Isolating Novel Hydrocarbon Degraders from Northwest Passage Beach Sediments and Assessing their Ability for Biodegradation

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Abstract

Due to the effect of global climate change, sea ice cover in the Arctic recedes further and for longer periods of time every year, opening the way to increased anthropogenic activity. The Northwest Passage is of special interest as it is expected to become an important sea route connecting the Pacific and Atlantic oceans through the islands and gulfs of northern Canada in the near future. With an increase in ship transit comes an increased risk of accidental spillage of ship fuels. Hydrocarbons, and especially their aromatic compounds, are of environmental concern due to their well-known toxic and carcinogenic properties. Once they leave the water column, these fuels are known to be particularly difficult to remove from Arctic beaches where they wash off due to their high viscosity, and the composition of Arctic beach sediments. One efficient method used to clean beaches of washed-up hydrocarbons is bioremediation, or the use of naturally occurring bacteria to degrade oil. In this study we isolated microbial strains from Northwest Passage beach sediments and determined their potential for degradation of various hydrocarbons through genetic and chemical methods. Moreover, the isolates' ability to grow under a wide range of temperatures and salinities common to the Arctic was assessed. By searching their genomes for the presence of cold and salt tolerance genes, hydrocarbon degradative pathways were recreated using KEGG database (Kanehisa et al. 2021) in 3 strains selected for their successful growth on Ultra Low Sulfur Fuel Oil (ULSFO) as sole carbon source under low temperature, their potential novelty based on 16S rRNA amplicon sequencing, or their unknown potential for hydrocarbon biodegradation. The extent of degradation of alkane and aromatics fractions of these strains was then assessed using Gas-Chromatography and Mass-Spectrometry. Our results show the widespread presence of marker hydrocarbon degradation genes in several microbial isolates from Northwest Passage beach sediments, and successfully mapped complete degradation pathways for alkanes and naphthalene

in several of those. Furthermore, the growth and genetic assays performed suggest a wide range of temperature and salt tolerance in the isolated strains, confirming their identity as psychrophiles and suggesting an ability for bioremediation under cold and highly saline environments. At the individual strain level, our results suggest that both *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T have potential in bioremediation in the events of an oil spill reaching the shores of the Northwest Passage, and that many more naturally occurring microbes could offer a potential response if such a spill was to reach the sediments of Northwest Passage beaches.

Résumé

Dû aux conséquences du réchauffement climatique, la calotte glacière arctique recède de plus en plus loin, pour de plus en plus longues périodes de temps chaque année, ouvrant du même coup la voie aux activités anthropogéniques. Le Passage du Nord-Ouest est d'intérêt particulier dû au fait que l'on s'attend à le voir devenir un important réseau de navigation commerciale naval connectant les océans Atlantique et Pacifique entre les îles et golfs de l'arctique Canadien d'ici la fin de la décennie. Avec une augmentation de l'activité navale vient une augmentation du risque d'incidents pouvant mener au déversement accidentel de carburant à navire. Les Hydrocarbures, et plus spécifiquement leurs composantes aromatiques, sont particulièrement inquiétants pour leur impact néfaste sur l'environnement et la santé humaine dû à leurs propriétés toxiques et carcinogènes bien connues. De plus, une fois qu'ils se sont échappés de la colonne d'eau, ils sont particulièrement complexes à nettoyer des plages où ils se sont échoués due aux conditions climatiques de l'arctique, et la composition des plages du Passage du Nord-Ouest. Une méthode efficace utilisée pour se débarrasser de ces hydrocarbures échoués est la bioremédiation, ou l'utilisation de bactéries naturellement présentes dans l'environnement, pour dégrader le carburant. Dans cette étude, nous avons isolé des souches de bactérienne depuis des sédiments obtenus sur une plage du Passage du Nord-Ouest et avons déterminé leur potentiel de dégradation d'hydrocarbures sur de multiples hydrocarbures avec des méthodes génétiques et chimiques. En outre, la capacité de croissance des isolats bactériens sous de multiples conditions de température et de salinité répliquant des conditions connues de l'arctique fut testée, en plus de rechercher leurs génomes pour la présence de gènes connus pour leurs rôles dans la tolérance à la température et la présence élevée de sel. Les voies de dégradation d`hydrocarbures variés furent recréées en utilisant la banque de données KEGG (Kanehisa et al. 2021) pour 3 souches bactériennes identifiées comme étant

particulièrement prometteuse grâce à l'étendue de leur capacité de prolifération en culture n'utilisant que le mazout à très faible teneur en soufre comme source de carbone sous basse températures, leur potentielle nouveauté basée sur leur séquence 16S, ou si l'espèce n'est pas connue pour pouvoir dégrader les hydrocarbures. L'étendu de la dégradation des fractions d'alkanes et d'hydrocarbures aromatiques de ces souches fût ensuite déterminée par Chromatographie Gazeuse couplée Spectromètre de Masse (GC-MS) Nos résultats démontrent une présence répandue de gênes marqueurs pour la dégradation des hydrocarbures dans de nombreux isolats obtenus depuis des sédiments de plages du Passage du Nord-Ouest, et permirent de reconstruire des voies de dégradation complète dans de nombreux isolats provenant de ces sédiments. De plus, les expériences de croissance et génétique performées suggèrent une habileté répandue des souches bactérienne isolées pour la croissance dans un environnement très froid et à salinité élevée, confirmant leur identité psychrophile et suggérant une habileté pour la dégradation des hydrocarbures dans ces conditions extrêmes de l'arctique. Au niveau des souches individuelles, *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T ont le potentiel pour être utilisées en bioremédiation, et plusieurs autre microbes naturellement présent pourraient aussi offrir une réponse si un déversement d'hydrocarbures devait atteindre les sédiments d'une plage du Passage du Nord-Ouest.

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Contribution of Authors

Chapter 1: Literature Review

Antoine-Olivier Lirette, the MSc candidate, wrote the literature review with guidance and editing provided by Dr. Ya-Jou Chen and Prof. Lyle G. Whyte

Chapter 2: Isolating Novel Hydrocarbon Degraders from Northwest Passage Beach Sediments and Assessing their Ability for Bioremediation

This manuscript was made in collaboration with Dr. Ya-Jou Chen, Dr. Nastasia Freyria, and Prof. Lyle G. Whyte from McGill university. Antoine-Olivier Lirette, The MSc candidate, conducted all laboratory work, data analysis, and wrote the manuscript. Dr. Ya-Jou Chen and Dr. Nastasia Freyria provided assistance with experimental design and data analysis and helped with the analysis software, Esteban Góngora provided assistance with the analysis software and data analysis. Dr. Ya-Jou Chen provided manuscript guidance. Prof. Lyle G. Whyte provided guidance and edited the manuscript.

Chapter 3: Discussion and Conclusion

Antoine-Olivier Lirette, the MSc candidate, wrote the discussion and conclusion with editing and counseling provided by Dr. Ya-Jou Chen and Prof. Lyle G. Whyte.

Introduction

The Northwest Passage (NWP) is a sea route connecting the Pacific and Atlantic oceans through the islands and gulfs of the Canadian High Arctic. Historically, it was widely inaccessible due to sea ice cover blocking access to ships for most of the year. As a result of climate change however, the sea ice cover blocking the NWP recedes further and for longer periods of time every year, to the extent that it is expected to become an economically favourable shipping route by mid-century (Smith & Stephenson 2013; Haas & Howell 2015). However, with an increase in anthropogenic activity, comes an increased risk of ship damage or incident resulting in an oil spill (Góngora et al. 2022, Ellis et al. 2022).

Oil spills have been demonstrated to have disastrous and persistent repercussions on the ecosystem they affect and their wildlife, lasting upwards of three decades in cold environments (Maselko et al. 2018). Hydrocarbons are known for their carcinogenic effects on humans (Mastrangelo et al. 1996), and long-term toxic effects of oil spills and the biproducts of their cleanup are well demonstrated (Beyer et al. 2016). This type of environmental contamination is thus of primary concern for their deleterious ecosystem effects and are important to address for conservation efforts. Due to their chemical properties, hydrocarbons which escape the water column and wash ashore onto marine beach sediments are very difficult to remediate; the rocky sediment of Arctic beaches coupled with their low temperature make them especially vulnerable to long term effects of hydrocarbon spills (Tarr et al. 2016). The inherent challenges of oil spills cleanup in the High Arctic where the NWP is located are exacerbated by the remoteness of the Arctic and its harsh climate (Li et al. 2016); in addition to the persistent nature of hydrocarbons, Arctic conditions force us to look for solutions which are easily deployed, require little manpower and resources, and are active over long periods of time on various hydrocarbons. Bioremediation is a candidate

solution that responds to all of these criteria. However, few studies so far have been conducted to examine the applicability of bioremediation techniques on Arctic beaches (Góngora et al. 2022), most of the literature on this subject coming from two studies: the "Baffin Island Oil Spill" (BIOS) project which occurred in Canada in 1980 (Sergy and Blackall. 1987) and the "Svalbard shoreline field trials" which occurred in Norway in 1997 (Guénette et al. 2003). Fortunately, several cold-adapted bacterial genera such as *Rhodococcus* and *Pseudomonas* include several well characterized species shown to biodegrade both alkanes and Polycyclic Aromatic Hydrocarbons (PAHs) on some terrestrial and marine ecosystems (Whyte et al. 1997, Whyte et al. 1998, Deppe et al. 2005; Ruberto et al. 2005). These findings lead us to believe that the potential for biodegradation of these hydrocarbons may be present in beaches of the NWP

In this study, we aimed to isolate and characterize hydrocarbon degrading bacterial strains from NWP beach sediments and assess their ability to degrade hydrocarbons in low temperature and high salinity. Bacterial strains were isolated from High Arctic beach sediments of the NWP using plates containing ULSFO, a ship fuel, as a sole carbon source. The isolates were identified by 16S rRNA amplicon sequencing, and their ability for hydrocarbon degradation was further investigated using chemical and genetic methods. Our first objective was addressed by a series of growth trials on medium containing various salt concentrations, or under various temperatures. The chemical component of our second objective was addressed by selecting promising isolates from preliminary growth trials and culturing them in minimal liquid medium containing ULSFO as sole carbon source over the course of 3 months. Total Petroleum Hydrocarbon (TPH) was performed on their medium at 3 time points and biomass was collected and frozen for future transcriptomics analysis. Finally, the genetic component of our second objective was addressed by performing whole genome sequencing of the isolates and comparing the resulting genomes to existing

databases in an effort to identify marker hydrocarbon degradation genes from their genomes. Biodegradation pathways for alkane fractions and certain PAHs were also mapped for the three isolated used for TPH analysis. The whole genomes were further screened for the presence of cold and salt tolerance genes in the isolates' genome to confirm their identity as cold or salt tolerant bacteria through genetic means and determine their potential for hydrocarbon degradation under highly saline or cold conditions. Given the results of this study, we hypothesized that bacterial species found on NWP beach sediments possess the potential for bioremediation under cold and saline conditions on NWP beaches in the events of an oil spill.

Chapter1. Literature Review

1.1 Introduction

The Northwest Passage (NWP) is a sea route connecting the Pacific and Atlantic oceans through the islands and gulfs of Canada's High Arctic (Figure 1; Britannica. 2021, Smith & Stephenson. 2013). Historically, it was widely inaccessible due to sea ice cover blocking access to ships for most of the year. As a result of climate change however, the sea ice cover blocking the NWP recedes faster and for longer periods of time every year, to the extent that it is expected to become an economically favourable shipping route by mid-century (Smith & Stephenson 2013; Haas & Howell 2015). The expected increase in anthropogenic activity threatens the NWP's ecosystem due to an increase in transit also increasing the probability of incidents leading to ship fuel spillage. Aftermaths of the Exxon Valdez oil spill which occurred in Prince William Sound, Alaska in 1989 demonstrated the dangers of spills in the Arctic and subArctic area. Nearly 30 years after the spill and widespread deployment of human resources and chemicals for cleaning, about 0.6% of the 10.8M gallons of oil that were spilled are still present in the surrounding beaches (Maselko et al. 2018). A spill in the NWP would represent an even greater challenge since not only the demonstrated persistence of the oil would have to be dealt with, but the remoteness of the region would represent an additional challenge for the deployment of human and material resources on site, which could take weeks to arrive and may experience long delay due to the unpredictable and extreme weather conditions of the High Arctic; all of this greatly increasing the response cost (Li et al. 2016). Moreover, the extreme Arctic environmental conditions pose a challenge of their own by increasing the viscosity of oil which may then become entrapped in the rocky sediments of Arctic beaches. Once the sea ice covers the beaches, these

