer	C00714	3	0	1.5
PM02	B01	N-Acetyl-D-Galactosamine	C-Source, carbohydrate	C01074	3	4	4
PM02	B02	N-Acetyl-Neuraminic acid	C-Source, carboxylic acid	C00270	1.5	3.5	4
PM02	B03	b-D-Allose	C-Source, carbohydrate	C01487	0	0	0
PM02	B04	Amygdalin	C-Source, carbohydrate	C08325	0	0	0
PM02	B05	D-Arabinose	C-Source, carbohydrate	C00216	1.5	0	2
PM02	B06	D-Arabitol	C-Source, carbohydrate	C01904	0	0	0
PM02	B07	L-Arabitol	C-Source, carbohydrate	C00532	0	0	0
PM02	B08	Arbutin	C-Source, carbohydrate	C06186	0	0	0
PM02	B09	2-Deoxy-D-Ribose	C-Source, carbohydrate	C01801	0	0	0.5

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM02	B10	i-Erythritol	C-Source, carbohydrate	C00503	0	0	0
PM02	B11	D-Fucose	C-Source, carbohydrate	C01018	0	0	0
PM02	B12	3-O-b-D-Galactopyranosyl-D-Arabinose	C-Source, carbohydrate		3	3.5	3
PM02	C01	Gentiobiose	C-Source, carbohydrate	C08240	0	0	0
PM02	C02	L-Glucose	C-Source, carbohydrate		0	0	0
PM02	C03	D-Lactitol	C-Source, carbohydrate		0	0	0
PM02	C04	D-Melezitose	C-Source, carbohydrate	C08243	0	0	0.5
PM02	C05	Maltitol	C-Source, carbohydrate	G00275	0	0	0
PM02	C06	a-Methyl-D-Glucoside	C-Source, carbohydrate		0	0	0
PM02	C07	b-Methyl-D-Galactoside	C-Source, carbohydrate	C03619	3	3.5	4
PM02	C08	3-Methylglucose	C-Source, carbohydrate		0	0	0
PM02	C09	b-Methyl-D-Glucuronic acid	C-Source, carboxylic acid	C08350	0.5	0	0
PM02	C10	a-Methyl-D-Mannoside	C-Source, carbohydrate		0	0	0
PM02	C11	b-Methyl-D-Xyloside	C-Source, carbohydrate		0.5	0	0
PM02	C12	Palatinose	C-Source, carbohydrate	C01742	0.5	0	0.5
PM02	D01	D-Raffinose	C-Source, carbohydrate	C00492	4	4	4
PM02	D02	Salicin	C-Source, carbohydrate	C01451	0	0	0
PM02	D03	Sedoheptulosan	C-Source, carbohydrate		0	0	0
PM02	D04	L-Sorbose	C-Source, carbohydrate	C00247	0.5	0	0
PM02	D05	Stachyose	C-Source, carbohydrate	C01613	0	0	0
PM02	D06	D-Tagatose	C-Source, carbohydrate	C00795	0	0.5	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM02	D07	Turanose	C-Source, carbohydrate	G03588	0.5	0	0
PM02	D08	Xylitol	C-Source, carbohydrate	C00379	0	0	0
PM02	D09	N-Acetyl-D-Glucosaminitol	C-Source, carbohydrate		0	0	0
PM02	D10	g-Amino-N-Butyric acid	C-Source, carboxylic acid	C00334	0	0	0
PM02	D11	d-Amino Valeric acid	C-Source, carboxylic acid	C00431	0	0	0
PM02	D12	Butyric acid	C-Source, carboxylic acid	C00246	0	0	0
PM02	E01	Capric acid	C-Source, carboxylic acid	C01571	0	0	0
PM02	E02	Caproic acid	C-Source, carboxylic acid	C01585	0	0	0
PM02	E03	Citraconic acid	C-Source, carboxylic acid	C02226	0	0	0
PM02	E04	Citramalic acid	C-Source, carboxylic acid	C00815	0	0	0
PM02	E05	D-Glucosamine	C-Source, carbohydrate	C00329	4	3.5	4
PM02	E06	2-Hydroxybenzoic acid	C-Source, carboxylic acid	C00805	0	0	0
PM02	E07	4-Hydroxybenzoic acid	C-Source, carboxylic acid	C00156	0	0	0
PM02	E08	b-Hydroxybutyric acid	C-Source, carboxylic acid	C01089	0	0	0
PM02	E09	g-Hydroxybutyric acid	C-Source, carboxylic acid	C00989	0	0	0
PM02	E10	a-Keto-Valeric acid	C-Source, carboxylic acid	C00567	0	0	0
PM02	E11	Itaconic acid	C-Source, carboxylic acid	C00490	0	0	0
PM02	E12	5-Keto-D-Gluconic acid	C-Source, carboxylic acid	C01062	0	0	0
PM02	F01	D-Lactic acid Methyl Ester	C-Source, ester		3.5	0	3.5
PM02	F02	Malonic acid	C-Source, carboxylic acid	C00383	0	0	0
PM02	F03	Melibionic acid	C-Source, carbohydrate		4	3.5	4
PM02	F04	Oxalic acid	C-Source, carboxylic acid	C00209	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM02	F05	Oxalomalic acid	C-Source, carboxylic acid	C01990	0	0	0
PM02	F06	Quinic acid	C-Source, carboxylic acid	C00296	0	0	0
PM02	F07	D-Ribono-1,4-Lactone	C-Source, carboxylic acid		0	0	0
PM02	F08	Sebacic acid	C-Source, carboxylic acid	C08277	0	0	0
PM02	F09	Sorbic acid	C-Source, carboxylic acid		0	0	0
PM02	F10	Succinamic acid	C-Source, carboxylic acid		0	0	0
PM02	F11	D-Tartaric acid	C-Source, carboxylic acid	C02107	0	0	0.5
PM02	F12	L-Tartaric acid	C-Source, carboxylic acid	C00898	0.5	0	0
PM02	G01	Acetamide	C-Source, amide	C06244	0	0	0
PM02	G02	L-Alaninamide	C-Source, amide		0	2.5	0
PM02	G03	N-Acetyl-L-Glutamic acid	C-Source, amino acid	C00624	0	0	0
PM02	G04	L-Arginine	C-Source, amino acid	C00062	0	0	0
PM02	G05	Glycine	C-Source, amino acid	C00037	0	0	0
PM02	G06	L-Histidine	C-Source, amino acid	C00135	0	0	0
PM02	G07	L-Homoserine	C-Source, amino acid	C00263	0	0	0
PM02	G08	Hydroxy-L-Proline	C-Source, amino acid	C01015	0	0	0.5
PM02	G09	L-Isoleucine	C-Source, amino acid	C00407	0	0	0
PM02	G10	L-Leucine	C-Source, amino acid	C00123	0	0	0
PM02	G11	L-Lysine	C-Source, amino acid	C00047	0	0	0
PM02	G12	L-Methionine	C-Source, amino acid	C00073	0.5	0	0
PM02	H01	L-Ornithine	C-Source, amino acid	C00077	0.5	0	0
PM02	H02	L-Phenylalanine	C-Source, amino acid	C00079	0	0	0
PM02	H03	L-Pyroglutamic acid	C-Source, amino acid	C02238	0	0	0.5

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM02	H04	L-Valine	C-Source, amino acid	C00183	0	0	0
PM02	H05	D,L-Carnitine	C-Source, carboxylic acid	C00487	0	0	0
PM02	H06	sec-Butylamine	C-Source, amine		0	0	0
PM02	H07	D,L-Octopamine	C-Source, amine	C04227	0	0	0
PM02	H08	Putrescine	C-Source, amine	C00134	0	0	0
PM02	H09	Dihydroxyacetone	C-Source, alcohol	C00184	0.5	0	0
PM02	H10	2,3-Butanediol	C-Source, alcohol	C03044	0	0	0.5
PM02	H11	2,3-Butanedione	C-Source, alcohol	C00741	0.5	0	0
PM02	H12	3-Hydroxy-2-buta	C-Source, alcohol	C00466	0	0	0
PM03	A01	Negative Control	N-Source, Negative control		0	0	0
PM03	A02	Ammonia	N-Source, inorganic	C00014	4	3	4
PM03	A03	Nitrite	N-Source, inorganic	C00088	1	0	1
PM03	A04	Nitrate	N-Source, inorganic	C00244	0.5	0	0.5
PM03	A05	Urea	N-Source, other	C00086	0	0	0
PM03	A06	Biuret	N-Source, other	C06555	0	0	0
PM03	A07	L-Alanine	N-Source, amino acid	C00041	4	3	4
PM03	A08	L-Arginine	N-Source, amino acid	C00062	3	3	2.5
PM03	A09	L-Asparagine	N-Source, amino acid	C00152	4	3	4
PM03	A10	L-Aspartic acid	N-Source, amino acid	C00049	4	3	4
PM03	A11	L-Cysteine	N-Source, amino acid	C00097	3	1.5	3
PM03	A12	L-Glutamic acid	N-Source, amino acid	C00025	2.5	2.5	3
PM03	B01	L-Glutamine	N-Source, amino acid	C00064	4	3	4
PM03	B02	Glycine	N-Source, amino acid	C00037	3	3	2.5
PM03	B03	L-Histidine	N-Source, amino acid	C00135	0	0	1
PM03	B04	L-Isoleucine	N-Source, amino acid	C00407	0	0	0
PM03	B05	L-Leucine	N-Source, amino acid	C00123	0	0	0
PM03	B06	L-Lysine	N-Source, amino acid	C00047	1	0.5	1

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM03	B07	L-Methionine	N-Source, amino acid	C00073	1.5	2	1.5
PM03	B08	L-Phenylalanine	N-Source, amino acid	C00079	2	2	1
PM03	B09	L-Proline	N-Source, amino acid	C00148	2	3	2.5
PM03	B10	L-Serine	N-Source, amino acid	C00065	4	3	4
PM03	B11	L-Threonine	N-Source, amino acid	C00188	2	1.5	2
PM03	B12	L-Tryptophan	N-Source, amino acid	C00078	1	1.5	2
PM03	C01	L-Tyrosine	N-Source, amino acid	C00082	1	0.5	2
PM03	C02	L-Valine	N-Source, amino acid	C00183	1.5	0.5	1
PM03	C03	D-Alanine	N-Source, amino acid	C00133	4	3	4
PM03	C04	D-Asparagine	N-Source, amino acid	C01905	0	0	0.5
PM03	C05	D-Aspartic acid	N-Source, amino acid	C00402	0	0	0
PM03	C06	D-Glutamic acid	N-Source, amino acid	C00217	0	0	0
PM03	C07	D-Lysine	N-Source, amino acid	C00739	0	0	0
PM03	C08	D-Serine	N-Source, amino acid	C00740	2	0	0
PM03	C09	D-Valine	N-Source, amino acid	C06417	1	0	0
PM03	C10	L-Citrulline	N-Source, amino acid	C00327	1	1.5	1
PM03	C11	L-Homoserine	N-Source, amino acid	C00263	1	0	0.5
PM03	C12	L-Ornithine	N-Source, amino acid	C00077	2	2.5	1.5
PM03	D01	N-Acetyl-L-Glutamic acid	N-Source, amino acid	C00624	0	0	0
PM03	D02	N-Phthaloyl-L-Glutamic acid	N-Source, amino acid		0	0.5	0
PM03	D03	L-Pyroglutamic acid	N-Source, amino acid	C02238	0	0.5	0
PM03	D04	Hydroxylamine	N-Source, other	C00192	0	0.5	0
PM03	D05	Methylamine	N-Source, other	C00218	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM03	D06	N-Amylamine	N-Source, other		0	0	0
PM03	D07	N-Butylamine	N-Source, other		0	0	0
PM03	D08	Ethylamine	N-Source, other	C00797	0	0	0
PM03	D09	Ethanolamine	N-Source, other	C00189	0	0	0
PM03	D10	Ethylenediamine	N-Source, other	C12511	0	0	0
PM03	D11	Putrescine	N-Source, other	C00134	0	0	0
PM03	D12	Agmatine	N-Source, other	C00179	0	0	0
PM03	E01	Histamine	N-Source, other	C00388	0	0	0
PM03	E02	b-Phenylethylamine	N-Source, other	C05332	0	0.5	0
PM03	E03	Tyramine	N-Source, other	C00483	0	0	0
PM03	E04	Acetamide	N-Source, other	C06244	0	0	0
PM03	E05	Formamide	N-Source, other	C00488	0	0.5	0
PM03	E06	Glucuronamide	N-Source, other	D01791	0	2	0.5
PM03	E07	DL-Lactamide	N-Source, other		0	0	0
PM03	E08	D-Glucosamine	N-Source, other	C00329	0.5	4	0
PM03	E09	D-Galactosamine	N-Source, other	C02262	0	0.5	0.5
PM03	E10	D-Mannosamine	N-Source, other	C03570	0.5	1	0
PM03	E11	N-Acetyl-D-Glucosamine	N-Source, other	C00140	0	3	1.5
PM03	E12	N-Acetyl-D-Galactosamine	N-Source, other	C01074	0	4	1.5
PM03	F01	N-Acetyl-D-Mannosamine	N-Source, other	C00645	1.5	3	1.5
PM03	F02	Adenine	N-Source, other	C00147	3	3	2
PM03	F03	Adenosine	N-Source, other	C00212	3	4	4
PM03	F04	Cytidine	N-Source, other	C00475	4	4	4
PM03	F05	Cytosine	N-Source, other	C00380	0.5	2	1
PM03	F06	Guanine	N-Source, other	C00242	0	1.5	0
PM03	F07	Guanosine	N-Source, other	C00387	0	2	0.5
PM03	F08	Thymine	N-Source, other	C00178	0.5	1	0.5
PM03	F09	Thymidine	N-Source, other	C00214	0	0	0
PM03	F10	Uracil	N-Source, other	C00106	0	0.5	0
PM03	F11	Uridine	N-Source, other	C00299	0	0	0
PM03	F12	Inosine	N-Source, other	C00294	0	0	0
PM03	G01	Xanthine	N-Source, other	C00385	2.5	3	2
PM03	G02	Xanthosine	N-Source, other	C01762	2.5	3	2
PM03	G03	Uric acid	N-Source, other	C00366	2	3	2
PM03	G04	Alloxan	N-Source, other	C07599	1.5	2	0.5
PM03	G05	Allantoin	N-Source, other	C01551	1	2	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM03	G06	Parabanic acid	N-Source, other		1	2	0.5
PM03	G07	DL-a-Amino-N-Butyric acid	N-Source, other	C02261	2.5	0	3
PM03	G08	g-Amino-N-Butyric acid	N-Source, other	C00334	2	3	2.5
PM03	G09	e-Amino-N-Caproic acid	N-Source, other	C02378	2	3	2
PM03	G10	DL-a-Amino-Caprylic acid	N-Source, other		2.5	1	2
PM03	G11	d-Amino-N-Valeric acid	N-Source, other	C00431	2	2	2
PM03	G12	a-Amino-N-Valeric acid	N-Source, other	C01826	1.5	0.5	0
PM03	H01	Ala-Asp	N-Source, peptide	C02871	4	4	4
PM03	H02	Ala-Gln	N-Source, peptide		4	4	4
PM03	H03	Ala-Glu	N-Source, peptide		4	4	4
PM03	H04	Ala-Gly	N-Source, peptide		4	4	4
PM03	H05	Ala-His	N-Source, peptide		4	2.5	4
PM03	H06	Ala-Leu	N-Source, peptide	C05035	3	3	4
PM03	H07	Ala-Thr	N-Source, peptide		3	3.5	4
PM03	H08	Gly-Asn	N-Source, peptide		4	3.5	4
PM03	H09	Gly-Gln	N-Source, peptide		4	4	4
PM03	H10	Gly-Glu	N-Source, peptide		3	3	4
PM03	H11	Gly-Met	N-Source, peptide		4	3	4
PM03	H12	Met-Ala	N-Source, peptide		4	3	4
PM04	A01	Negative Control	P-Source, Negative control		0	0	0
PM04	A02	Phosphate	P-Source, inorganic	C00009	4	3	4
PM04	A03	Pyrophosphate	P-Source, inorganic	C00013	4	3	4
PM04	A04	Trimetaphosphate	P-Source, inorganic	C02466	4	3	4
PM04	A05	Tripolyphosphate	P-Source, inorganic	C00536	3	3	4
PM04	A06	Triethyl Phosphate	P-Source, organic		0	0	0
PM04	A07	Hypophosphite	P-Source, inorganic		0	0	0
PM04	A08	Adenosine 2`-Monophosphate	P-Source, organic	C00946	4	3	4
PM04	A09	Adenosine 3`-Monophosphate	P-Source, organic	C01367	4	3.5	4
PM04	A10	Adenosine 5`-Monophosphate	P-Source, organic	C00020	4	3.5	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM04	A11	Adenosine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	3.5	4
PM04	A12	Adenosine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00575	2	3	3
PM04	B01	Thiophosphate	P-Source, inorganic		4	1.5	4
PM04	B02	Dithiophosphate	P-Source, inorganic		4	3	4
PM04	B03	DL-a-Glycerol Phosphate	P-Source, organic	C00093	4	4	4
PM04	B04	b-Glycerol Phosphate	P-Source, organic	C02979	4	3.5	4
PM04	B05	Carbamyl Phosphate	P-Source, organic	C00169	4	3	4
PM04	B06	D-2-Phospho-Glyceric acid	P-Source, organic	C00631	4	3	4
PM04	B07	D-3-Phospho-Glyceric acid	P-Source, organic	C00197	4	3	4
PM04	B08	Guanosine 2`-Monophosphate	P-Source, organic		4	3	4
PM04	B09	Guanosine 3`-Monophosphate	P-Source, organic	C06193	4	3	4
PM04	B10	Guanosine 5`-Monophosphate	P-Source, organic	C00144	4	4	4
PM04	B11	Guanosine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	3	4
PM04	B12	Guanosine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00942	0	0	0.5