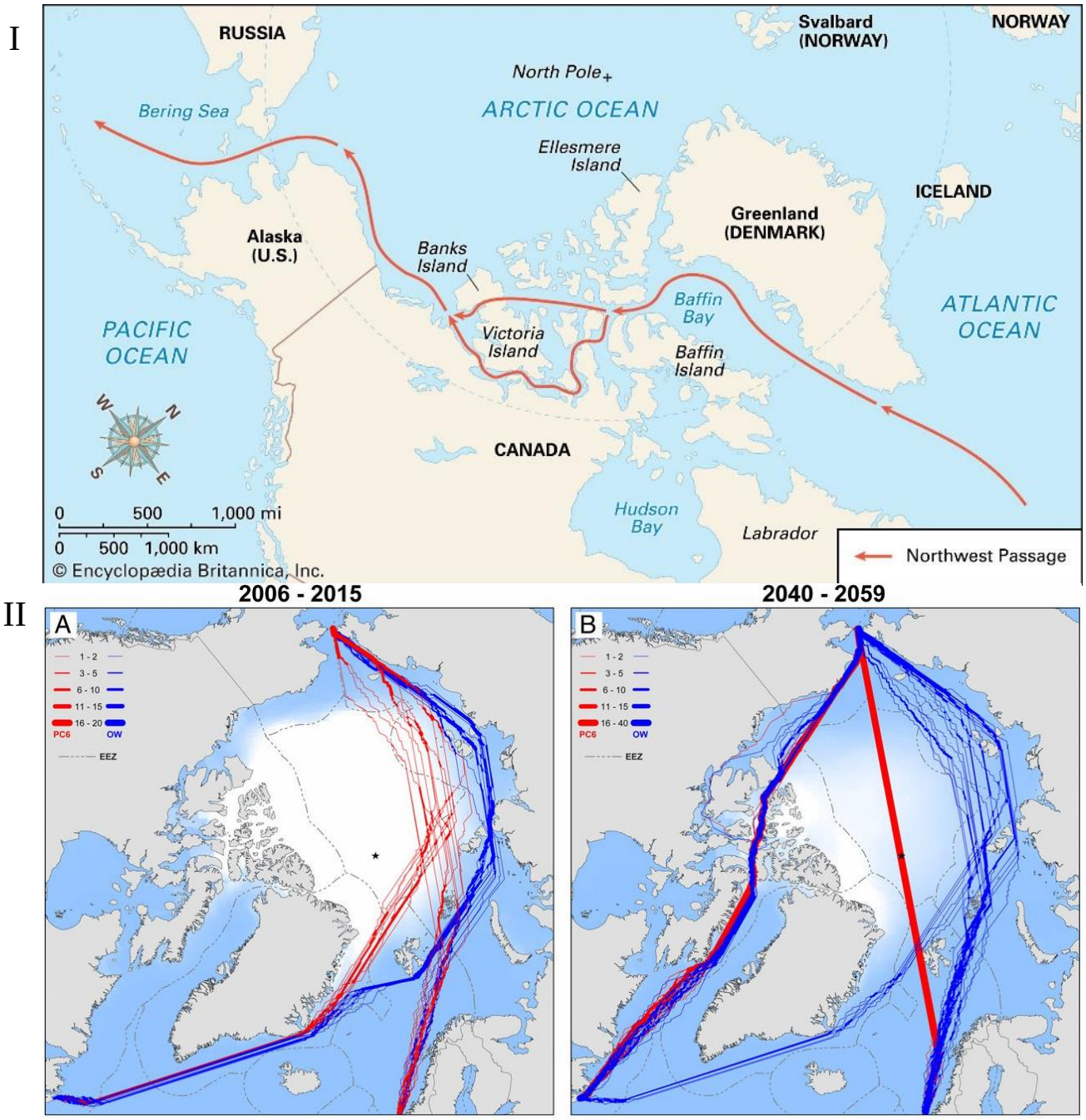

Figure 1. Path and Sea Routes of The Canadian Northwest Passage.

I Map of Canada's Northwest Passage (Encyclopædia Britannica); II September navigation routes as per medium-low climate scenarios on sea ice concentration and thickness, highlighting changes in ship traffic (red and blue lines) through the Northwest Passage by mid century (Smith and Stephenson 2013)

sediments then become virtually inaccessible until the next year's thaw meaning the oil could be entrapped for extended periods of time if not dealt with quickly.

Bioremediation, or using the metabolic ability for hydrocarbon biodegradation of naturally occurring bacteria for remediation of fuel oil (Agamuthu et al. 2013), presents itself as an efficient response (Atlas and Hazen 2011) which may circumvent these issues. Using endemic microbes which are already adapted to the environment offers an advantage in the face of the harsh Arctic conditions as they are well adapted for such conditions. Moreover, the usage of local microbial communities for cleanup avoids the introduction of chemicals or biproducts of *in-situ* burnings (Fingas, 2011, Okoh et al. 2020, Macaulay & Rees 2014; Prince 1997; Tremblay et al. 2017, 2019; Ribicic et al. 2018a). Another advantage is the need for relatively few resources, both human and material, required for implementation (Okoh et al. 2020).

Unfortunately, biodegradative microbial communities inhabiting the High Arctic beaches remain largely uninvestigated and their ability to degrade hydrocarbons must be assessed before an efficient response to oil spills can be deployed. In oceans, bacteria have demonstrated their ability to degrade fuels, with the help of environmental factors such as waves and currents which emulsify the fuel and increases its availability to microbes for degradation, photooxidation and evaporation which cause the loss of low molecular weight and volatile compounds, and sunlight which degrades the fuel (Nunziata et al. 2018;Tarr et al. 2016; Wang et al. 2020; Bacosa et al. 2015); however, once the spilled hydrocarbons reach the shore many of these factors become unavailable to assist in degradation and the environmental characteristics of the beaches may even complicate degradation further. Once the hydrocarbons reach the shores, they may stick to the sediments present and becomes much more difficult to remove. This is especially true in Arctic regions where the low temperature causes the oil to solidify forming a viscous, nearly solid, mass which can mix with and become trapped within the sediments of the beaches. Once entrapped, the hydrocarbons also risk becoming buried, making it even more arduous to physically remove or wash off (Tarr et

al. 2016). In the face of these challenges, bioremediation offers a possible solution. However, since bacterial communities of High Arctic beaches have been neglected by previous studies on bioremediation and possess unique compositions and characteristics to the communities of oceanic or terrestrial Arctic communities, these differences must be explored for appropriate oil spills responses. Only few studies have been conducted to examine the applicability of bioremediation techniques in Arctic beach environments (Góngora et al. 2022, Ellis et al. 2022) but research about marine bacteria capable of hydrocarbon biodegradation have been conducted in the waters of the NWP (Garneau et al. 2016, Greer et al. 2014) which are relevant to this study as the same waters wash on our surveyed beaches and thus bacterial strains isolated from the sediments might be taxonomically related to those found in the marine environment with similar biodegradative properties. This study aims at filling some of the knowledge gaps currently present in the literature.

1.2 Abiotic factors affecting biodegradation on Arctic beaches and cold environments

1.2.1 Temperature

A great challenge to the biodegradation of hydrocarbons is temperature; Arctic weather conditions are among the harshest in the world, with temperatures below freezing for most of the year, low water availability, and long periods of constant exposure to UV radiation from sunlight or total darkness. Despite being well adapted to the cold conditions and maintaining a certain level of metabolic activity below freezing (Margesin & Schinner. 2001), Arctic microorganisms have optimal growth temperatures generally much higher than 0°C (Morita, 1975). Lower temperatures are also inherently associated with decreased rates of hydrocarbon biodegradation due to a decreased rate of enzymatic activity (Atlas. 1991; Sharma & Schiewer. 2016). The presence of sea

ice covering beaches for most days of the year in the Canadian high Arctic (Prince et al. 2002) also contributes to the challenging nature of hydrocarbon biodegradation by delaying the thawing of snow, ice, and subsurface frost-table, processes which requires surface ice cover to recede allowing sea water infiltration before beginning, and on which microbial metabolic activity depends (Owens et al. 1977). Moreover, temperature not only affects microbial activity, but also the chemical properties of the oil. At lower temperature, oil becomes increasingly viscous and decreasingly volatile for short chain alkanes (Atlas. 1991). Studies of cold temperatures on oil tend to demonstrate that their increased viscosity under low temperatures decreases their biological availability which might presents itself a greater challenge to biodegradation than the reduced metabolic activity it also induces (Nordam et al. 2020).

1.2.2 Nutrients availability and salinity

Much of the high Arctic consists of nutrient poor waters and sediments (Wells & Deming, 2003) for which microorganisms have evolved an oligotrophic lifestyle. Although global warming and thawing of permafrost is expected to increase nutrient availability such as carbon in the near future (Zhang et al. 2021), nutrient availability remains a major limiting factor to the rate of metabolic activity of Arctic microbial communities, especially in the case of nitrogen and phosphorus (Prince 1993, Boufadel et al. 2010). The metabolism of ship fuels requires the intake from the environment of mineral nutrient, namely nitrogen, phosphorus, and iron (Atlas, 1988) to degrade the large amount of carbon introduced by the fuel. However, the addition of the carbon rich ship fuel to the environment does not increase the availability of these other nutrients, making them a limiting factor to the rate of degradation. Furthermore, oxygen is required for the aerobic biodegradation of hydrocarbons via the use of oxygenases or as a terminal electron acceptor for aerobic metabolism (Van Hamme et al. 2003). However, oxygen was found be limited in sub-surface

Arctic sediments as observed following the *Exxon Valdez* spill (Boufadel et al. 2010), suggesting hydrocarbons that reach these depths might require anaerobic metabolism for degradation. Salinity also seems to play a role in the biodegradation of hydrocarbons; it was observed that Arctic shoreline sediments with high salinity were more successful in their removal than those with lower salinities (Sharma & Schiewer. 2016)

1.2.3 Properties of ship fuels used in Arctic Sea routes

Ship fuels such as ULSFO, Bunker C, or marine diesel consist of several different hydrocarbons, the majority of which are alkanes and naturally occur in various forms such as linear, branched, or cyclic, and a small quantity of PAHs which are composed of more than one benzene rings fused together (Abrajano Jr. et al. 2007; Cerniglia 1993). Due to their simplicity, alkanes are generally degraded quickly by hydrocarbon degraders, with the exception of circular alkanes which may be degraded more slowly than low molecular weight PAHs (Perry 1984). Although they represent the minority in the composition of hydrocarbons, PAHs are particularly persistent and difficult to degrade, and are especially toxic thus being of special concern and posing a challenge in remediation (Cerniglia 1993; Abrajano Jr. et al. 2007; Chandra et al. 2013). After entering the marine environment, several weathering processes immediately begin altering the chemical composition of the oil; these processes include photooxidation and evaporation which rid most of the low molecular weight, volatile compounds, often short chain alkanes, leaving the more viscous residuals behind (Tarr et al. 2016, Wang et al. 2020). Emulsification of the oil by waves and other physical forces increase its bioavailability for local oceanic microorganisms capable to metabolize their components (Nunziata et al. 2018). Finally, sunlight and the presence of oxygen modifies the oil further through photo-oxidation (Albaigés et al. 2016). However, physical processes such as the mixing of oil with sediments or detritus leading to the formation of sinking aggregates that

may become trapped on the seabed or beach may reduce natural attenuation from these processes (Warnock et al. 2015, Tarr et al. 2016). Barrier to these weathering processes such as the absence of sunlight during the winter season, or the presence of sea ice covering the surface oil should also be considered when evaluating the impact of those weathering processes.

1.3 Methods of hydrocarbon remediation in Arctic conditions

1.3.1 Assessing oil biodegradation using chemical methods

Assessing oil biodegradation requires measuring the extent of removal and transformation of the hydrocarbon components of the ship fuel. This removal and those transformation consist of steps in the metabolism of specific hydrocarbons. An approach to quantifying oil biodegradation is through the concentration of TPH, a method which uses gas chromatography and mass spectrometry to distinguish the grouping of chemical compounds of a substance, in this case several aliphatic and aromatic hydrocarbons (Reddy & Quinn. 1999, Prince & Douglas. 2005). This type of analysis provides information about the change in chemical composition of the medium, which is informative to assess biodegradation, and helps in recreating metabolic pathways by providing information regarding the fate of specific compounds (Balba et al. 1998). Their usefulness is made even greater when coupled with genetic or transcriptomic data.

1.3.2 Genetic approach to assessing potential for oil biodegradation in Arctic beach isolates

Modern sequencing technologies and analysis software allow for efficient data gathering and processing when assessing the potential for hydrocarbon degradation of communities, or of specific organisms therein. Whole genomes sequencing allows for the screening of marker genes involved in the biodegradation of alkane and aromatic hydrocarbons. These genes, which are either already experimentally characterized or putative, are stored into databases which can be queried by matching their genetic sequence to that of the genome we are analyzing. Among the most common alkane degradation genes, *alkB* and *CYP153* have been identified in 369 and 87 genomes respectively in aerobic terrestrial, marine, and freshwater environments (Nie et al. 2014). Genes *masD* and *assA* have also been identified for their involvement in the anaerobic degradation of alkanes in cold sediments (Gittel et al. 2015). Besides alaknes, genes involved in the aerobic degradation of PAHs such as *ndoB,* involved in the degradation of naphthalene, have also been previously identified in cold adapted microorganisms isolated from AntArctic soils (Panicker et al. 2010). Anaerobic degradation of PAHs has also been observed in both ground water and sediment samples with gene *ncr*, another naphthalene biodegradation gene (Morris et al. 2014). Recent hydrocarbon biodegradation studies have often focused on determining community structure and assessing communities' potential for hydrocarbon degradation through the use of metadata and marker gene sequencing (Bidja Abena et al. 2020). Through metagenomics, function can be determined based on the presence of metabolic genes, and community taxonomy can be elucidated, and metatranscriptomics allows for the determination of activity in the community by observing gene expression at a single point in time (Narasimhan et al. 2016; Tremblay et al. 2019). An issue however in the identification of these marker genes is that several known hydrocarbon

degradation genes share sequence similarity with genes from other metabolic pathways and are thus often missed on non-specialized assays (Callaghan et al. 2008, Khelifi et al. 2014). To circumvent this issue, a team in Calgary, Canada, identified 37 marker genes of hydrocarbon bioremediation (Table 1) and developed a specialized software capable of detecting their presence from whole genome sequences (Khot et al. 2021). The Calgary approach to ANnoTating HYDrocarbon (CANT-HYD) degradation genes allows for efficient characterization of microbial communities or isolates by searching their genomes for the presence of the identified 37 key hydrocarbon degradation genes with better accuracy than previous, non-specialized, processing tools (Khot et al. 2021). Genes targeted by CANT-HYD such as *alkB* and *ndoB* contain the amino sequence encoding the catalytic subunit of enzymes involved in hydrocarbon degradation and their presence often correlates with the presence of the full metabolic pathway for their respective substrate (Khot et al. 2021) further increasing the usefulness of this tool.

1.4 Existing literature on Arctic hydrocarbon degradation on Arctic beaches

1.4.1 Bioremediation as a solution to the challenges of hydrocarbon remediation in the Arctic

Bioremediation is the employment of supplementary processes to enhance or facilitate the natural metabolic processes found in the microorganisms present in the environment to degrade introduced pollutants (Atlas and Cerniglia 1995). These processes include biostimulation in which nutrients or oxygen are supplied to the system to help the microbes present grow (Leahy & Colwell 1990, Lindstrom et al. 1991, Mrozik & Piotrowska-Seget. 2010), or bioaugmentation in which the local community is enriched with a hydrocarbon degrading microbial consortium to enhance its

Table1. Key Hydrocarbon Degradation Genes used for Identification (adapted from Khot et al. 2021)

Genes	Respiration	Hydrocarbon Group	Enzyme	Substrate
alkB	aerobic	alkane	AlkB-type alkane hydroxylase	C_5-C_{13} Alkane
almA_GroupI			Flavin-binding monooxygenase	C_{20}-C_{32} Alkane
almA_GroupIII			Flavin-binding monooxygenase	C_{20}-C_{32} Alkane
cyp153			Cytochrome P450 alkane hydroxylase	C_5-C_{13} Alkane
ladA_alpha			Long-chain alkane monooxygenase	C_{15}-C_{36} Alkane
ladA_beta			Long-chain alkane monooxygenase	C_{15}-C_{36} Alkane
ladB			Long-chain alkane monooxygenase	C_{15}-C_{36} Alkane
prmA			Propane 2-monoxygenase	Propane
prmC			Propane 2-monoxygenase	Propane
bmoC			butane monooxygenase (particulate)	Butane
bmoA			butane monooxygenase (particulate)	Butane
bmoB			butane monooxygenase (particulate)	Butane
bmoX			butane monooxygenase (soluble) complex	Butane
bmoY			butane monooxygenase (soluble) complex	Butane
bmoZ			butane monooxygenase (soluble) complex	Butane
dmpO		aromatic	Phenol hydroxylase	Phenol
dszC (soxC)			Dibenzothiophene desulfurization enzyme C	Dibenzothiophene
bphA/tcbA/ipbA/bnzA			Monoaromatic dioxygenase alpha subunit	Monoaromatics(Benzene, Toluene, Isopropyl Benzene)/Biphenyl
tcbAb/todC2/bphAb			Monoaromatic dioxygenase beta subunit	Monoaromatics(Benzene, Toluene, Isopropyl Benzene)/Biphenyl
ndoB			Naphthalene 1,2-dioxygenase alpha	Polyaromatics(Naphthalene, Phenanthrene)
ndoC			Naphthalene 1,2-dioxygenase beta subunit	Polyaromatics(Naphthalene, Phenanthrene)
non ndoB type naphthalene dioxygenase alpha			Naphthalene 1,2-dioxygenase alpha	Naphthalene/Polyaromatics
tmoA/bmoA			Toluene 4-monooxygenase	Toluene/Benzene
tmoB/bmoB			Toluene 4-monooxygenase	Toluene/Benzene
tmoE			Toluene 4-monooxygenase	Toluene
tomA1			Toluene 2-monooxygenase/Phenol Hydroxylase	Toluene
tomA3			Toluene 2-monooxygenase/Phenol Hydroxylase	Toluene
tomA4			Toluene 2-monooxygenase/Phenol Hydroxylase	Toluene
ahyA	anaerobic	alkane	Alkane C2 methylene hydroxylase (putative)	Alkane
masD/assA			1-methylalkyl (alkyl) succinate synthase	Alkane
abcA1		aromatic	Benzene carboxylase	Benzene
abcA2			Benzene carboxylase	Benzene
bssA/ibsA			Benzyl/(4-Isopropylbenzyl) succinate synthase	Benzene/Isopropyl Benzene
cmdA			p-cymene dehydrogenase	p-Cymene
ebdA			Ethylbenzene dehydrogenase	EthylBenzene
K27540			Naphthalene carboxylase	Naphthalene
nmsA			Naphthyl 2-methylsuccinate synthase	Naphthalene

hydrocarbon degrading potential (Tyagi et al. 2011). The method has demonstrated its effectiveness in prior studies where microorganisms successfully metabolized various hydrocarbon compounds from oil (Atlas and Hazen 2011). This process works thanks to a variety of genes found across microbial species which encodes enzymes able to degrade various hydrocarbon compounds found in oil (Van Hamme et al. 2003). Despite a vast literature on hydrocarbon biodegradation, most of the research efforts were concentrated in warmer regions of the globe, where the current ship routes exist, and which possess different microbial communities than the Arctic (Ribicic et al. 2018b) and thus these communities remain largely unexplored. Fortunately, hydrocarbon degradation genes are found across 175 prokaryotic genera (Hazen et al. 2016) many of which are found in Arctic regions, offering a prospective solution to oil spills. Several of those genera found in the Arctic are known to possess genes capable of degrading a wide array of PAHs and are thus being of special interest in bioremediation (Dong et al. 2015). Furthermore, several cold-adapted bacteria such as *Rhodococcus* and *Pseudomonas* species have already demonstrated an ability to degrade both alkanes and PAHs on some terrestrial and oceanic ecosystems (Deppe et al. 2005; Ruberto et al. 2005). *Rhodococcus* species are of special interest to us due to the result of recent studies (Ellis et al. 2022, Góngora et al. unpublished) by our research team which discovered many members of this genera on Arctic shorelines. These findings lead us to believe that the potential for biodegradation of these compounds may be present on Arctic beaches of the NWP.

Microbial communities of the High Arctic are known to have a slow metabolism due to their environment's extreme conditions; these include extreme colds, changing temperatures, transient presence of ice, and a nutrient-limited environment (Atlas 1988). Thus, not only must the presence of hydrocarbon degradation genes be demonstrated, but the metabolic activity of these

communities must be at a level which is substantial enough for bioremediation to be implemented as a cleaning strategy in the events of a spill. Most knowledge of biodegradation on high Arctic beaches comes from "the Baffin Island Oil Spill" (BIOS) which occurred in Canada in 1980 (Sergy and Blackall. 1987) and the "Svalbard shoreline field trials" which occurred in Norway in 1997 (Guénette et al. 2003). The BIOS trials mostly focused on evaluating the fate and effects of a medium gravity oil spill washing on an Arctic shoreline environment, and evaluate cleanup strategies such as burning, mixing, chemical surfactant, solidifying agents (Owens et al. 1987), and flushing, and the use of nutrients (Eimjhellen et al. 1982). Results of those trials demonstrated the vulnerability of high Arctic shorelines to long term effects of oil spills (Owens et al. 1987; Prince et al. 2002). The remediation strategies tested at Svalbard included bioremediation, mixing and sediment relocation (Guénette et al. 2003) and highlighted the effects of nutrient-fertilizer on the rate of degradation on Arctic shorelines (Sergy et al. 2003) and increase in biomass (Prince et al. 2003). However, the results on microbial communities were limited due to their low resolution and were spatially limited to beaches of Norway which might not reflect the reality of the Canadian NWP.

1.4.2 Culture-based approachs used to study hydrocarbon degrading bacteria

With modern advancements in molecular technologies, recent studies have often focused on the use of metadata, microbial diversity characterization, and have assessed the potential for biodegradation through marker-gene sequencing (Bidja Abena et al. 2020). However, for the purpose of this study we were interested in a hands-on approach by isolating specific members of the community and observing their ability to metabolise hydrocarbons directly rather than assessing the metabolic potential of a community through genetics means alone. Culture-based approaches were successful in isolating hydrocarbon degrading microorganisms and assessing

these isolate's ability to degrade PAHs and alkanes (Crisafi et al. 2016, Whyte et al. 1998) in the past and were used in studies of both marine and terrestrial (Michaud et al. 2004, Bej et al, 2000) ecosystems, highlighting their versatility. Moreover, this approach was successful in investigating soil microbes' ability to degrade hydrocarbons in microcosms studies of whole communities, but also of individual bacterium in prior studies (Eriksson et al. 2001, Whyte et al. 1997) demonstrating further their broad range of utility which contributed to the selection of this method. Several cold-adapted bacterial genera ubiquitous to the Arctic have been identified in laboratory studies as possessing hydrocarbon degrading abilities; namely *Loktanella, Sulfitobacter, Sphingopyxis, Sphingomonas, Alteromonas, Glaciecola, Marinobacter, Colwellia, Thalassomonas, Moritella, Algicola, Pseudoalteromonas, Psychromonas, Shewanella, Alcanivorax, Marinomonas, Oleispira, Halomonas, Psychrobacter, Pseudomonas, Cycloclasticus, Arcobacter, Cytophagia, Ulvibacter, Polaribacter, Rhodococcus, Agreia and Arthrobacter* (Table 2; Brakstad et al. 2017). Most of these genera are members of the Proteobacteria phylum which consist of Gram-negative organisms divided in 3 classes: Alphaproteobacteria, Gammaproteobacteria, and Epsilonproteobacteria (Prince et al. 2018). Proteobacteria are well known for their ability to degrade a broad range of hydrocarbon substrates such as alkanes, phenanthrene, dibenzofuran, crude oil, gas oil, biphenyl, and PAHs (Prince et al. 2018). Many of the listed genera also belong to the phylum Actinobacteria, a phylum of Gram-positive microbes which has demonstrated an ability for biodegradation of various hydrocarbon substrates such as n-alkanes, phenol, anthracene, and pyrene under cold temperatures (Margesin et al. 2013). Genera *Rhodococcus*, *Sphingomonas,* and *Pseudomonas* were prevalent in Arctic soils previously contaminated with hydrocarbons (Aislabie et al. 2006) and their ability to degrade various chain length alkanes, aromatics, and PAHs at low temperatures is

Table2. Taxonomic Classification of Polar oil-degrading bacteria (adapted from Brakstad et al. 2017)