PM04	C01	Phosphoenol Pyruvate	P-Source, organic	C00074	4	3	4
PM04	C02	Phospho-Glycolic acid	P-Source, organic	C00988	4	3	4
PM04	C03	D-Glucose-1-Phosphate	P-Source, organic	C00103	2	3	4
PM04	C04	D-Glucose-6-Phosphate	P-Source, organic	C00092	2	3	4
PM04	C05	2-Deoxy-D-Glucose 6-Phosphate	P-Source, organic	C06369	0	0	0
PM04	C06	D-Glucosamine-6-Phosphate	P-Source, organic	C00352	4	4	4
PM04	C07	6-Phospho-Gluconic acid	P-Source, organic		4	3	4
PM04	C08	Cytidine 2`-Monophosphate	P-Source, organic	C03104	4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM04	C09	Cytidine 3`-Monophosphate	P-Source, organic	C05822	0.5	4	0
PM04	C10	Cytidine 5`-Monophosphate	P-Source, organic	C00055	3	4	4
PM04	C11	Cytidine 2`,3`-Cyclic Monophosphate	P-Source, organic		4	3.5	4
PM04	C12	Cytidine 3`,5`-Cyclic Monophosphate	P-Source, organic	C00941	0.5	3	0.5
PM04	D01	D-Mannose-1-Phosphate	P-Source, organic	C00636	4	4	4
PM04	D02	D-Mannose-6-Phosphate	P-Source, organic	C00275	2.5	3.5	4
PM04	D03	Cysteamine-S-Phosphate	P-Source, organic		4	2.5	4
PM04	D04	Phospho-L-Arginine	P-Source, organic	C05945	4	3	4
PM04	D05	O-Phospho-D-Serine	P-Source, organic	C02532	3	3	4
PM04	D06	O-Phospho-L-Serine	P-Source, organic	C01005	3	3	4
PM04	D07	O-Phospho-L-Threonine	P-Source, organic		3	3	4
PM04	D08	Uridine 2`-Monophosphate	P-Source, organic	C03031	4	3.5	4
PM04	D09	Uridine 3`-Monophosphate	P-Source, organic	C01368	4	4	4
PM04	D10	Uridine 5`-Monophosphate	P-Source, organic	C00105	4	4	4
PM04	D11	Uridine 2`,3`-Cyclic Monophosphate	P-Source, organic	C02355	4	4	4
PM04	D12	Uridine 3`,5`-Cyclic Monophosphate	P-Source, organic		0	0	0
PM04	E01	O-Phospho-D-Tyrosine	P-Source, organic	C06501	4	3	4
PM04	E02	O-Phospho-L-Tyrosine	P-Source, organic	C06501	4	3	4
PM04	E03	Phosphocreatine	P-Source, organic	C02305	4	3	4
PM04	E04	Phosphoryl Choline	P-Source, organic	C00588	4	3	4
PM04	E05	O-Phosphoryl-Ethanolamine	P-Source, organic	C00346	3	3	3.5
PM04	E06	Phosphono Acetic acid	P-Source, organic	C05682	0.5	0	0
PM04	E07	2-Aminoethyl Phosphonic acid	P-Source, organic	C03557	0	2	0
PM04	E08	Methylene Diphosphonic acid	P-Source, organic		0.5	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM04	E09	Thymidine 3`-Monophosphate	P-Source, organic		4	3	4
PM04	E10	Thymidine 5`-Monophosphate	P-Source, organic	C00364	4	3	4
PM04	E11	Inositol Hexaphosphate	P-Source, organic	C01204	2	3	4
PM04	E12	Thymidine 3`,5`-Cyclic Monophosphate	P-Source, organic		0	0	0
PM04	F01	Negative Control	S-Source, Negative control		0	0	0
PM04	F02	Sulfate	S-Source, inorganic	C00059	3	3	3
PM04	F03	Thiosulfate	S-Source, inorganic	C00320	4	2	4
PM04	F04	Tetrathionate	S-Source, inorganic	C02084	3.5	2.5	4
PM04	F05	Thiophosphate	S-Source, inorganic		3.5	1.5	4
PM04	F06	Dithiophosphate	S-Source, inorganic		4	2.5	4
PM04	F07	L-Cysteine	S-Source, organic	C00097	4	3	4
PM04	F08	D-Cysteine	S-Source, organic	C00793	3.5	3	4
PM04	F09	Cys-Gly	S-Source, organic		1.5	3	1
PM04	F10	L-Cysteic acid	S-Source, organic	C00506	0	0.5	0
PM04	F11	Cysteamine	S-Source, organic	C01678	0	1.5	0
PM04	F12	L-Cysteine Sulfinic acid	S-Source, organic	C00606	3	2.5	3.5
PM04	G01	N-Acetyl-L-Cysteine	S-Source, organic	C06809	0.5	0	0
PM04	G02	S-Methyl-L-Cysteine	S-Source, organic	C03540	0	0	0
PM04	G03	Cystathionine	S-Source, organic	C00542	1.5	2.5	2.5
PM04	G04	Lanthionine	S-Source, organic		3.5	3	3.5
PM04	G05	Glutathione	S-Source, organic	C00051	2.5	2.5	3
PM04	G06	DL-Ethionine	S-Source, organic	C11227	0	0.5	0
PM04	G07	L-Methionine	S-Source, organic	C00073	0	2.5	1.5
PM04	G08	D-Methionine	S-Source, organic	C00855	0	2.5	0
PM04	G09	Gly-Met	S-Source, organic		0	2.5	1.5
PM04	G10	N-Acetyl-D,L-Methionine	S-Source, organic	C02712	0	2.5	1.5
PM04	G11	L-Methionine Sulfoxide	S-Source, organic	C02989	0	2.5	1.5
PM04	G12	L-Methionine Sulfone	S-Source, organic		0	1	0
PM04	H01	L-Djenkolic acid	S-Source, organic	C08275	4	3	4
PM04	H02	Thiourea	S-Source, organic	C14415	1	1	0
PM04	H03	1-Thio-b-D-Glucose	S-Source, organic		1	0	0
PM04	H04	DL-Lipoamide	S-Source, organic	C00248	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM04	H05	Taurocholic acid	S-Source, organic	C05122	0	1	0
PM04	H06	Taurine	S-Source, organic	C00245	3	2.5	3
PM04	H07	Hypotaurine	S-Source, organic	C00519	3	3	3
PM04	H08	p-Aminobenzene Sulfonic acid	S-Source, organic	C00568	0	0	0
PM04	H09	Butane Sulfonic acid	S-Source, organic		0	0	0
PM04	H10	2-Hydroxyethane Sulfonic acid	S-Source, organic	C05123	0	0	0
PM04	H11	Methane Sulfonic acid	S-Source, organic	C11145	0	0	0
PM04	H12	Tetramethylene Sulfone	S-Source, organic		0	0	0
PM05	A01	Negative Control	Nutritional supplement, Negative control		0	0	0
PM05	A02	Positive Control	Nutritional supplement, Positive control		0	0.5	0
PM05	A03	L-Alanine	Nutritional supplement	C00041	0	0.5	0
PM05	A04	L-Arginine	Nutritional supplement	C00062	0	0	0
PM05	A05	L-Asparagine	Nutritional supplement	C00152	0	0.5	0
PM05	A06	L-Aspartic acid	Nutritional supplement	C00049	0.5	0.5	0
PM05	A07	L-Cysteine	Nutritional supplement	C00097	0	0.5	0
PM05	A08	L-Glutamic acid	Nutritional supplement	C00025	0	0.5	0
PM05	A09	Adenosine 3`,5`-Cyclic Monophosphate	Nutritional supplement	C00575	0	0.5	0
PM05	A10	Adenine	Nutritional supplement	C00147	0	0.5	0
PM05	A11	Adenosine	Nutritional supplement	C00212	0	0	0
PM05	A12	2`-Deoxyadenosine	Nutritional supplement	C00559	0	0	0
PM05	B01	L-Glutamine	Nutritional supplement	C00064	0	0.5	0
PM05	B02	Glycine	Nutritional supplement	C00037	0	0	0
PM05	B03	L-Histidine	Nutritional supplement	C00135	0	0	0
PM05	B04	L-Isoleucine	Nutritional supplement	C00407	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM05	B05	L-Leucine	Nutritional supplement	C00123	0	0	0
PM05	B06	L-Lysine	Nutritional supplement	C00047	0	0.5	0
PM05	B07	L-Methionine	Nutritional supplement	C00073	0	0.5	0
PM05	B08	L-Phenylalanine	Nutritional supplement	C00079	0	0	0
PM05	B09	Guanosine 3`,5`-Cyclic Monophosphate	Nutritional supplement	C00942	0	0	0
PM05	B10	Guanine	Nutritional supplement	C00242	0	0.5	0
PM05	B11	Guanosine	Nutritional supplement	C00387	0	0.5	0
PM05	B12	2`-Deoxyguanosine	Nutritional supplement	C00330	0	0	0