Class	Family	Genus	Source[a]	Degraded Hydrocabon	References[b]
Alphaproteobacteria	Rhodobacteraceae	Loktanella	Ar, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1
		Sulfitobacter	Ar, SW	Aliphatics, Aromatics, low molecular weight PAHs	2
	Sphingomonadaceae	Sphingopyxis	Ar, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1
		Sphingomonas	An, SW	Aliphatics, Aromatics, PAHs	3
Gammaproteobacteria	Alteromonadaceae	Alteromonas	SW	Alkanes, low molecular weight PAHs	4
		Glaciecola	Ar, SI	Alkanes, low molecular weight PAHs	5
		Marinobacter	An, Ar, SI, SW	Aliphatics, Aromatics, low molecular weight PAHs	3, 6, 7
	Colwelliaceae	Colwellia	An, Ar, S, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 2, 3, 4, 4, 5, 8, 9, 10
		Thalassomonas	SW	Alkanes, low molecular weight PAHs	4
	Moritellaceae	Moritella	Ar, S, SI, SW	Aliphatics, Aromatics, low molecular weight PAHs	2, 8, 9
	Pseudoalteromonadaceae	Algicola	Ar, SI	Aliphatics, Aromatics, low molecular weight PAHs	2
		Pseudoalteromonas	An, Ar, S, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 5, 6, 8, 9, 10, 11
	Psychromonadaceae	Psychromonas	Ar, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 11
	Shewanellaceae	Shewanella	An, Ar, S, SI, SW	Alkanes, Aromatics, low and high molecular weight PAHs	3, 4, 6, 7, 9, 10
	Alcanivoracaceae	Alcanivorax	Ar, S, SW	Aliphatics, Aromatics, low molecular weight PAHs	2, 10
	Oceanospirillaceae	Marinomonas	An, Ar, S, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs	3, 5, 10
		Oleispira	Ar, An, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 2, 3, 4, 5, 11
	Halomonadaceae	Halomonas	An, Ar, S, SI SW	Aliphatics, Aromatics, low and high molecular weight PAHs	3, 7, 10
	Moraxellaceae	Psychrobacter	Ar, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 6
	Pseudomonadaceae	Pseudomonas	An, Ar, S, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs	3, 6, 7, 10
	Piscirickettsiaceae	Cycloclasticus	Ar, S, SW	Alkanes, low and high molecular weight PAHs	4, 10
Epsilonproteobacteria	Campylobacteraceae	Arcobacter	An, Ar, SW	Aliphatics, Aromatics, low molecular weight PAHs	3, 8, 11
Bacteroidetes	Cytophagales	Cytophagia	An, SW	Aliphatics, Aromatics, low molecular weight PAHs	3, 11
Flavobacteriia	Flavobacteriaceae	Ulvibacter	Ar, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1
		Polaribacter	Ar, SI, SW	Aliphatics, Aromatics, low and high molecular weight PAHs up to chrysene	1, 7, 8
Actinobacteria	Nocardiaceae	Rhodococcus	An, SW	Aliphatics, Aromatics	3, 12
	Microbacteriaceae	Agreia	Ar, SI, SW	Aliphatics, Aromatics, PAHs	6, 7
	Micrococcaceae	Arthrobacter	An, SW	Aliphatics, Aromatics	12, 13, 14

[a]An AntArctic, Ar Arctic, S sediment, SI sea ice, SW seawater

[b]1, McFarlin et al. (2014); 2, Garneau et al. (2016); 3, Yakimov et al. (2004); 4, Lofthus et al. (2015); 5, Brakstad et al. (2008); 6, Deppe et al. (2005); 7, Gerdes et al. (2005); 8, Bagi et al. (2014); 9, Bowman and McCuaig (2003); 10, Dong et al. (2015); 11, Brakstad and Bonaunet (2006); 12, Michaud et al. (2004); 13, Abdulrasheed et al. (2020); 14, Lee et al. 2018

well established (Whyte et al. 1997, Whyte et al. 1998, Baraniecki et al. 2002). Isolates of these genera were successfully obtained from Arctic soil samples and subsequently cultivated on plates in the laboratory, and their abilities to degrade a wide range of hydrocarbons such as hexadecane, toluene, or naphthalene was successfully assessed *ex-situ* (Saul et al. 2005). There are however limitations and challenges to this approach; only a minority of microorganisms found in environmental samples are successfully cultured by following standard laboratory techniques (Amann et al. 1995) Which can be explained by several factors such as absence of nutrients, temperature, pH, presence of other organisms, and many more (Vartoukian et al. 2010). The ability of bacteria to utilize and degrade hydrocarbons is also highly dependent on environmental conditions such as temperature, pH, salinity, and presence of nitrogen (Habib et al. 2018). Furthermore, certain bacterial communities are known to work cooperatively to degrade hydrocarbons fully with each members possessing part of the full degradative pathway (Guo et al. 2017), meaning that isolates involved in the process might not show any degradation on their own. Thus, it is a challenge to find a suitable condition to grow these microorganisms and to test their degradation ability at low temperatures.

1.4.3 Research objectives

In this study, I aimed to isolate and characterize hydrocarbon degrading bacterial strains from NWP beach sediments and assess their ability to degrade hydrocarbons in low temperature and high salinities. Bacterial strains were isolated from High Arctic beach sediments of the NWP using plates containing hydrocarbons as a sole carbon source. The isolates were identified through 16S rRNA amplicon sequencing, and their abilities for hydrocarbon degradation was investigated using chemical and genetic methods.

-The first goal was addressed by a series of growth trials on medium containing various salt concentrations ranging from 0% v/v to 10% v/v sea salt, or in temperatures ranging from 37 ℃, to -5 ℃.

-The second objective was addressed by selecting promising isolates from a preliminary growth test and culturing them in minimal liquid medium containing ULSFO, a ship fuel, as sole carbon source over the course of 3 months. TPH analysis was performed on the growth medium of these three strains, which contained the fuel, at 3 time points and biomass was collected and kept frozen in RNA shield for future transcriptomics analysis.

-Finally, the third objective was addressed by performing whole genome sequencing of the isolates and comparing the resulting genomes to existing databases to locate marker hydrocarbon degradation genes in their genomes. Biodegradative pathways for alkanes and certain PAHs were also mapped for the three isolated used in TPH analysis. The whole genomes were further screened for the presence of cold and salt tolerance genes in the isolates' genome to confirm their identity as cold or salt tolerant bacteria, and to establish their potential for hydrocarbon degradation under highly saline or cold conditions.

1.4.4 Contribution of this study to bridging the knowledge gap of bioremediation on Arctic beaches

To bridge the knowledge gap regarding hydrocarbon degradation capabilities of microbial communities on Arctic beach sediments, we focused on the isolation and characterization of bacteria from NWP beach sediments treated with ULSFO and fertilizers. The main goal of this study was to isolate bacterial strains from NWP beach sediments, and investigate their potential for hydrocarbon biodegradation under Arctic conditions. This was achieved through the use of

using growth on selective medium plates, whole genome sequencing (WGS), and TPH analysis. The findings of this project can provide insight on the abilities of microbial communities of NWP beaches to respond to ship fuel spillage and be used in bioremediation or natural attenuation. Furthermore, the results of WGS and TPH analysis deepen our understanding of the biodegradation of Arctic beach communities of the NWP by detecting the presence of degradation genes and pathways in bacteria of those beaches, and by providing evidence of the degradation of the hydrocarbons found in ship fuels.

Connecting text

The research objectives of isolating novel hydrocarbon degrading bacteria from NWP beach sediments and characterizing their ability and potential for hydrocarbon degradation through chemical and genetic methods were addressed within one manuscript. This manuscript titled "Isolating Novel Hydrocarbon Degraders from NWP Beach Sediments and Assessing their Ability for Bioremediation" is presented in *chapter 2* of this thesis and will be submitted following the deposition of this thesis.

Chapter 2: Isolating Novel Hydrocarbon Degraders from Northwest Passage Beach Sediments and Assessing their Ability for Bioremediation.

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Abstract

Due to the effect of global climate change, sea ice cover in the Arctic recedes further and for longer periods of time every year, opening the way to increased anthropogenic activity. The Northwest Passage is of special interest as it is expected to become an important sea route connecting the Pacific and Atlantic oceans through the islands and gulfs of northern Canada in the near future. With an increase in ship transit comes an increased risk of accidental spillage of ship fuels. Hydrocarbons, and especially their aromatic compounds, are of environmental concern due to their well-known toxic and carcinogenic properties. Once they leave the water column, these fuels are known to be particularly difficult to remove from Arctic beaches where they wash off due to their high viscosity, and the composition of Arctic beach sediments. One efficient method used to clean beaches of washed-up hydrocarbons is bioremediation, or the use of naturally occurring bacteria to degrade oil. In this study we isolated microbial strains from Northwest Passage beach sediments and determined their potential for degradation of various hydrocarbons through genetic and chemical methods. Moreover, the isolates' ability to grow under a wide range of temperatures and salinities common to the Arctic was assessed, while also searching their genomes for the presence of cold and salt tolerance genes. Hydrocarbon degradative pathways were recreated using KEGG

database (Kanehisa et al. 2021) in 3 strains selected for their successful growth on Ultra Low Sulfur Fuel Oil (ULSFO) as sole carbon source under low temperature, their potential novelty based on 16S rRNA amplicon sequencing, or their unknown status as hydrocarbon degraders. The extent of degradation of alkane and aromatics fractions of these strains was then assessed using Gas-Chromatography and Mass-Spectrometry. Our results show the widespread presence of marker hydrocarbon degradation genes in several microbial isolates from Northwest Passage beach sediments, and successfully mapped complete degradation pathway for alkanes and naphthalene in several of those. Furthermore, the growth and genetic assays performed suggest a wide range of temperature and salt tolerance in the isolates, confirming their identity as psychrophiles and suggesting an ability for bioremediation under a wide range of extreme Arctic conditions. At the individual strain level, our results suggest that both *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T have potential in bioremediation in the events of an oil spill reaching the shores of the Northwest Passage, and that many more naturally occurring microbes could offer a potential response if such a spill was to reach the sediments of Northwest Passage beaches

2.1 Introduction

The Northwest Passage (NWP) is a sea route connecting the Pacific and Atlantic oceans through the islands and gulfs of the Canadian High Arctic. Historically, it was widely inaccessible due to sea ice cover blocking access to ships for most of the year. As a result of climate change however, the sea ice cover blocking the NWP recedes further and for longer periods of time every year, to the extent that it is expected to become an economically favourable shipping route by mid-century (Smith & Stephenson 2013; Haas & Howell 2015). With an increase in anthropogenic activity,

comes an increased risk of ship damage or incident resulting in an oil spill (Góngora et al. 2022, Ellis et al. 2022).

Oil spills have been demonstrated to have disastrous and persistent repercussions on the ecosystem they affect and their wildlife, lasting upwards of three decades in cold environments (Maselko et al. 2018). Hydrocarbons are known for their carcinogenic effects on humans (Mastrangelo et al. 1996), and long-term toxic effects of oil spills and the biproducts of their cleanup are well demonstrated (Beyer et al. 2016). This type of environmental contamination is thus of primary concern for their deleterious ecosystem effects and are important to address for conservation efforts. Due to their chemical properties, hydrocarbons which escape the water column and wash ashore onto marine beach sediments are very difficult to remediate; the rocky sediment of Arctic beaches coupled with their low temperature make them especially vulnerable to long term effects of hydrocarbon spills (Tarr et al. 2016). The inherent challenges of oil spills cleanup in the High Arctic where the NWP is located are made exacerbated by the remoteness of the Arctic and its harsh climate (Li et al. 2016); in addition to the persistent nature of hydrocarbons, Arctic conditions force us to look for solutions which are easily deployed, require little manpower and resources, and are active over long periods of time on various hydrocarbons. Bioremediation is a candidate solution that responds to all of these criteria. However, few studies so far have been conducted to examine the applicability of bioremediation techniques on high Arctic beaches (Góngora et al. 2022), most of the literature on this subject derived from two studies: the “Baffin Island Oil Spill” (BIOS) project which occurred in Canada in 1980 (Sergy and Blackall. 1987) and the “Svalbard shoreline field trials” which occurred in Norway in 1997 (Guénette et al. 2003). Fortunately, several cold-adapted bacterial genera such as *Rhodococcus* and *Pseudomonas* include several well characterized species shown to biodegrade both alkanes and Polycyclic Aromatic

Hydrocarbons (PAHs) on some terrestrial and oceanic ecosystems (Whyte et al. 1997, Whyte et al. 1998, Deppe et al. 2005; Ruberto et al. 2005). These findings lead us to believe that the potential for biodegradation of these hydrocarbons may be present in beaches of the NWP

In this study, we isolated and characterized hydrocarbon degrading bacterial strains from NWP beach sediments and assessed their ability to degrade hydrocarbons in low temperature and high salinity. Isolation was performed using plates containing hydrocarbons as a sole carbon source. The isolates were identified by 16S rRNA amplicon sequencing, and their ability for hydrocarbon degradation was further investigated using chemical and genetic methods. Growth abilities of the isolates were assessed a series of trials performed on medium containing various salt concentrations, or under various temperatures. Chemical analysis to determine fuel mineralisation was addressed by selecting promising isolates from a preliminary growth test and culturing them in minimal liquid medium containing Ultra Low Sulfur Fuel Oil (ULSFO) as sole carbon source over the course of 3 months. Total Petroleum Hydrocarbon (TPH) was performed on their medium at 3 time points and biomass was collected and frozen for future transcriptomics analysis. Finally, genomics analysis was performed on the isolates. Whole genome sequencing was performed on all isolates and the resulting genomes were compared existing databases in an effort to identify marker hydrocarbon degradation genes. Biodegradation pathways for alkane fractions and certain PAHs were also mapped for the three isolated used for TPH analysis. The whole genomes were further screened for the presence of cold and salt tolerance genes in the isolates' genome to further assess their potential hydrocarbon biodegradation in cold and saline conditions. Given the results of this study, we hypothesized that bacterial species found on NWP beach sediments possess the ability for hydrocarbon biodegradation under cold and saline conditions in the events of an oil spill.

2.2 Materials & Methods

2.2.1 Site description and sample collection

Resolute bay (74.6973° N, 94.8297° W) is an Inuit community located on the Island of Cornwallis in Nunavut, Canada, and is located within the NWP. Its elevation is of 67.7m and its yearly temperature average is of -15.7°C with precipitations averaging 59.5mm yearly for rain, and 111.2mm for snow (Environment and Climate Change Canada). Average temperature in August ranges from 4.2 to -0.3°C, with an average rainfall of 23.2mm and snowfall of 10.9mm (Environment and Climate Change Canada). Environmental parameters were measured using the ProQuatro handheld multiparameter meter (YSI) (fyi:https://www.ysi.com/proquatro) on site during the field season of 2021. Water samples were also collected during the 2021 field season and analyzed using CHEMetrics instrumental kit (K-6913, K-8513, and K-9203).

Sediment samples were obtained during the 2019 field season from Tupirvik beach (74.74542° N, 95.03954° W) near Resolute Bay (Cornwallis Island), Nunavut, Canada. (Figure 2.). The sediment samples were kept in a cooler immediately and stored at -20°C until their transported back to Montreal for following experiments, where they were stored at -20°C. The sediment samples were used in a column experiment study (unpublished data) prior to this study. Briefly, 600 g of sediments were added into each column with 6000 ppm of ULSFO (Shell Trading Canada, 002D4509) to simulate oil contamination on an Arctic beach. Detailed information on the ULSFO can be found from the Shell website (https://www.shell.com/business-customers/marine/fuel/marine157safety-data-sheets.html). ULSFO contains saturated, aromatic, and olefinic hydrocarbons in the C9 to C50 range, and PHCs mostly containing 3 rings, but going up to 6 (Shell Trading Canada, 002D4509).

Figure 2. Location of Resolute Bay on the Northwest passage.

Resolute Bay is the town located near Tupirvik beach; the site at which sample collection occurred during the summer field season of 2019 (Google, n.d.).

Inorganic and oleophilic fertilizers were used to investigate their effects on the biodegradation pathway of hydrocarbons. Inorganic fertilizer, NH_4PO_4 (monoammonium phosphate, MAP; Sigma), was shown to facilitate the biodegradation process of oil on Arctic soils (Bell et al. 2013). S-200 OilGone (International Environmental Products, llc) is an oleophilic nitrogen-phosphorus nutrient and has been applied on some of the main oil spills such as the Prestige oil spill and the Exxon Valdez spill (Jiménez et al. 2006; Pritchard et al. 1992). Approximately 0.15 g of MAP and

4 ml of S-200 OilGone were added in each treated column. The concentration of two fertilizers were determined based on the previous studies (Bell et al. 2013; Jiménez et al. 2006). All treatments were performed in triplicate at 8°C for 32 days (2020/9/24 to 2020/10/26). Autoclaved artificial seawater (2% v/v sea salt) was introduced to the columns at 12 h intervals to simulate the tidal cycle on a beach.

2.2.2 Isolation of bacterial strains

To isolate and characterize NWP beach hydrocarbon degraders strains from the beach sediment columns, five grams of sediments from the column experiment were sampled and deposited into 50mL falcon tubes to which sterilized glass beads and 3 times the weight of the sediment of water (1:4 dilution) was added. The process was repeated for each column and the tubes were then vortexed for 2 minutes with glass beads, allowing the sediments to break down. 1mL of each solution was subsequently added to 9mL of sterilized water (10-fold dilution), serially diluted, and 200µL of supernatant from the 10^{-3}, 10^{-4}, and 10^{-5} dilutions subsequently spread-plated onto R2A medium (a low nutrient medium for culturing bacteria from oligotrophic environments and which enhances the growth of slower growing microorganisms (Reasoner & Geldreich. 1985)) (BD) solidified with Gellan (Alfa Aesar) and onto a MSM medium composed of Gellan gum supplemented with 500 ppm ULSFO and 0.67g/L ammonium sulfate as the nitrogen source (Figure 3 A.). The plates were subsequently incubated at 5°C for one month. Following the growth period, colonies were counted on each plate to determine bacterial counts of the original sediment sample, and colonies were selected for isolation based on their successful growth on ULSFO as sole carbon source and colony morphology to maximize diversity. The selected strains were then subcultured by streaking on the same type of plates described above containing 500ppm ULSFO at 5°C for

another 4 weeks. A set of plates amended with ULSFO and ammonium sulfate was also prepared to detect any growth that would result from a contamination of our fuel but none was detected.

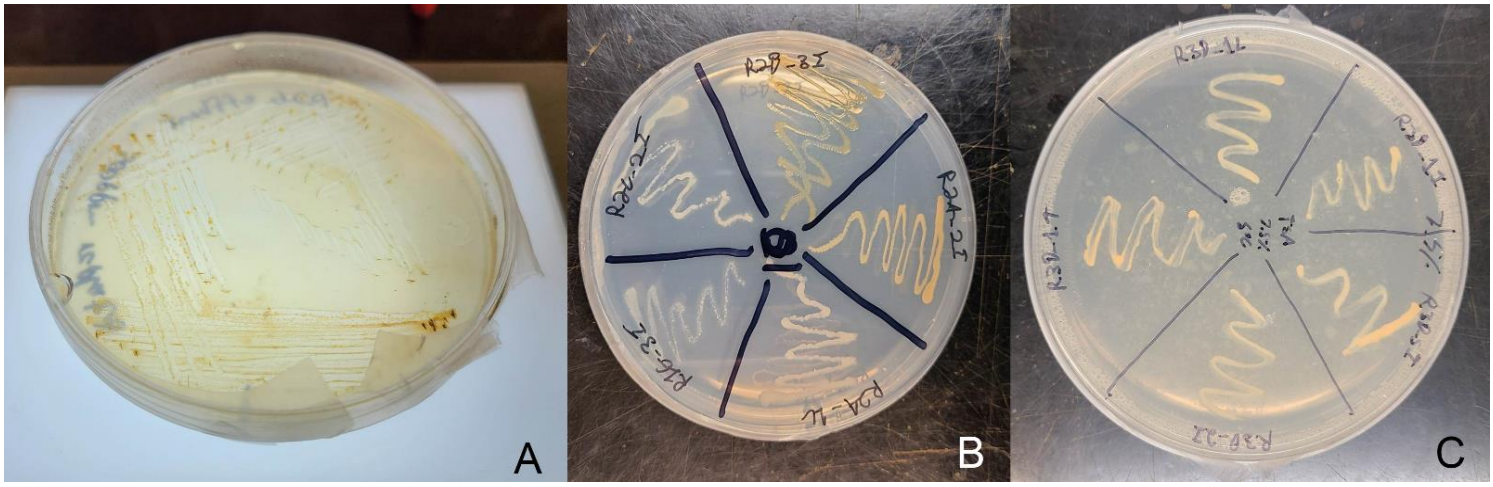


Figure 3. Solid Culture Medium Used in Isolation and Growth Trials of Isolates Obtained from Northwest Passage Beach Sediments

A. Isolation plate on selective medium containing 500ppm of Ultra Low Sulfur Fuel Oil (ULSFO) as sole carbon source. The medium was made using source water from Tupirvik, 2% gellan gum instead of agar and was amended with ammonium sulfate as a nitrogen source. B. Temperature growth trial medium employing 1/10TSA as nutrient source; the one illustrated was for 5 strains at 10 ℃. The plates were made with source water from Tupirvik, 2% gellan gum, and amended with ammonium sulfate as an N source. C. Salinity growth trial plate employing 1/10TSA as nutrient source; all plates were grown at 5℃ and contained 5 or 6 strains. The medium is made with source water from Tupirvik, 2% gellan gum, and amended with ammonium sulfate as an N source.

2.2.3 16S rRNA gene sequencing and identification.

Isolate bacterial strains were identified by 16S rRNA sequencing and phylogenetic analyses as follows. Single, Colonies from pure cultures were transferred to clean 1.5mL microfuge tubes containing 250µL PCR grade dH_2O (Invitrogen). The tubes were vortexed and microwaved for 3 minutes for cell lysis. Isolates which failed to provide DNA through this method were lysed following QIAGEN DNeasy PowerLyzer PowerSoil Kit (QIAGEN) to extract DNA. PCR amplification was performed using universal bacterial primers 27F (AGRGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) with 1µL of cell

lysis extract and Hot Star Taq Master Mix (Qiagen). The PCR cycle conditions were as follow: 94°C for 12 minutes, 35 cycles of 94°C for 1minute, 50°C for 1 minute, and 72°C for 2 minutes, then 72°C for 10 minutes after the last cycle, held at 4°C until removed from the thermal cycler. PCR amplified samples were analyzed by gel electrophoresis in SB (sodium borate) buffer at a voltage of 100V for 1 hour to confirm amplicon was produced.

In preparation for sequencing, 5µL of unpurified 16s rRNA PCR product from each isolate with a concentration of 200 ng/µL, verified by Qubit fluorometer (Invitrogen) were loaded into 96 well plates (Eppendorf twin.tec, Full Skirt, Cat# 951020401). Sanger sequencing was performed at the "Plateforme d'analyses génomiques" of the "Institut de Biologie Intégrative et des Systèmes" (PAG-IBIS, Université Laval, Québec, Canada). Sequencing reactions were performed using the BDT v3.1 chemistry (Applied biosystems) and then loaded on an ABI 3500 (Applied Biosystems) equipped with 50 cm capillaries. Obtained 16S rRNA sequences were analysed using Sequencing Analysis Software 7 (Applied Biosystems). Raw sequencing reads obtained from IBIS were first cleaned manually using the software FinchTV v. 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) to remove low quality sequences and ends. The cleaned reverse reads were then reverse complement using Sequence Manipulation Suite's (SMS) Reverse Complement tool (Stothard P. 2000) and subsequently merged with the cleaned forward reads using EMBOSS merger v. 6.6.0.0 (Rice et al. 2000). The resulting sequences were submitted to EZbiocloud (Yoon et al. 2017), a platform dedicated to the taxonomy, phylogeny, and genomics of prokaryotes, for identification.

2.2.4 Growth condition assay and Gram staining

A total of 22 strains were selected for growth condition assays. For the temperature and salinity growth assay. For these trials, all strains were grown on 1/10 TSA (tryptone soya agar) (BD) plates

with gellan gum added to obtain proper solidity. TSA is a nutritious medium containing enzymatic digest of casein and soybeans which provide amino acids and glucose for energy, and dipotassium sulfate as a buffer to maintain pH; it allows for the growth of various microorganisms including cold growing microbes (Subramanian et al. 2014). Preliminary tests were made to confirm all isolates were able to grow on TSA in the same salinity and temperature conditions they had grown on 2% gellan plates; furthermore, gellan has been shown more suitable for the growth of novel microbial species (Tamaki et al. 2009) and thus it was also necessary to make sure the isolates were able to grow in the presence of agar as well before using them for these assays. For the temperature trials, the TSA plates were made using sea water collected from Tupirvik to which 0.67g/L ammonium sulfate (MP Biomedicals) was added and were incubated at temperatures of either 37°C, 25°C, 10°C, 0°C, or -10°C for a duration of 4 weeks after which growth was assessed (Figure 3. B.). As for salinity assay, 1/10 TSA plates were also used, but instead of source water we used autoclaved distilled water with 0%, 3%, 5%, 7.5%, or 10% v/v sea salt added, and 0.67g/L ammonium sulfate (Figure 3. C.). The salinity assay plates were grown at 5°C for 4 weeks followed by growth assessment. Following the salinity growth assay, 3 sets of plates were created to assess hydrocarbon metabolism at 5 °C, 0 °C, and -5°C. The 5 °C and 0 °C used minimum growth medium plates made with 2% gellan gum and sea water from Tupirvik, amended with 500ppm ULSFO as sole carbon source, and incubated for the period of one month. For the -5°C trials, minimum growth medium plates were made using 2% gellan gum, with autoclaved water amended with 5%v/v sea salt; 500ppm ULSFO was added as sole carbon source and the plates were grown for 2 months.

Gram staining was performed as per Smith, A., & Hussey, M. (2005). A loopful of colony was added to a drop of sterile water before being air dried and heat fixed. Once fixed the slide was flooded with crystal violet staining reagent for 1 minute before being washed gently with an

indirect flow of tap water for 2 seconds. The slide was then flooded with Gram's iodine for a minute before being washed again with an indirect flow of tap water for two seconds. The chosen decolorizing agent was 95% Ethanol, which was added at this point drop by drop until it ran clear off the side of the slide. The slide was then flooded with counterstaining agent safranin for 30 to 60 seconds before being washed with an indirect flow of tap water until no more color appeared in the effluent and was then blotted dry. Once all isolates had been stained, they were observed under oil immersion using a Brightfield microscope (Nikon eclipse 80i). Pictures of all stained isolates were kept as reference.