PM05	C01	L-Proline	Nutritional supplement	C00148	0	0	0
PM05	C02	L-Serine	Nutritional supplement	C00065	0	0	0
PM05	C03	L-Threonine	Nutritional supplement	C00188	0	0	0
PM05	C04	L-Tryptophan	Nutritional supplement	C00078	0	0	0
PM05	C05	L-Tyrosine	Nutritional supplement	C00082	0	0	0
PM05	C06	L-Valine	Nutritional supplement	C00183	0	0	0
PM05	C07	L-Isoleucine + L-Valine	Nutritional supplement		0	0	0
PM05	C08	Hydroxy-L-Proline	Nutritional supplement	C01015	0	0	0
PM05	C09	(5) 4-Amino-Imidazole-4(5)-Carboxamide	Nutritional supplement	C04051	0	0	0
PM05	C10	Hypoxanthine	Nutritional supplement	C00262	0	0	0
PM05	C11	Inosine	Nutritional supplement	C00294	0	0	0
PM05	C12	2`-Deoxyinosine	Nutritional supplement	C05512	0	0	0
PM05	D01	L-Ornithine	Nutritional supplement	C00077	0	0.5	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM05	D02	L-Citrulline	Nutritional supplement	C00327	0	0	0
PM05	D03	Chorismic acid	Nutritional supplement	C00251	0	0	0
PM05	D04	(-)Shikimic acid	Nutritional supplement	C00493	0	0	0
PM05	D05	L-Homoserine Lactone	Nutritional supplement		0	0	0
PM05	D06	D-Alanine	Nutritional supplement	C00133	0	0	0
PM05	D07	D-Aspartic acid	Nutritional supplement	C00402	0	0	0
PM05	D08	D-Glutamic acid	Nutritional supplement	C00217	0	0	0
PM05	D09	DL-Diamino-a,e-Pimelic acid	Nutritional supplement	C00680	0	0	0
PM05	D10	Cytosine	Nutritional supplement	C00380	0	0.5	0
PM05	D11	Cytidine	Nutritional supplement	C00475	0	0.5	0
PM05	D12	2`-Deoxycytidine	Nutritional supplement	C00881	0	0.5	0
PM05	E01	Putrescine	Nutritional supplement	C00134	0	1	0
PM05	E02	Spermidine	Nutritional supplement	C00315	0	0	0
PM05	E03	Spermine	Nutritional supplement	C00750	0	0	0
PM05	E04	Pyridoxine	Nutritional supplement	C00314	0	0	0
PM05	E05	Pyridoxal	Nutritional supplement	C00250	0	0	0
PM05	E06	Pyridoxamine	Nutritional supplement	C00534	0	0	0
PM05	E07	b-Alanine	Nutritional supplement	C00099	0	0	0
PM05	E08	D-Pantothenic acid	Nutritional supplement	C00864	0	0	0
PM05	E09	Orotic acid	Nutritional supplement	C00295	0	0	0
PM05	E10	Uracil	Nutritional supplement	C00106	0	0	0
PM05	E11	Uridine	Nutritional supplement	C00299	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM05	E12	2`-Deoxyuridine	Nutritional supplement	C00526	0	0.5	0
PM05	F01	Quinolinic acid	Nutritional supplement	C03722	0	1	0
PM05	F02	Nicotinic acid	Nutritional supplement	C00253	0	0	0
PM05	F03	Nicotinamide	Nutritional supplement	C00153	0	0	0
PM05	F04	b-Nicotinamide Adenine Dinucleotide	Nutritional supplement	C00003	1.5	0	0
PM05	F05	d-Amino-levulinic acid	Nutritional supplement	C00431	0	0	0
PM05	F06	Hematin	Nutritional supplement		0	0	0
PM05	F07	Deferoxamine	Nutritional supplement	C06940	0	0	0
PM05	F08	a-D-Glucose	Nutritional supplement	C00031	0	0.5	0
PM05	F09	N-Acetyl-D-Glucosamine	Nutritional supplement	C00140	0	0.5	0
PM05	F10	Thymine	Nutritional supplement	C00178	0	0.5	0
PM05	F11	Glutathione	Nutritional supplement	C00051	0	0	0
PM05	F12	Thymidine	Nutritional supplement	C00214	0	0.5	0
PM05	G01	Oxaloacetic acid	Nutritional supplement	C00036	0	1	0
PM05	G02	D-Biotin	Nutritional supplement	C00120	0	0.5	0
PM05	G03	Cyanocobalamin	Nutritional supplement	C02823	0	0.5	0
PM05	G04	p-Amino-Benzoic acid	Nutritional supplement	C00568	0	0.5	0
PM05	G05	Folic acid	Nutritional supplement	C00504	0	0.5	0
PM05	G06	Inosine + Thiamine	Nutritional supplement		0	0	0
PM05	G07	Thiamine	Nutritional supplement	C00378	0	0.5	0
PM05	G08	Thiamine Pyrophosphate	Nutritional supplement	C00068	0	0.5	0
PM05	G09	Riboflavin	Nutritional supplement	C00255	0	0.5	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM05	G10	Pyrrolo-Quinoline Qui	Nutritional supplement	C00113	0	0.5	0
PM05	G11	Menadione	Nutritional supplement	C05377	0	0.5	0
PM05	G12	m-Inositol	Nutritional supplement	C00137	0	0	0
PM05	H01	Butyric acid	Nutritional supplement	C00246	0	0	0
PM05	H02	a-Hydroxybutyric acid	Nutritional supplement	C05984	0	0.5	0
PM05	H03	a-Ketobutyric acid	Nutritional supplement	C00109	0	0.5	0
PM05	H04	Caprylic acid	Nutritional supplement	C06423	0	1	0
PM05	H05	DL-Thioctic acid	Nutritional supplement	C00725	0	0.5	0
PM05	H06	DL-Mevalonic acid Lactone	Nutritional supplement	C02104	0	0.5	0
PM05	H07	DL-Carnitine	Nutritional supplement	C00487	0	0.5	0
PM05	H08	Choline	Nutritional supplement	C00114	0	0.5	0
PM05	H09	Tween 20	Nutritional supplement	C11624	0	0.5	0
PM05	H10	Tween 40	Nutritional supplement		0	0.5	0
PM05	H11	Tween 60	Nutritional supplement		0	0.5	0
PM05	H12	Tween 80	Nutritional supplement	C11625	0	0.5	0
PM06	A01	Negative Control	N-Source, Negative control		0	0	0
PM06	A02	L-Glutamine	N-Source, amino acid	C00064	3	3	4
PM06	A03	Ala-Ala	N-Source, peptide		3	3	3
PM06	A04	Ala-Arg	N-Source, peptide	C05034	3	3	4
PM06	A05	Ala-Asn	N-Source, peptide		4	3	4
PM06	A06	Ala-Glu	N-Source, peptide		4	3	4
PM06	A07	Ala-Gly	N-Source, peptide		3.5	3	4
PM06	A08	Ala-His	N-Source, peptide		3	2	3.5
PM06	A09	Ala-Leu	N-Source, peptide	C05035	3	3	4
PM06	A10	Ala-Lys	N-Source, peptide	C05036	3	2	3
PM06	A11	Ala-Phe	N-Source, peptide		3	3	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM06	A12	Ala-Pro	N-Source, peptide		4	3	4
PM06	B01	Ala-Ser	N-Source, peptide		3.5	3	4
PM06	B02	Ala-Thr	N-Source, peptide		3	3	3
PM06	B03	Ala-Trp	N-Source, peptide	C02732	2	3	3
PM06	B04	Ala-Tyr	N-Source, peptide	C05039	4	3	4
PM06	B05	Arg-Ala	N-Source, peptide		3.5	2	4
PM06	B06	Arg-Arg	N-Source, peptide	C05034	4	2	4
PM06	B07	Arg-Asp	N-Source, peptide	C02871	4	3	4
PM06	B08	Arg-Gln	N-Source, peptide		4	3	4
PM06	B09	Arg-Glu	N-Source, peptide		4	3	4
PM06	B10	Arg-Ile	N-Source, peptide		3	1	3.5
PM06	B11	Arg-Leu	N-Source, peptide	C05035	3.5	0.5	4
PM06	B12	Arg-Lys	N-Source, peptide	C05036	4	2	4
PM06	C01	Arg-Met	N-Source, peptide		4	0.5	4
PM06	C02	Arg-Phe	N-Source, peptide		4	2	4
PM06	C03	Arg-Ser	N-Source, peptide		4	3	4
PM06	C04	Arg-Trp	N-Source, peptide	C02732	3	2.5	3.5
PM06	C05	Arg-Tyr	N-Source, peptide	C05039	4	3	4
PM06	C06	Arg-Val	N-Source, peptide		3	0.5	3.5
PM06	C07	Asn-Glu	N-Source, peptide		4	3	4
PM06	C08	Asn-Val	N-Source, peptide		4	2	4
PM06	C09	Asp-Asp	N-Source, peptide	C02871	2	2	2