2.2.5 Whole genome sequencing and bioinformatics

DNA extraction was performed using Qiagen DNeasy PowerSoil DNA extraction kit (Qiagen) following the manufacturer's instructions. DNA shotgun sequencing (with PCR) was performed at Genome Quebec using Illumina NovaSeq 6000 S4 PE150 – 35M reads following the sample preparation guidelines; 50µL of solution containing 150ng of nucleic acid was send in a 98 well plate (Eppendorf twin.tec, Full Skirt, Cat# 951020401). Concentrations were verified using Qubit fluorometer (Thermo Fisher Scientific) and OD 260/280 and 260/230 ratios were measured using Nanodrop (Thermo Scientific). Raw reads were trimmed using Trimmomatic v0.36 (Bolger et al. 2014) and assembly was performed using Spades v3.15.4 (Bankevich et al. 2012) ran on python v3.8.12 (Van Rossum & Drake Jr. 1995). Assembled reads were subsequently annotated through 3 different methods. Firstly, the assembled reads were annotated using METAerg (Dong & Strous. 2019) to obtain immediate results and KEGG (Kanehisa et al. 2021) pathways. The presence of key hydrocarbon degradation genes was annotated using the Calgary approach to ANnoTating HYDrocarbon degradation genes (CANT-HYD) (Khot et al. 2021) with a noise cut-off of E-value at 0.01. Using this approach, we can identify and annotate 37 critical genes (Table 1) associated

with aerobic and anaerobic hydrocarbon degradation pathways, including genes for the degradation of propane monooxygenase (*prmAC*), butane monooxygenase *(bmoABC* and *bmoXYZ*), alkane hydroxylase *(alkB* and *CYP153*), flavin binding monooxygenase (*almA*), long-chain alkane hydroxylase (*ladA* and *ladB*), toluene-4-monooxygenase (t*moA, tmoB* and *tmoE*), monoaromatic dioxygenase (MAH_alpha and MAH_beta), toluene-ortho-monooxygenase/phenol hydroxylase (*tomA1*, *tomA3* and *tomA4*/*dmpO*), Naphthalene-1,2 dioxygenase (*ndoB, ndoC* and non-*ndoB*), dibenzothiophene monooxygenase (*dszC*), alkylsuccinate synthase (*assA*), putative alkane C2 methylene hydroxylase (*ahyA*), ethylbenzene dehydrogenase (*edbA* and *cmdA*), benzylsuccinate synthase (*bssA*), 2-naphthylmethyl-succinate synthase (*nmsA*), benzene carboxylase (*abcA*) and naphthalene carboxylase (K27540) (Khot et al. 2021). Finally, assembled reads were sent to JGI (Nordberg et al. 2014) for annotation and addition into the Integrated Microbial Genomes (IMG) online database (Markowitz et al. 2012). Besides Hydrocarbon degradation genes, the annotated whole genome sequences of all 22 isolates obtained from Tupirvik beach sediments were searched for the presence of cold adaptation genes; these include cold shock proteins, osmotic and oxidative stress response proteins, carbon storage and starvation proteins, membrane or cell wall alteration proteins, and other general stress response proteins (Table 3). The list of genes was put together

Table 3. List of Known Cold Tolerance Proteins Queried in the Isolated Bacterial Strains' Genomes

Protein	Role
Cold Shock protein (cspA)	cold shock protein
Cold Shock protein (cshA)	cold shock protein
cold shock-induced ribonuclease R	cold shock protein
Molecular chaperone (HtpG)	cold shock protein
Sodium/proton antiporter, CPA1 family	Osmotic stress/oxidative stress
Sodium/proton antiporter, NhaA family	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit A	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit C	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit D	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit E	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit F	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit G	Osmotic stress/oxidative stress
Choline oxidase	Osmotic stress/oxidative stress
Trehalose 6-phosphate synthase	Osmotic stress/oxidative stress
Trehalose 6-phosphatase	Osmotic stress/oxidative stress
Ectoine synthase	Osmotic stress/oxidative stress
Osmosensitive K+ channel histidine kinase	Osmotic stress/oxidative stress
Osmoprotectant transporter ProP	Osmotic stress/oxidative stress
Peroxiredoxin, OsmC and Ohr subfamilies	Osmotic stress/oxidative stress
Catalase/peroxidase HPI	Osmotic stress/oxidative stress
K+ uptake TrkH	Osmotic stress/oxidative stress
K+ uptake TrkA	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit A	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit C	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit D	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit E	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit F	Osmotic stress/oxidative stress
multicomponent Na+:H+ antiporter subunit G	Osmotic stress/oxidative stress
choline/glycine/proline betaine transport protein	Osmotic stress/oxidative stress
sorbitol/mannitol transport system substrate-binding protein	Osmotic stress/oxidative stress
sorbitol/mannitol transport system permease protein	Osmotic stress/oxidative stress
sorbitol/mannitol transport system permease protein	Osmotic stress/oxidative stress
glycerol-3-phosphate O-acyltransferase	Membrane/ cell wall alteration
1-acylglycerol-3-phosphate O-acyltransferase	Membrane/ cell wall alteration
Fatty Acid desaturases	Membrane/ cell wall alteration
Fatty Acid desaturases	Membrane/ cell wall alteration
Fatty Acid desaturases	Membrane/ cell wall alteration
Phytoene desaturase	Membrane/ cell wall alteration
Phytoene synthase	Membrane/ cell wall alteration
carbon starvation protein	Carbon storage/ starvation
glycogen debranching enzyme	Carbon storage/ starvation
Glycogen synthase (ADP-glucose)	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Diacylglycerol acyltransferase	Carbon storage/ starvation
Glycerol-3-phosphate O-acyltransferase	Carbon storage/ starvation
SOS-response transcriptional repressor, LexA	General stress response
Phage shock protein C (PspC) family protein	General stress response
Phage shock protein A (PspA) family protein	General stress response

through a thorough literature search (Quabron. 2018, Goordial et al. 2016, Khaleque et al. 2019, Gunde-Cimerman et al. 2018, Chen et al. 2017, Maggiori et al. 2021) and compared to the isolate's genomes for identification. Further identification using whole genome sequences for isolates with low 16S percent identity was performed using software GTDB-TK v2.1.0 (Chaumeil et al. 2020) which assigns taxonomic classification to bacterial and archaeal genomes using the Genome Database Taxonomy (GTDB) (Parks DH, et al. 2018, Parks DH, et al. 2020.).

2.2.6 Chemical and molecular hydrocarbon degradation assay

3 microbial strains previously isolated and identified through 16S ribosomal RNA sequencing were selected for TPH analysis. The selected isolates were *Rhodococcus* sp.R1B_2T, *Flavobacterium* sp.R2B_3I, and *Pseudarthrobacter* R2D_1T. The strains were grown in flasks over the course of 3 months at 5ºC with a triplicate being sacrificed for each strain, at each time point for analysis. The time points were: directly after inoculation (T0), after 1 month(T1), and after the 3 months (T2) (Figure 4). 250mL of liquid culture of each isolate was prepared by adding $\frac{1}{10}$ R2A and 0.35g/L ammonium sulfate to 250g of source water from Tupirvik beach which was then autoclaved. Following sterilization, a scoop of culture was added to the medium and grown over 2 weeks. For two time points at 1- and 3-months intervals, flasks were prepared for each bacterium in triplicates. Each flask contained 600mL of autoclaved artificial sea water (3%) to which was amended 0.35g/L ammonium sulfate (MP Biomedicals) as nitrogen source and 500ppm of the commercial ship fuel ULSFO. These flasks were inoculated with the previously prepared liquid cultures of the respective isolate (50mL for *Pseudarthrobacter* sp.R2D_1T, *Rhodococcus* sp.R1B_2T, and 25mL *Flavobacterium* sp.R2B_3I) after the medium cooled down to room

temperature. The flasks were kept on shakers rotating at 100rpm in a 5◦C incubator for 1 (T1) or 3 months (T2). A time zero flask was also prepared for each strain and collected right away to determine the base level of gene expression of hydrocarbon degradation genes in each strain, and an inoculated control flask was prepared for each time point. After each incubation period was completed, the medium was heated at 60◦C to allow the ULSFO, which was stuck to the glass flask, to dissolve back in the medium before being transferred to glass bottles provided by the analysis company (SGS Canada) and stored at -20 ℃ until the experiment was completed at which point all samples would be sent for TPH analysis at once. The hydrocarbon analysis was carried out following SGS Canada's protocols. Briefly, for aqueous samples, semi-volatile organic compounds (SVOCs) are extracted using liquid/liquid extraction using dichloromethane to remove analytes from the water quantitatively and the determination of SVOCs was conducted following the United States Environmental

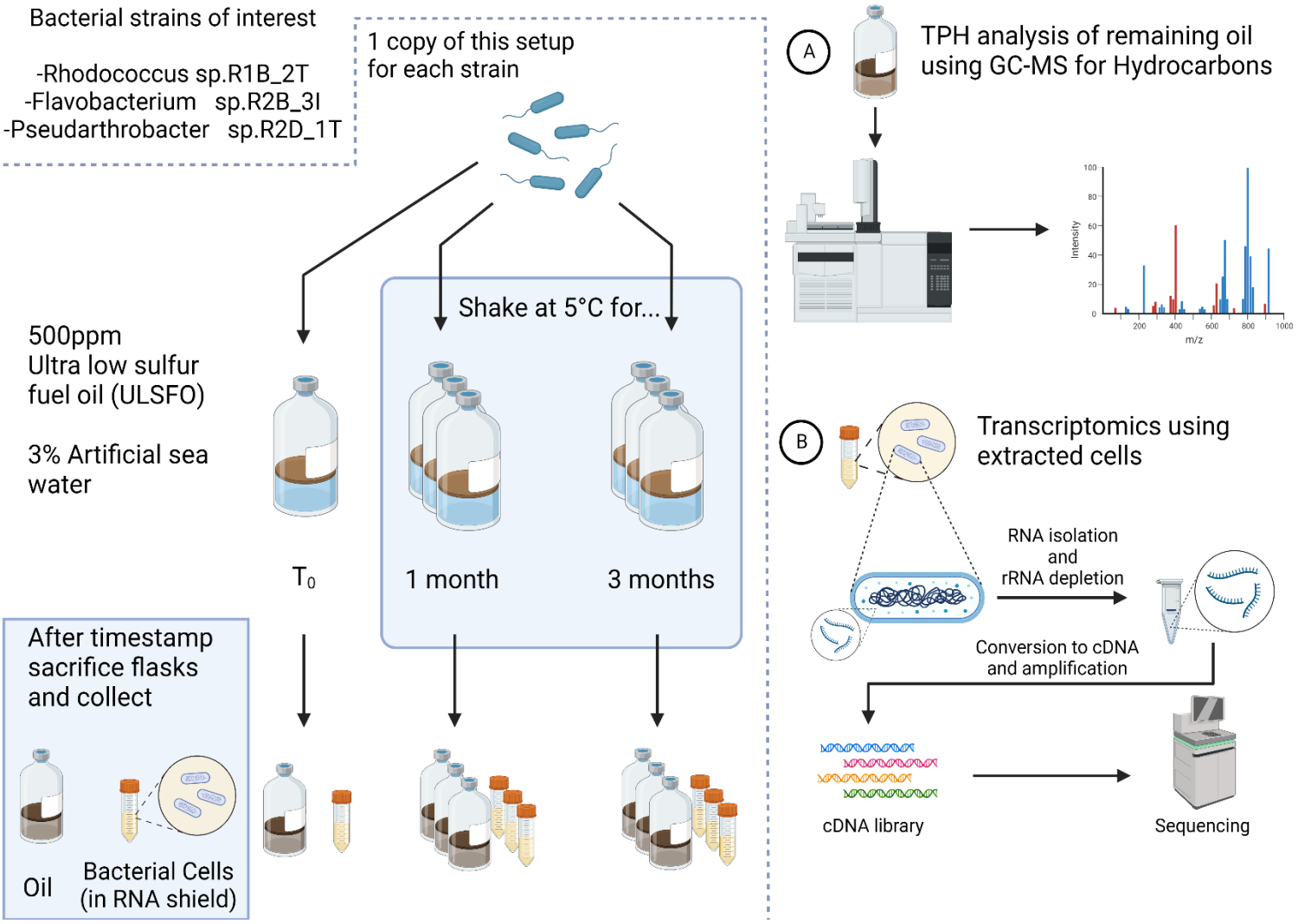


Figure 4. Schematic Diagram of the Experimental Design and Steps for the Hydrocarbon Degradation Assay Performed Using Three Hydrocarbon Degrading Bacteria of Interest Isolated from Northwest Passage Beach Sediments Used in a Column Experiment

A. Samples at each time points were frozen in glass vials at -20°C until the final time point (T2) before being thawed and transferred into the analysis company's provided vials containing chemicals and shipped all at once. Samples were extracted by SGS on July 05, 2022, and GC-MS analysis was performed between the 7th and the 20th of July 2022. B. Bacterial biomass was extracted from each flask before the hydrocarbons were stored, up to 200mL of culture were centrifugated until a sizeable pellet was formed. The supernatant was then removed, and the bacteria were covered in RNA shield. The samples are stored at -20°C for transcriptomic analysis in a follow up study.

Protection Agency (EPA) Method 8270E (U.S.EPA, 2014.). The analysis of petroleum hydrocarbons, such as BTEX and certain PAH compounds, was conducted following the CCME Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbons in Soil – Tier 1 (CCME, 2002.).

Past 4 (v4.12) (Hammer et al. 2001), R (4.1.1), and R studio (2022.02.3+492) were used to process the statistical analyses and generate figures. The bubble plot graph was constructed using R packages "ggplot2" and "phyloseq", the TPH result graphs were also built in "ggplot2" with additional packages "tidyverse" and "scales". Tables were formatted in Microsoft Excel (16.0.13901.20400) before being converted to PDF and Figures to SVS format. Biodegradation pathway maps and the experimental design diagram were generated using BioRender.com.

2.3 Results

2.3.1 Isolation of hydrocarbon degrading bacteria from oil-enriched Arctic sediments

A total of 34 bacterial strains were isolated from Arctic beach sediments used in a column enrichment experiment in October 2020. Sediment samples were collected in August 2019 from Tupirvik beach near Resolute Bay, in Nunavut, Canada. Data collected on site on the 5th of August 2021 indicated the temperature of the water on the beach was of 1.3°C with a DO (mg/L) of 10.25, pH of 8.2, PSU of 32.46, and ORP of 123.1 with concentration of Nitrate at 0.005ppm, of Phosphate at 1.18ppm, and sulfate at 1538 ppm. The 34 isolates obtained from these sediments grown on selective media containing 500 ppm ULSFO as sole carbon source. The bacterial strains isolated this way belonged to 7 different orders and 9 genera, the most abundant being *Rhodococcus* which consisted of 32.35% of all isolates, followed by *Flavobacterium* at 26.47%, and *Pseudomonas* at 11.76%. Despite the large number of isolates obtained from the genus

Flavobacterium, little diversity was observed between based on 16S rRNA amplicon sequencing results with 8 of the 9 strains belonging to the same phylotype, *Flavobacterium* AHKF_s. The closest related species of this phylotype, *Flavobacterium frigoris*, is also the closest relative of the 9th *Flavobacterium* isolate as determined by 16S rRNA sequencing. *Rhodococcus* on the other hand possessed the highest diversity within a single genus with 7 different species and phylotypes across 11 obtained isolates. Of these 34 isolates, 22 were successfully sub-cultured for further characterization and genomic analyses. The isolates which were not successfully sub-cultured included 4 strains of the genus *Rhodococcus*, 2 of *Flavobacterium*, 2 of *Pseudomonas*, 2 of *Sulfitobacter*, 1 of *Loktanella*, and 1 of *Arthrobacter*.

Relative abundance of bacterial orders from the 22 isolates was mapped according to which treatment the sediments they were extracted from were subjected to during the column experiment (Figure 5). Results show that Mycobacteriales dominated in the control trials and ULSFO only trials, however they were isolated less often in the other treatments columns. Flavobacteriales on the other hand were not isolated from the control columns, but were found in each other treatments, most of them being isolated in the columns containing oil and organic fertilizer. Sphingomonadales, Rhodobacterales, were mostly isolated from the columns containing both oil and inorganic fertilizer in which some Pseudomonadales also appeared. Micrococcales were only isolated from the columns containing oil and organic fertilizer. Pseudomonadales, Mycobacteriales, and Flavobacteriales were the only 3 bacterial orders obtained from the columns containing oil and both organic and inorganic fertilizer.

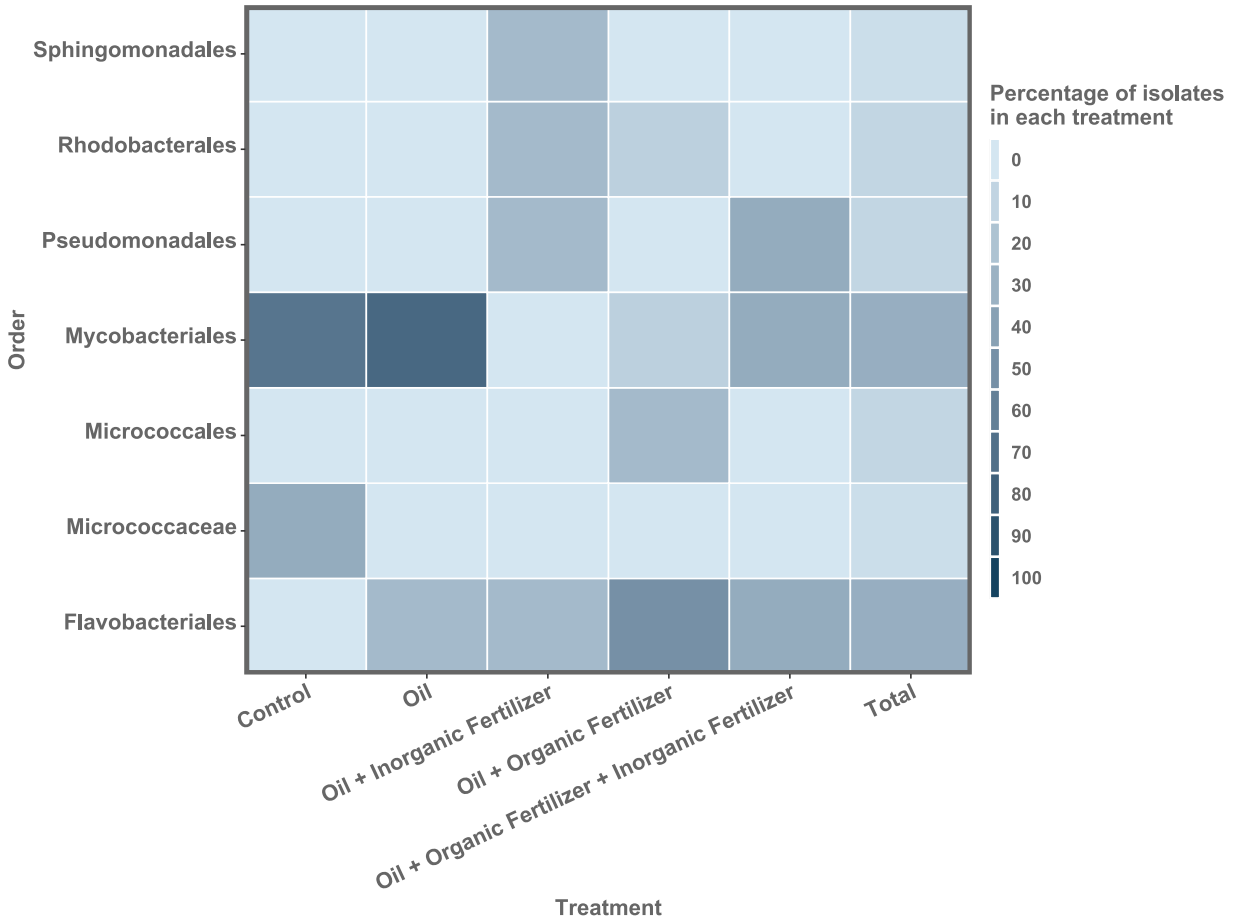


Figure 5. Relative Abundance of Orders of Bacterial Isolates from Northwest Passage Beach Sediments Used in a Column Experiment by Treatment Type

Percentage of bacterial strains isolates from each column experiment belonging to each of the seven orders identified. The treatments range from just sediments on the leftmost column, sediments with ULSFO, sediments with ULSFO and inorganic fertilizer, sediments with ULSFO and organic fertilizer, Sediments with ULSFO and both organic and Inorganic fertilizer, and finally the proportions of each order isolated from each treatment.

These isolates' identity, previously determined by 16S rRNA amplicon sequencing, was recorded and later tested using Average Nucleotide Identity (ANI) of their WGS to determine their potential novelty and confirm their identity (Table 4). Most isolated bacteria were able to grow successfully at 0℃, and more than half were also successful at sub-zero temperatures. Most strains were able to grow between 0 ℃ and 25 ℃ with the exception of *Flavobacteriup* sp. R1C_1I which was not

able to grow at 0 ℃ and 25 ℃. Two strains, *Pseudarthrobacter* sp. R2D_1L and *Rhodococcus* sp. R3D_1T, were also able to grow at 37 ℃. Similarly, most isolates grew at salinities between 0% to 7.5% with the exception of *Flavobacterium* sp.R3D_2I, *Flavobacterium* sp. R3D_1L, and *Flavobacterium* sp. R1C_1I which did not exhibit growth at 0%v/v sea salt, and *Flavobacterium* sp. R2B_3I, *Rhodococcus* sp. R1B_1T, and *Rhodococcus* sp. R1E_2I which showed no growth at 7.5%v/v. Isolates *Arthrobacter* sp.R3A_1T, *Flavobacterium* sp.R3D_5I, *Pseudarthrobacter* sp. R2D_1T, and *Rhodococcus* sp.R3D_1T grew at the highest salinity of those trials which was 10%v/v. (Table 5.). All isolated strains successfully grew on plates containing ULSFO as sole carbon source at temperatures of 5 ℃ and 0 ℃. Strains R3D_5I, R3D_2I, R3D_1T, R3D_1L, R3D_1I, R3A_1T, R2E_4I, R2D_1T, R2D_1L, R2D_1I exhibited growth at -5℃ on plates containing ULSFO as sole carbon source and with a salinity of 5% (Table 6.).

2.3.2 Presence of hydrocarbon degradation key genes

Overall aerobic hydrocarbon degradation pathways were more widespread than anaerobic ones in the 22 isolates (Figure 6). The most common aerobic degradation genes found were *almA*_GroupI and *almA*_GroupIII, a long-chain alkane degrading gene with a wide diversity of sequences occurring with high frequency in hydrocarbon degrading bacteria (Wang et al. 2012) and which encode flavin-binding monooxygenase, which degrades alkanes of C_{20} to C_{32} length. Also common was *ndoB*, which encodes the catalytic alpha subunit of Naphthalene 1,2-dioxygenase, and *dszC* which is involved in dibenzothiopene degradation. Those enzyme encoding genes were observed in all isolates. Genes *ladAα*, *ladAβ* and *ladB*, which are involved in the degradation of long-chain-alkanes, were also consistently found across most isolates with the exception of *Flavobacterium* sp.R2B_3I and *Flavobacterium* sp. R3D_2I which had none of them. The most commonly found anaerobic hydrocarbon degradation genes were *ahyA*, which encodes alkane hydroxylase, *ebdA*

Table 4. Identity of Bacterial Strains Isolated from Northwest Passage Beach Sediments Used in a Column Study Determined by Closest Relative Using16S rRNA Sequencing

Strain_ID	16S_closest_hit	Order	Similarity to closest strain (%)
R3A_1T	*Arthrobacter ginsengisoli*	Micrococcales	98.74
R3D_5I	*Flavobacterium* AHKF_s	Flavobacteriales	99.35
R3D_2I	*Flavobacterium* AHKF_s	Flavobacteriales	99.13
R3D_1L	***Flavobacterium* AHKF_s**	**Flavobacteriales**	**99.06**
R3D_1I	***Flavobacterium* AHKF_s**	**Flavobacteriales**	**98.99**
R2E_4I	*Flavobacterium* AHKF_s	Flavobacteriales	99.35
R1C_1I	*Flavobacterium* AHKF_s	Flavobacteriales	99.13
R2B_3I	***Flavobacterium frigoris***	**Flavobacteriales**	**97.76**
R2D_1I	*Loktanella salsilacus*	Rhodobacterales	99.47
R1C_2I	*Loktanella salsilacus*	Rhodobacterales	99.62
R2D_1T	*Pseudarthrobacter psychrotolerans*	Micrococcales	99.42
R2D_1L	***Pseudarthrobacter sulfonivorans***	**Micrococcales**	**98.47**
R2C_2I	*Pseudomonas* CP019947_s	Pseudomonadales	99.78
R1E_3I	*Pseudomonas* CP019947_s	Pseudomonadales	99.93
R2A_1L	*Rhodococcus baikonurensis*	Mycobacteriales	98.46
R2A_2I	*Rhodococcus cerastii*	Mycobacteriales	99.62
R1B_1T	***Rhodococcus cerastii***	**Mycobacteriales**	**95.37**
R1B_2T	*Rhodococcus cercidiphylli*	Mycobacteriales	99.48
R1B_2I	*Rhodococcus cercidiphylli*	Mycobacteriales	99.93
R3D_1T	*Rhodococcus fascians*	Mycobacteriales	99.76
R1E_2I	*Rhodococcus globerulus*	Mycobacteriales	99.68
R1C_3I	*Pntixanthobacter sediminis*	Sphingomonadales	99.26

Strains highlighted in bold were determined as potentially novel based on Average Nucleotide Identity from their whole genome sequences.

encoding ethylbenzene dehydrogenase, and *cmdA* encoding p-cymene dehydrogenase. All 3 were found in all isolated strain except in strain *Pseudarthrobacter* sp.R2D_1L which did not possess *cmdA.* Overall, more alkane degradation pathways were observed than aromatic degradation pathways. The most common hydrocarbon degradation gene found in all isolates was *dszC*, encoding dibenzothiophene (DBT) monooxygenase, which may be a result of our sediment samples used in the column experiment. *Pseudarthrobacter* sp.R2D_1T harbours genes for a wide range of enzymes involved