PM06	C10	Asp-Glu	N-Source, peptide		1	2	2.5
PM06	C11	Asp-Leu	N-Source, peptide	C05035	2	2	2.5
PM06	C12	Asp-Lys	N-Source, peptide	C05036	2	2	2
PM06	D01	Asp-Phe	N-Source, peptide		3	2	2.5
PM06	D02	Asp-Trp	N-Source, peptide	C02732	1	2	0.5
PM06	D03	Asp-Val	N-Source, peptide		2	2	2.5
PM06	D04	Cys-Gly	N-Source, peptide		4	2	4
PM06	D05	Gln-Gln	N-Source, peptide		4	3	4
PM06	D06	Gln-Gly	N-Source, peptide		4	3.5	4
PM06	D07	Glu-Asp	N-Source, peptide	C02871	4	2	4
PM06	D08	Glu-Glu	N-Source, peptide		2	2	2.5
PM06	D09	Glu-Gly	N-Source, peptide		0.5	2.5	1.5
PM06	D10	Glu-Ser	N-Source, peptide		4	3	4
PM06	D11	Glu-Trp	N-Source, peptide	C02732	2	2.5	2
PM06	D12	Glu-Tyr	N-Source, peptide	C05039	3	2	3
PM06	E01	Glu-Val	N-Source, peptide		4	2	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM06	E02	Gly-Ala	N-Source, peptide		4	3	4
PM06	E03	Gly-Arg	N-Source, peptide	C05034	4	3	4
PM06	E04	Gly-Cys	N-Source, peptide		2	2	1
PM06	E05	Gly-Gly	N-Source, peptide		2	3	3.5
PM06	E06	Gly-His	N-Source, peptide		2	2.5	2
PM06	E07	Gly-Leu	N-Source, peptide	C05035	3	2.5	3.5
PM06	E08	Gly-Lys	N-Source, peptide	C05036	1.5	2	1.5
PM06	E09	Gly-Met	N-Source, peptide		4	3	4
PM06	E10	Gly-Phe	N-Source, peptide		2.5	3	4
PM06	E11	Gly-Pro	N-Source, peptide		2	3	2.5
PM06	E12	Gly-Ser	N-Source, peptide		2	3	4
PM06	F01	Gly-Thr	N-Source, peptide		3	2	3
PM06	F02	Gly-Trp	N-Source, peptide	C02732	2	3	2
PM06	F03	Gly-Tyr	N-Source, peptide	C05039	2	3	3.5
PM06	F04	Gly-Val	N-Source, peptide		3.5	2	4
PM06	F05	His-Asp	N-Source, peptide	C02871	2	2	3
PM06	F06	His-Gly	N-Source, peptide		2	2	2
PM06	F07	His-Leu	N-Source, peptide	C05035	0	0	0.5
PM06	F08	His-Lys	N-Source, peptide	C05036	0	0	0
PM06	F09	His-Met	N-Source, peptide		0	0	0
PM06	F10	His-Pro	N-Source, peptide		0	0.5	0
PM06	F11	His-Ser	N-Source, peptide		2	2	2.5
PM06	F12	His-Trp	N-Source, peptide	C02732	0	1.5	1
PM06	G01	His-Tyr	N-Source, peptide	C05039	0.5	0	0
PM06	G02	His-Val	N-Source, peptide		0	0	0.5
PM06	G03	Ile-Ala	N-Source, peptide		3	2	3.5
PM06	G04	Ile-Arg	N-Source, peptide	C05034	3	1.5	3.5
PM06	G05	Ile-Gln	N-Source, peptide		4	3	4
PM06	G06	Ile-Gly	N-Source, peptide		2	2	3
PM06	G07	Ile-His	N-Source, peptide		0.5	0	0
PM06	G08	Ile-Ile	N-Source, peptide		0	0	0.5
PM06	G09	Ile-Met	N-Source, peptide		0	0	0
PM06	G10	Ile-Phe	N-Source, peptide		0	0	0
PM06	G11	Ile-Pro	N-Source, peptide		0	1	0
PM06	G12	Ile-Ser	N-Source, peptide		3.5	2	4
PM06	H01	Ile-Trp	N-Source, peptide	C02732	1.5	1	1
PM06	H02	Ile-Tyr	N-Source, peptide	C05039	0.5	0	2
PM06	H03	Ile-Val	N-Source, peptide		0	0	1

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM06	H04	Leu-Ala	N-Source, peptide		3	1.5	4
PM06	H05	Leu-Arg	N-Source, peptide	C05034	3	2	3.5
PM06	H06	Leu-Asp	N-Source, peptide	C02871	3.5	2.5	4
PM06	H07	Leu-Glu	N-Source, peptide		2	3	2.5
PM06	H08	Leu-Gly	N-Source, peptide		3	1	3.5
PM06	H09	Leu-Ile	N-Source, peptide		0	0	0
PM06	H10	Leu-Leu	N-Source, peptide	C05035	0	0	0
PM06	H11	Leu-Met	N-Source, peptide		2.5	1	3
PM06	H12	Leu-Phe	N-Source, peptide		0	1	1
PM07	A01	Negative Control	N-Source, Negative control		0	0	0
PM07	A02	L-Glutamine	N-Source, amino acid	C00064	4	3	4
PM07	A03	Leu-Ser	N-Source, peptide		4	3	4
PM07	A04	Leu-Trp	N-Source, peptide	C02732	1	1.5	1.5
PM07	A05	Leu-Val	N-Source, peptide		0.5	1	0
PM07	A06	Lys-Ala	N-Source, peptide		2	2	3
PM07	A07	Lys-Arg	N-Source, peptide	C05034	3.5	3	4
PM07	A08	Lys-Glu	N-Source, peptide		2	3	2.5
PM07	A09	Lys-Ile	N-Source, peptide		2	0	2
PM07	A10	Lys-Leu	N-Source, peptide	C05035	0	0.5	1
PM07	A11	Lys-Lys	N-Source, peptide	C05036	0.5	0	0.5
PM07	A12	Lys-Phe	N-Source, peptide		0	0	0
PM07	B01	Lys-Pro	N-Source, peptide		2	2.5	2
PM07	B02	Lys-Ser	N-Source, peptide		3.5	3	3
PM07	B03	Lys-Thr	N-Source, peptide		2	2	1
PM07	B04	Lys-Trp	N-Source, peptide	C02732	0	3	0
PM07	B05	Lys-Tyr	N-Source, peptide	C05039	0.5	1.5	0.5
PM07	B06	Lys-Val	N-Source, peptide		0.5	0	0
PM07	B07	Met-Arg	N-Source, peptide	C05034	4	3	4
PM07	B08	Met-Asp	N-Source, peptide	C02871	4	3	4
PM07	B09	Met-Gln	N-Source, peptide		4	4	4
PM07	B10	Met-Glu	N-Source, peptide		4	3.5	4
PM07	B11	Met-Gly	N-Source, peptide		4	2	4
PM07	B12	Met-His	N-Source, peptide		1	0	3
PM07	C01	Met-Ile	N-Source, peptide		2	2	1
PM07	C02	Met-Leu	N-Source, peptide	C05035	2	2	2.5
PM07	C03	Met-Lys	N-Source, peptide	C05036	2	2	2.5
PM07	C04	Met-Met	N-Source, peptide		3	0	3

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM07	C05	Met-Phe	N-Source, peptide		0	2	0
PM07	C06	Met-Pro	N-Source, peptide		0	3.5	3
PM07	C07	Met-Trp	N-Source, peptide	C02732	0.5	3	0
PM07	C08	Met-Val	N-Source, peptide		1	1.5	1.5
PM07	C09	Phe-Ala	N-Source, peptide		4	3	4
PM07	C10	Phe-Gly	N-Source, peptide		2	3	3
PM07	C11	Phe-Ile	N-Source, peptide		0	1	0
PM07	C12	Phe-Phe	N-Source, peptide		0.5	0	1
PM07	D01	Phe-Pro	N-Source, peptide		2	2	2
PM07	D02	Phe-Ser	N-Source, peptide		3	3	4
PM07	D03	Phe-Trp	N-Source, peptide	C02732	2	2.5	2
PM07	D04	Pro-Ala	N-Source, peptide		4	4	4
PM07	D05	Pro-Asp	N-Source, peptide	C02871	1	3	0
PM07	D06	Pro-Gln	N-Source, peptide		0	3	0
PM07	D07	Pro-Gly	N-Source, peptide		0.5	3	0
PM07	D08	Pro-Hyp	N-Source, peptide		0	0	0.5
PM07	D09	Pro-Leu	N-Source, peptide	C05035	0	4	0
PM07	D10	Pro-Phe	N-Source, peptide		0	2	0
PM07	D11	Pro-Pro	N-Source, peptide		0	3	0
PM07	D12	Pro-Tyr	N-Source, peptide	C05039	2	2	2
PM07	E01	Ser-Ala	N-Source, peptide		4	4	4
PM07	E02	Ser-Gly	N-Source, peptide		4	4	4
PM07	E03	Ser-His	N-Source, peptide		0.5	3	1.5
PM07	E04	Ser-Leu	N-Source, peptide	C05035	4	4	4
PM07	E05	Ser-Met	N-Source, peptide		4	3	4
PM07	E06	Ser-Phe	N-Source, peptide		0	3	0
PM07	E07	Ser-Pro	N-Source, peptide		2	4	4
PM07	E08	Ser-Ser	N-Source, peptide		4	1	4
PM07	E09	Ser-Tyr	N-Source, peptide	C05039	2	3	3
PM07	E10	Ser-Val	N-Source, peptide		2.5	3	3.5
PM07	E11	Thr-Ala	N-Source, peptide		3.5	3	4
PM07	E12	Thr-Arg	N-Source, peptide	C05034	4	3	4
PM07	F01	Thr-Glu	N-Source, peptide		4	3	4
PM07	F02	Thr-Gly	N-Source, peptide		2	3	2
PM07	F03	Thr-Leu	N-Source, peptide	C05035	2	3	3
PM07	F04	Thr-Met	N-Source, peptide		4	3	4
PM07	F05	Thr-Pro	N-Source, peptide		1	4	0
PM07	F06	Trp-Ala	N-Source, peptide		4	3	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM07	F07	Trp-Arg	N-Source, peptide	C05034	2	3	3.5
PM07	F08	Trp-Asp	N-Source, peptide	C02871	0	3	3
PM07	F09	Trp-Glu	N-Source, peptide		0	3	3
PM07	F10	Trp-Gly	N-Source, peptide		3	3	4
PM07	F11	Trp-Leu	N-Source, peptide	C05035	0	0.5	0
PM07	F12	Trp-Lys	N-Source, peptide	C05036	2	2	2.5
PM07	G01	Trp-Phe	N-Source, peptide		2	1	2
PM07	G02	Trp-Ser	N-Source, peptide		3.5	3	4
PM07	G03	Trp-Trp	N-Source, peptide	C02732	0.5	3	1
PM07	G04	Trp-Tyr	N-Source, peptide	C05039	2	3	2.5
PM07	G05	Tyr-Ala	N-Source, peptide		4	3	4
PM07	G06	Tyr-Gln	N-Source, peptide		4	3	4
PM07	G07	Tyr-Glu	N-Source, peptide		0	3	3
PM07	G08	Tyr-Gly	N-Source, peptide		3	3	4
PM07	G09	Tyr-His	N-Source, peptide		0	0	0.5
PM07	G10	Tyr-Leu	N-Source, peptide	C05035	0	0	0.5
PM07	G11	Tyr-Lys	N-Source, peptide	C05036	2	1	0
PM07	G12	Tyr-Phe	N-Source, peptide		1	0	1
PM07	H01	Tyr-Trp	N-Source, peptide	C02732	2	0	2
PM07	H02	Tyr-Tyr	N-Source, peptide	C05039	1	2	2
PM07	H03	Val-Arg	N-Source, peptide	C05034	4	3	4
PM07	H04	Val-Asn	N-Source, peptide		3	3	4
PM07	H05	Val-Asp	N-Source, peptide	C02871	3.5	3	4
PM07	H06	Val-Gly	N-Source, peptide		3	3	3.5
PM07	H07	Val-His	N-Source, peptide		0	0	0.5
PM07	H08	Val-Ile	N-Source, peptide		0	0	0
PM07	H09	Val-Leu	N-Source, peptide	C05035	0	0	0
PM07	H10	Val-Tyr	N-Source, peptide	C05039	0	0	2
PM07	H11	Val-Val	N-Source, peptide		0	0	0
PM07	H12	g-Glu-Gly	N-Source, peptide		0	0	0
PM08	A01	Negative Control	N-Source, Negative control		0	0	0
PM08	A02	L-Glutamine	N-Source, amino acid	C00064	3.5	3	4
PM08	A03	Ala-Asp	N-Source, peptide	C02871	4	3.5	4
PM08	A04	Ala-Gln	N-Source, peptide		4	4	4
PM08	A05	Ala-Ile	N-Source, peptide		4	3	4
PM08	A06	Ala-Met	N-Source, peptide		4	3	4
PM08	A07	Ala-Val	N-Source, peptide		3	3	3

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM08	A08	Asp-Ala	N-Source, peptide		4	3	4
PM08	A09	Asp-Gln	N-Source, peptide		4	3	4
PM08	A10	Asp-Gly	N-Source, peptide		3	3	3
PM08	A11	Glu-Ala	N-Source, peptide		4	4	4
PM08	A12	Gly-Asn	N-Source, peptide		4	3.5	4
PM08	B01	Gly-Asp	N-Source, peptide	C02871	4	3	4
PM08	B02	Gly-Ile	N-Source, peptide		3	3	3
PM08	B03	His-Ala	N-Source, peptide		2.5	2	2.5
PM08	B04	His-Glu	N-Source, peptide		0	3	0
PM08	B05	His-His	N-Source, peptide		0.5	1.5	1.5
PM08	B06	Ile-Asn	N-Source, peptide		4	3	4
PM08	B07	Ile-Leu	N-Source, peptide	C05035	2	2.5	2
PM08	B08	Leu-Asn	N-Source, peptide		4	3.5	4
PM08	B09	Leu-His	N-Source, peptide		2	1.5	2
PM08	B10	Leu-Pro	N-Source, peptide		2	4	2
PM08	B11	Leu-Tyr	N-Source, peptide	C05039	2	3	2.5
PM08	B12	Lys-Asp	N-Source, peptide	C02871	3	3	3
PM08	C01	Lys-Gly	N-Source, peptide		3	2	3
PM08	C02	Lys-Met	N-Source, peptide		3	1	3
PM08	C03	Met-Thr	N-Source, peptide		4	3	4
PM08	C04	Met-Tyr	N-Source, peptide	C05039	2.5	2.5	3
PM08	C05	Phe-Asp	N-Source, peptide	C02871	2	3	3
PM08	C06	Phe-Glu	N-Source, peptide		1.5	3	2
PM08	C07	Gln-Glu	N-Source, peptide		4	3	4
PM08	C08	Phe-Met	N-Source, peptide		3	2	3
PM08	C09	Phe-Tyr	N-Source, peptide	C05039	2	3	2
PM08	C10	Phe-Val	N-Source, peptide		2	2	2
PM08	C11	Pro-Arg	N-Source, peptide	C05034	4	3	4
PM08	C12	Pro-Asn	N-Source, peptide		4	3.5	4
PM08	D01	Pro-Glu	N-Source, peptide		2	2	2
PM08	D02	Pro-lle	N-Source, peptide		1	2.5	0
PM08	D03	Pro-Lys	N-Source, peptide	C05036	1	2.5	1
PM08	D04	Pro-Ser	N-Source, peptide		0.5	3.5	3
PM08	D05	Pro-Trp	N-Source, peptide	C02732	0	2	0.5
PM08	D06	Pro-Val	N-Source, peptide		2	3	1.5
PM08	D07	Ser-Asn	N-Source, peptide		4	4	4
PM08	D08	Ser-Asp	N-Source, peptide	C02871	4	3.5	4
PM08	D09	Ser-Gln	N-Source, peptide		4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM08	D10	Ser-Glu	N-Source, peptide		4	3.5	4
PM08	D11	Thr-Asp	N-Source, peptide	C02871	4	3	4
PM08	D12	Thr-Gln	N-Source, peptide		4	3.5	4
PM08	E01	Thr-Phe	N-Source, peptide		2	2	2
PM08	E02	Thr-Ser	N-Source, peptide		4	3	4
PM08	E03	Trp-Val	N-Source, peptide		1	2.5	1
PM08	E04	Tyr-Ile	N-Source, peptide		0.5	0	1
PM08	E05	Tyr-Val	N-Source, peptide		0.5	0.5	1.5
PM08	E06	Val-Ala	N-Source, peptide		3	3	3.5
PM08	E07	Val-Gln	N-Source, peptide		3.5	3	4
PM08	E08	Val-Glu	N-Source, peptide		2	3	1
PM08	E09	Val-Lys	N-Source, peptide	C05036	1.5	0	2
PM08	E10	Val-Met	N-Source, peptide		2	2	2
PM08	E11	Val-Phe	N-Source, peptide		2	2	2
PM08	E12	Val-Pro	N-Source, peptide		2	3	2.5
PM08	F01	Val-Ser	N-Source, peptide		3	3	3.5
PM08	F02	b-Ala-Ala	N-Source, peptide		1	0	1
PM08	F03	b-Ala-Gly	N-Source, peptide		0	0	0
PM08	F04	b-Ala-His	N-Source, peptide		0	0	0
PM08	F05	Met-b-Ala	N-Source, peptide		0	0	0.5
PM08	F06	b-Ala-Phe	N-Source, peptide		0	0	0
PM08	F07	D-Ala-D-Ala	N-Source, peptide		0.5	0	0.5
PM08	F08	D-Ala-Gly	N-Source, peptide		2	2	1.5
PM08	F09	D-Ala-Leu	N-Source, peptide	C05035	0	0	0
PM08	F10	D-Leu-D-Leu	N-Source, peptide		0	0	0
PM08	F11	D-Leu-Gly	N-Source, peptide		0	0	0
PM08	F12	D-Leu-Tyr	N-Source, peptide	C05039	0	0	0
PM08	G01	g-Glu-Gly	N-Source, peptide		0	0	0
PM08	G02	g-D-Glu-Gly	N-Source, peptide		0	0	0
PM08	G03	Gly-D-Ala	N-Source, peptide		0	0	0.5
PM08	G04	Gly-D-Asp	N-Source, peptide		0	0	0
PM08	G05	Gly-D-Ser	N-Source, peptide		0	0	0.5
PM08	G06	Gly-D-Thr	N-Source, peptide		0	0	0
PM08	G07	Gly-D-Val	N-Source, peptide		0	0	0
PM08	G08	Leu-b-Ala	N-Source, peptide		0	0	0
PM08	G09	Leu-D-Leu	N-Source, peptide		0	0	0
PM08	G10	Phe-b-Ala	N-Source, peptide		0	0	0.5
PM08	G11	Ala-Ala-Ala	N-Source, peptide		3	3	3

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM08	G12	D-Ala-Gly-Gly	N-Source, peptide		2	2	3
PM08	H01	Gly-Gly-Ala	N-Source, peptide		4	3	4
PM08	H02	Gly-Gly-D-Leu	N-Source, peptide		0	2	0.5
PM08	H03	Gly-Gly-Gly	N-Source, peptide		2.5	2	3
PM08	H04	Gly-Gly-Ile	N-Source, peptide		3	3	3
PM08	H05	Gly-Gly-Leu	N-Source, peptide	C05035	3	3	4
PM08	H06	Gly-Gly-Phe	N-Source, peptide		3	3	3
PM08	H07	Val-Tyr-Val	N-Source, peptide		0	0	1
PM08	H08	Gly-Phe-Phe	N-Source, peptide		2	3	2.5
PM08	H09	Leu-Gly-Gly	N-Source, peptide		3	2	4
PM08	H10	Leu-Leu-Leu	N-Source, peptide	C05035	1	1	1.5
PM08	H11	Phe-Gly-Gly	N-Source, peptide		3	3	3
PM08	H12	Tyr-Gly-Gly	N-Source, peptide		4	3	4
PM09	A01	1% NaCl	osmotic sensitivity, NaCl	C13563	4	4	4
PM09	A02	2% NaCl	osmotic sensitivity, NaCl	C13563	4	4	4
PM09	A03	3% NaCl	osmotic sensitivity, NaCl	C13563	4	4	4
PM09	A04	4% NaCl	osmotic sensitivity, NaCl	C13563	3	3	4
PM09	A05	5% NaCl	osmotic sensitivity, NaCl	C13563	3	2	3
PM09	A06	5.5% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A07	6% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A08	6.5% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A09	7% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A10	8% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A11	9% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	A12	10% NaCl	osmotic sensitivity, NaCl	C13563	0	0	0
PM09	B01	6% NaCl	osmotic sensitivity, NaCl control		0	1	0
PM09	B02	6% NaCl + Betaine	osmolyte, betaine	C00719	1	2	1
PM09	B03	6% NaCl + NN Dimethyl Glycine	osmolyte, dimethylglycine	C01026	0	3	0.5

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM09	B04	6% NaCl + Sarcosine	osmolyte, sarcosine	C00213	2	2	0
PM09	B05	6% NaCl + Dimethyl Sulphonyl Propionate	osmolyte, dimethyl sulphonyl propionate		1	1	1
PM09	B06	6% NaCl + MOPS	osmolyte, MOPS		1	1.5	0.5
PM09	B07	6% NaCl + Ectoine	osmolyte, ectoine	C06231	0	0.5	0
PM09	B08	6% NaCl + Choline	osmolyte, choline	C00114	0	1	0
PM09	B09	6% NaCl + Phosphorylcholine	osmolyte, phosphorylcholine	C00588	0.5	3	0
PM09	B10	6% NaCl + Creatine	osmolyte, creatine	C00300	0	1.5	0
PM09	B11	6% NaCl + Creatinine	osmolyte, creatinine	C00791	0	0	0
PM09	B12	6% NaCl + L-Carnitine	osmolyte, carnitine	C00318	0	0	0
PM09	C01	6% NaCl + KCl	osmolyte, KCl	C13567	0	0.5	0
PM09	C02	6% NaCl + L-Proline	osmolyte, proline	C00148	0.5	3	0
PM09	C03	6% NaCl + N-Acetyl-L-Glutamine	osmolyte, acetyl glutamine		0.5	3	0.5
PM09	C04	6% NaCl + b-Glutamic acid	osmolyte, b-glutamate		0.5	1	0.5
PM09	C05	6% NaCl + g-Amino-N-Butyric acid	osmolyte, g-amino butyric acid		0.5	3	0.5
PM09	C06	6% NaCl + Glutathione	osmolyte, glutathione	C00051	1.5	1	0.5
PM09	C07	6% NaCl + Glycerol	osmolyte, glycerol	C00116	0.5	1	0.5
PM09	C08	6% NaCl + Trehalose	osmolyte, trehalose	C01083	0.5	2	0.5
PM09	C09	6% NaCl + Trimethylamine-N-Oxide	osmolyte, trimethylamine-N-oxide	C01104	0.5	1	0.5
PM09	C10	6% NaCl + Trimethylamine	osmolyte, trimethylamine	C00565	0.5	1	0
PM09	C11	6% NaCl + Octopine	osmolyte, octopine	C04137	0.5	2	0
PM09	C12	6% NaCl + Trigonelline	osmolyte, trigonelline	C01004	0	0	0
PM09	D01	3% Potassium Chloride	osmotic sensitivity, KCl	C13567	4	4	4
PM09	D02	4% Potassium chloride	osmotic sensitivity, KCl	C13567	4	3	4
PM09	D03	5% Potassium Chloride	osmotic sensitivity, KCl	C13567	3.5	3	4
PM09	D04	6% Potassium chloride	osmotic sensitivity, KCl	C13567	3	3	3
PM09	D05	2% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM09	D06	3% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	4	4	4
PM09	D07	4% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	4	4	4
PM09	D08	5% Sodium Sulfate	osmotic sensitivity, Na2SO4	C13199	4	4	4
PM09	D09	5% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4	4
PM09	D10	10% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4	4
PM09	D11	15% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4	4
PM09	D12	20% Ethylene Glycol	osmotic sensitivity, ethylene glycol	C01380	4	4	4
PM09	E01	1% Sodium Formate	osmotic sensitivity, sodium formate		4	4	4
PM09	E02	2% Sodium Formate	osmotic sensitivity, sodium formate		4	3	3
PM09	E03	3% Sodium Formate	osmotic sensitivity, sodium formate		3	3	3
PM09	E04	4% Sodium Formate	osmotic sensitivity, sodium formate		2	1	1
PM09	E05	5% Sodium Formate	osmotic sensitivity, sodium formate		1	1	1
PM09	E06	6% Sodium Formate	osmotic sensitivity, sodium formate		1	2	1
PM09	E07	2% Urea	osmotic sensitivity, urea	C00086	4	4	4
PM09	E08	3% Urea	osmotic sensitivity, urea	C00086	4	4	4
PM09	E09	4% Urea	osmotic sensitivity, urea	C00086	3.5	3	3.5
PM09	E10	5% Urea	osmotic sensitivity, urea	C00086	1.5	1.5	0
PM09	E11	6% Urea	osmotic sensitivity, urea	C00086	0	0	0
PM09	E12	7% Urea	osmotic sensitivity, urea	C00086	0	0	0
PM09	F01	1% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	4	4	4
PM09	F02	2% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	4	4	3.5
PM09	F03	3% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	3	4	1

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM09	F04	4% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0.5	3	0
PM09	F05	5% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3	0
PM09	F06	6% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	2	0
PM09	F07	7% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	0.5	0
PM09	F08	8% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3	0
PM09	F09	9% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	2	0
PM09	F10	10% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	1	0
PM09	F11	11% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	1.5	0
PM09	F12	12% Sodium Lactate	osmotic sensitivity, sodium lactate	C13960	0	3	0
PM09	G01	20mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	4	4
PM09	G02	50mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	4	4
PM09	G03	100mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	4	4
PM09	G04	200mM Sodium Phosphate pH 7	osmotic sensitivity, sodium phosphate		4	4	4
PM09	G05	20mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	4	4	3
PM09	G06	50mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	1	2	1
PM09	G07	100mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	0	0	0
PM09	G08	200mM Sodium Benzoate pH 5.2	toxicity, benzoate	D02277	0	0	0
PM09	G09	10mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4	4
PM09	G10	20mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4	4
PM09	G11	50mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4	4
PM09	G12	100mM Ammonium Sulfate pH 8	toxicity, ammonia		4	4	4
PM09	H01	10mM Sodium Nitrate	toxicity, nitrate	C00244	4	4	4
PM09	H02	20mM Sodium Nitrate	toxicity, nitrate	C00244	4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM09	H03	40mM Sodium Nitrate	toxicity, nitrate	C00244	4	3.5	4
PM09	H04	60mM Sodium Nitrate	toxicity, nitrate	C00244	3	2.5	2
PM09	H05	80mM Sodium Nitrate	toxicity, nitrate	C00244	3	3	3
PM09	H06	100mM Sodium Nitrate	toxicity, nitrate	C00244	3	3	3
PM09	H07	10mM Sodium Nitrite	toxicity, nitrite	C00088	4	4	4
PM09	H08	20mM Sodium Nitrite	toxicity, nitrite	C00088	4	4	4
PM09	H09	40mM Sodium Nitrite	toxicity, nitrite	C00088	4	4	4
PM09	H10	60mM Sodium Nitrite	toxicity, nitrite	C00088	4	3.5	4
PM09	H11	80mM Sodium Nitrite	toxicity, nitrite	C00088	4	3	3
PM09	H12	100mM Sodium Nitrite	toxicity, nitrite	C00088	3	2	3
PM10	A01	pH 3.5	pH, growth at 3.5		0	0	0
PM10	A02	pH 4	pH, growth at 4		0	0	0
PM10	A03	pH 4.5	pH, growth at 4.5		3	2	0
PM10	A04	pH 5	pH, growth at 5		4	4	4
PM10	A05	pH 5.5	pH, growth at 5.5		4	4	4
PM10	A06	pH 6	pH, growth at 6		4	4	4
PM10	A07	pH 7	pH, growth at 7		4	4	4
PM10	A08	pH 8	pH, growth at 8		4	4	4
PM10	A09	pH 8.5	pH, growth at 8.5		4	4	4
PM10	A10	pH 9	pH, growth at 9		4	4	4
PM10	A11	pH 9.5	pH, growth at 9.5		4	4	4
PM10	A12	pH 10	pH, growth at 10		4	4	4
PM10	B01	pH 4.5	pH, decarboxylase control		0	3	0
PM10	B02	pH 4.5 + L-Alanine	pH, decarboxylase	C00041	0	2.5	0
PM10	B03	pH 4.5 + L-Arginine	pH, decarboxylase	C00062	0	2	0
PM10	B04	pH 4.5 + L-Asparagine	pH, decarboxylase	C00152	1.5	2.5	0
PM10	B05	pH 4.5 + L-Aspartic acid	pH, decarboxylase	C00049	0.5	2	0
PM10	B06	pH 4.5 + L-Glutamic acid	pH, decarboxylase	C00025	0.5	2	0
PM10	B07	pH 4.5 + L-Glutamine	pH, decarboxylase	C00064	3	0	1
PM10	B08	pH 4.5 + Glycine	pH, decarboxylase	C00037	3	3	0
PM10	B09	pH 4.5 + L-Histidine	pH, decarboxylase	C00135	0	2.5	0.5
PM10	B10	pH 4.5 + L-Isoleucine	pH, decarboxylase	C00407	0	0	0
PM10	B11	pH 4.5 + L-Leucine	pH, decarboxylase	C00123	0	0	0
PM10	B12	pH 4.5 + L-Lysine	pH, decarboxylase	C00047	4	4	3

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM10	C01	pH 4.5 + L-Methionine	pH, decarboxylase	C00073	0	2	0
PM10	C02	pH 4.5 + L-Phenylalanine	pH, decarboxylase	C00079	0	2.5	0
PM10	C03	pH 4.5 + L-Proline	pH, decarboxylase	C00148	1	3	0
PM10	C04	pH 4.5 + L-Serine	pH, decarboxylase	C00065	1	3	0.5
PM10	C05	pH 4.5 + L-Threonine	pH, decarboxylase	C00188	1.5	3	0.5
PM10	C06	pH 4.5 + L-Tryptophan	pH, decarboxylase	C00078	0	0	0
PM10	C07	pH 4.5 + L-Citrulline	pH, decarboxylase	C00082	1	3	0
PM10	C08	pH 4.5 + L-Valine	pH, decarboxylase	C00183	1.5	3	0
PM10	C09	pH 4.5 + Hydroxy-L-Proline	pH, decarboxylase	C01015	2.5	3	0
PM10	C10	pH 4.5 + L-Ornithine	pH, decarboxylase	C00077	3	4	3
PM10	C11	pH 4.5 + L-Homoarginine	pH, decarboxylase		1	2	0
PM10	C12	pH 4.5 + L-Homoserine	pH, decarboxylase	C00263	0	2.5	0
PM10	D01	pH 4.5 + Anthranilic acid	pH, decarboxylase	C00108	0	0	0
PM10	D02	pH 4.5 + L-Norleucine	pH, decarboxylase	C01933	0	0	0
PM10	D03	pH 4.5 + L-Norvaline	pH, decarboxylase		3	4	3
PM10	D04	pH 4.5 + a-Amino-N-Butyric acid	pH, decarboxylase		3	3	1
PM10	D05	pH 4.5 + p-Aminobenzoate	pH, decarboxylase		0	0	0
PM10	D06	pH 4.5 + L-Cysteic acid	pH, decarboxylase	C00506	3	3.5	3
PM10	D07	pH 4.5 + D-Lysine	pH, decarboxylase	C00739	3	3	1
PM10	D08	pH 4.5 + 5-Hydroxy-L-Lysine	pH, decarboxylase	C01211	3	3	1
PM10	D09	pH 4.5 + 5-Hydroxy-L-Tryptophan	pH, decarboxylase		0	0	0
PM10	D10	pH 4.5 + DL-Diamino-a,e-Pimelic acid	pH, decarboxylase	C00680	3	3.5	1
PM10	D11	pH 4.5 + Trimethylamine-N-Oxide	pH, decarboxylase	C01104	0	1.5	0
PM10	D12	pH 4.5 + Urea	pH, decarboxylase	C00086	2.5	3	1.5
PM10	E01	pH 9.5	pH, deaminase control		4	4	4

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM10	E02	pH 9.5 + L-Alanine	pH, deaminase	C00041	4	4	4
PM10	E03	pH 9.5 + L-Arginine	pH, deaminase	C00062	4	4	4
PM10	E04	pH 9.5 + L-Asparagine	pH, deaminase	C00152	4	4	4
PM10	E05	pH 9.5 + L-Aspartic acid	pH, deaminase	C00049	4	4	4
PM10	E06	pH 9.5 + L-Glutamic acid	pH, deaminase	C00025	4	4	4
PM10	E07	pH 9.5 + L-Glutamine	pH, deaminase	C00064	4	4	4
PM10	E08	pH 9.5 + Glycine	pH, deaminase	C00037	4	4	4
PM10	E09	pH 9.5 + L-Histidine	pH, deaminase	C00135	4	4	4
PM10	E10	pH 9.5 + L-Isoleucine	pH, deaminase	C00407	3.5	4	3.5
PM10	E11	pH 9.5 + L-Leucine	pH, deaminase	C00123	4	4	4
PM10	E12	pH 9.5 + L-Lysine	pH, deaminase	C00047	4	4	4
PM10	F01	pH 9.5 + L-Methionine	pH, deaminase	C00073	4	4	4
PM10	F02	pH 9.5 + L-Phenylalanine	pH, deaminase	C00079	3.5	4	3.5
PM10	F03	pH 9.5 + L-Proline	pH, deaminase	C00148	4	4	4
PM10	F04	pH 9.5 + L-Serine	pH, deaminase	C00065	4	4	4
PM10	F05	pH 9.5 + L-Threonine	pH, deaminase	C00188	4	4	4
PM10	F06	pH 9.5 + L-Tryptophan	pH, deaminase	C00078	0	0.5	0
PM10	F07	pH 9.5 + L-Tyrosine	pH, deaminase	C00082	3	3	3
PM10	F08	pH 9.5 + L-Valine	pH, deaminase	C00183	4	4	3.5
PM10	F09	pH 9.5 + Hydroxy-L-Proline	pH, deaminase	C01015	4	4	4
PM10	F10	pH 9.5 + L-Ornithine	pH, deaminase	C00077	4	4	4
PM10	F11	pH 9.5 + L-Homoarginine	pH, deaminase		4	4	4
PM10	F12	pH 9.5 + L-Homoserine	pH, deaminase	C00263	4	4	4
PM10	G01	pH 9.5 + Anthranilic acid	pH, deaminase	C00108	4	4	4
PM10	G02	pH 9.5 + L-Norleucine	pH, deaminase	C01933	3.5	0	3.5
PM10	G03	pH 9.5 + L-Norvaline	pH, deaminase		4	4	4
PM10	G04	pH 9.5 + Agmatine	pH, deaminase	C00179	4	4	4
PM10	G05	pH 9.5 + Cadaverine	pH, deaminase	C01672	3.5	0	2.5
PM10	G06	pH 9.5 + Putrescine	pH, deaminase	C00134	4	4	4
PM10	G07	pH 9.5 + Histamine	pH, deaminase	C00388	0	0	0

Plate	Well	Chemical	Mode of action	Co_id	WT-O157	ZZ b2	ZZ b4
PM10	G08	pH 9.5 + b-Phenylethylamine	pH, deaminase	C05332	0	4	0
PM10	G09	pH 9.5 + Tyramine	pH, deaminase	C00483	0	0	0
PM10	G10	pH 9.5 + Creatine	pH, deaminase	C00300	4	4	4
PM10	G11	pH 9.5 + Trimethylamine-N-Oxide	pH, deaminase	C01104	4	4	4
PM10	G12	pH 9.5 + Urea	pH, deaminase	C00086	4	4	4
PM10	H01	X-Caprylate	caprylate esterase		4	4	4
PM10	H02	X-a-D-Glucoside	a-D-glucosidase		4	4	4
PM10	H03	X-b-D-Glucoside	b-D-glucosidase		4	4	4
PM10	H04	X-a-D-Galactoside	a-D-galactosidase		4	4	4
PM10	H05	X-b-D-Galactoside	b-D-galactosidase		4	4	4
PM10	H06	X-a-D-Glucuronide	a-D-glucuronidase		4	4	4
PM10	H07	X-b-D-Glucuronide	b-D-glucuronidase		4	4	4
PM10	H08	X-b-D-Glucosaminide	b-D-glucosaminidase		4	4	4
PM10	H09	X-b-D-Galactosaminide	b-D-galactosaminidase		4	4	4
PM10	H10	X-a-D-Mannoside	a-D-mannosidase		4	4	4
PM10	H11	X-PO4	aryl phosphatase		4	4	4
PM10	H12	X-SO4	aryl sulfatase		4	4	4

*. Compound ID in the KEGG database.