Table 5. Identities and Growth Condition Ranges of Bacterial Strains Isolated from Northwest Passage Beach Sediments Used in a Column Experiment

Strain ID	Temperature						Salt Concentration				
	37°C	25°C	10°C	5°C	0°C	-5°C	10%	7.50%	5%	3%	no salt
Arthrobacter sp.R3A_1T	-	+	+	+	+	+	+	+	+	+	+
Flavobacterium sp.R3D_5I	-	+	+	+	+	+	+	+	+	+	+
Flavobacterium sp.R3D_2I	-	-	+	+	+	+	-	+	+	+	-
Flavobacterium sp.R3D_1L	-	+	+	+	+	+	-	+	+	+	-
Flavobacterium sp.R3D_1I	-	+	+	+	+	+	-	+	+	+	+
Flavobacterium sp.R2E_4I	-	+	+	+	+	+	-	+	+	+	+
Flavobacterium sp.R1C_1I	-	-	+	+	-	-	-	+	+	+	-
Flavobacterium sp.R2B_3I	-	+	+	+	+	-	-	-	+	+	+
Loktanella sp.R2D_1I	-	+	+	+	+	+	-	+	+	+	+
Loktanella sp.R1C_2I	-	+	+	+	+	-	-	+	+	+	+
Pseudarthrobacter sp.R2D_1T	-	+	+	+	+	+	+	+	+	+	+
Pseudarthrobacter sp.R2D_1L	+	+	+	+	+	+	-	+	+	+	+
Pseudomonas sp.R2C_2I	-	+	+	+	+	-	-	+	+	+	+
Pseudomonas sp.R1E_3I	-	+	+	+	+	-	-	+	+	+	+
Rhodococcus sp.R2A_1L	-	+	+	+	+	-	-	+	+	+	+
Rhodococcus sp.R2A_2I	-	+	+	+	+	-	-	+	+	+	+
Rhodococcus sp.R1B_1T	-	+	+	+	+	-	-	-	+	+	+
Rhodococcus sp.R1B_2T	-	+	+	+	+	-	-	+	+	+	+
Rhodococcus sp.R1B_2I	-	+	+	+	+	-	-	+	+	+	+
Rhodococcus sp.R3D_1T	+	+	+	+	+	+	+	+	+	+	+
Rhodococcus sp.R1E_2I	-	+	+	+	+	-	-	-	+	+	+
Pontixanthobacter sp.R1C_3I	-	+	+	+	+	+	-	+	+	+	+

Strains highlighted in bold were used in the TPH biodegradation study.

in degrading aliphatic hydrocarbons, including *alkB, almA*, and *ladA*. It also possesses several aromatic hydrocarbon degradation enzymes such as MAH_alpha and MAH_beta for monoaromatics, but also including *dszC, ndoB,* and *ndoC,* which are involved in the degradation of PAHs DBT and naphthalene respectively. It also possesses 3 different anaerobic hydrocarbon degradation pathways; *ahyA*, for n-alkanes, *ebdA*, for ethylbenzene, and *cmdA*, for p-Cymene biodegradation. The bacterial strains obtained from NWP beach sediments possessed genes to degrade a wide array of alkanes. All except two possessed *alkB* or *CYP153* (or both) which are responsible for the degradation of small chain alkanes (C_5 to C_{16}). All except 2 also contained one

Table 6. Temperature range at which Bacterial Strains Isolated from Northwest Passage Beach Sediments Used in a Column Experiment Grew on Ship Fuel as Sole Carbon Source

Strain ID	Temperature		
	5°C	0°C	-5°C
Arthrobacter sp.R3A_1T	+	+	+
Flavobacterium sp.R3D_5I	+	+	+
Flavobacterium sp.R3D_2I	+	+	+
Flavobacterium sp.R3D_1L	+	+	+
Flavobacterium sp.R3D_1I	+	+	+
Flavobacterium sp.R2E_4I	+	+	+
Flavobacterium sp.R1C_1I	+	-	-
Flavobacterium sp.R2B_3I	+	+	-
Loktanella sp.R2D_1I	+	+	+
Loktanella sp.R1C_2I	+	+	-
Pseudarthrobacter	+	+	+
Pseudarthrobacter	+	+	+
Pseudomonas sp.R2C_2I	+	+	-
Pseudomonas sp.R1E_3I	+	+	-
Rhodococcus sp.R2A_1L	+	+	-
Rhodococcus sp.R2A_2I	+	+	-
Rhodococcus sp.R1B_1T	+	+	-
Rhodococcus sp.R1B_2T	+	+	-
Rhodococcus sp.R1B_2I	+	+	-
Rhodococcus sp.R3D_1T	+	+	+
Rhodococcus sp.R1E_2I	+	+	-
Pontixanthobacter sp.R1C_3I	+	+	+

Strains highlighted in bold were used in the TPH biodegradation study

or more copies of *ladAα*, *ladAβ,* and *ladB* which are responsible for the degradation of medium to long chain alkanes in the C_{15} to C_{36} fraction. Long chain alkane degradation genes *almA*_GroupI and *almA*_GroupIII which encode Flavin binding monooxygenase were also present in all isolates and are responsible for the degradation of C_{20} to C_{32}. Isolates *Pseudarthrobacter* sp.R2D_1T and *Pontixanthobacter* sp.R1C_3I possessed gene *bmoB* for butane degradation; Isolates *Rhodococcus* sp.R1E_2I, *Rhodococcus* sp.R3D_1T, *Pseudomonas* sp.R1E_3I, and *Flavobacterium* sp. R3D_2I possessed genes for the degradation of propane and butane, namely, *prmA* and *prmC* for propane

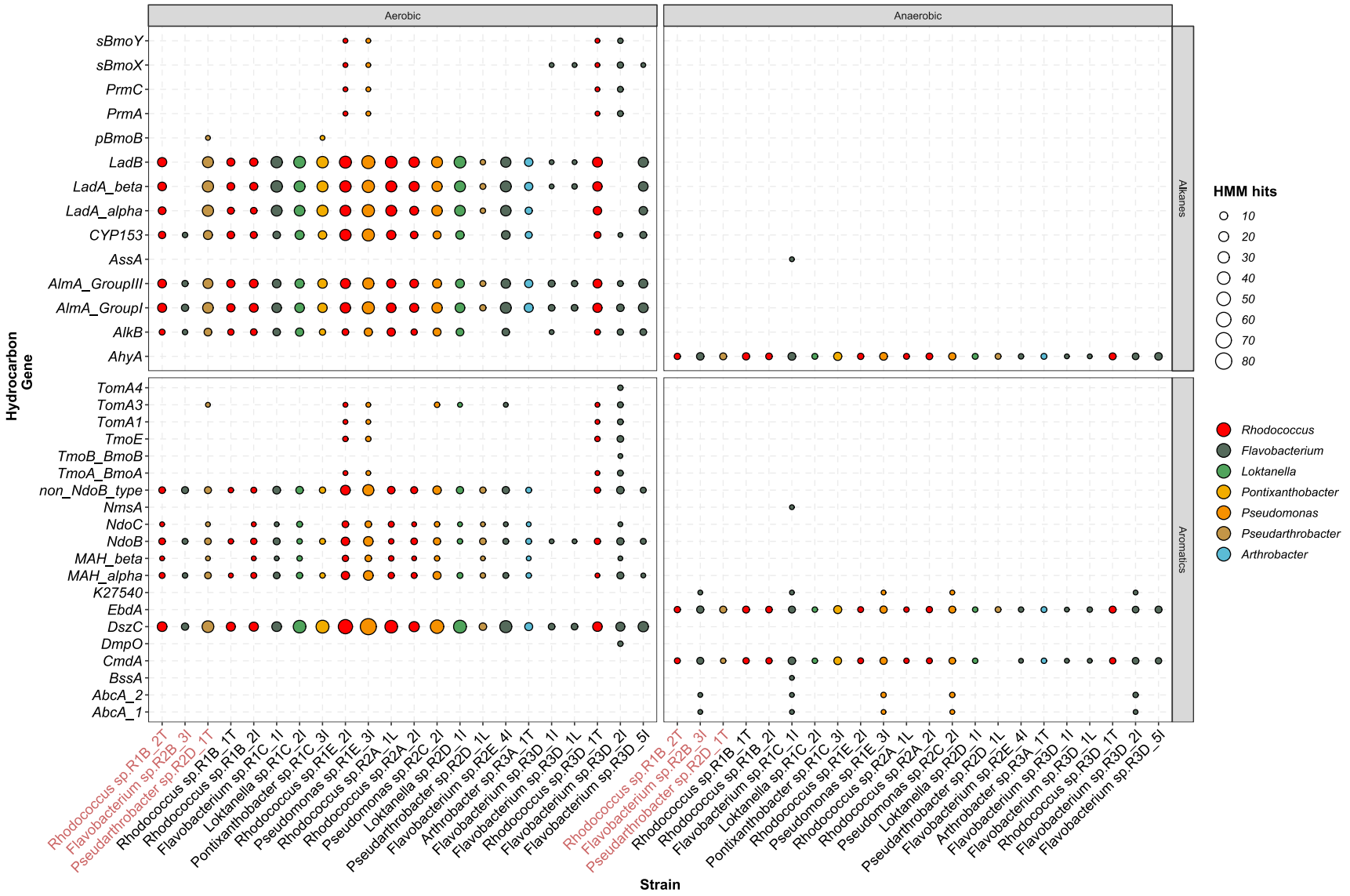

Aerobic
Anaerobic
Alkanes
Aromatics
Hydrocarbon Gene
sBmoY
sBmoX
PrmC
PrmA
pBmoB
LadB
LadA_beta
LadA_alpha
CYP153
AssA
AlmA_GroupIII
AlmA_GroupI
AlkB
AhyA
TomA4
TomA3
TomA1
TmoE
TmoB_BmoB
TmoA_BmoA
non_NdoB_type
NmsA
NdoC
NdoB
MAH_beta
MAH_alpha
K27540
EbdA
DszC
DmpO
CmdA
BssA
AbcA_2
AbcA_1
Rhodococcus sp.R1B_2T
Flavobacterium sp.R2B_3I
Pseudarthrobacter sp.R2D_1T
Rhodococcus sp.R1B_1T
Rhodococcus sp.R1B_2I
Flavobacterium sp.R1C_1I
Loktanella sp.R1C_2I
Pontixanthobacter sp.R1C_3I
Rhodococcus sp.R1E_2I
Pseudomonas sp.R1E_3I
Rhodococcus sp.R2A_1L
Pseudomonas sp.R2C_2I
Loktanella sp.R2D_1I
Pseudarthrobacter sp.R2D_1L
Flavobacterium sp.R2E_4I
Arthrobacter sp.R3A_1T
Flavobacterium sp.R3D_1I
Rhodococcus sp.R3D_1L
Flavobacterium sp.R3D_1T
Rhodococcus sp.R3D_2I
Flavobacterium sp.R3D_5I
Strain
HMM hits
10
20
30
40
50
60
70
80
Rhodococcus
Flavobacterium
Loktanella
Pontixanthobacter
Pseudomonas
Pseudarthrobacter
Arthrobacter

Figure 6. Presence of Aerobic and Anaerobic Alkane and Aromatic Hydrocarbons Degradation Genes Identified in Bacterial Strains Isolated from Northwest Passage Beach Sediments Used in a Column Experiment Using HMM in Software CANT-HYD

Presence of hydrocarbon degradation marker genes in whole genome sequences of selected isolates. Genes for the key enzymes of hydrocarbon degradation pathways (y-axis) are separated by aerobic or anaerobic metabolism, and by the identity of the hydrocarbon they degrade (Alkane or Aromatics). Isolates are represented on the x-axis. The first three isolates (colored in red) were the strains used for TPH analysis. The size of each circle represents the abundance of individual genes in each isolate, and the color represents the genus of the isolate in which the gene was found.

and *bmoX* and *bmoY* for butane biodegradation. Strains *Flavobacterium* sp. R3D_1I, *Flavobacterium* sp. R3D_1L, *Flavobacterium* sp. R3D_2I also possessed gene *bmoX*.

2.3.3 Genomic survey of hydrocarbon degradation pathways and stress adaptation mechanisms

In the reconstructed hydrocarbon degradation pathways, *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T both possessed complete or nearly complete degradation pathways for alkane degradation, *Pseudarthrobacter* spR2D_1T missing only a protein for the step converting (S)-3-Hydroxyhexadecanoyl-COA into 3-Oxyhexadecanoyl-COA. *Flavobacterium* sp.R2B_3I lacks 3 genes including *hcaD,* encoding Rubredoxin-NAD+ reductase, which is involved in the activity of *alkB,* ADH which encodes alcohol dehydrogenase, ACOX which encodes acyl-CoA oxidase (but possessed ACADM), and the genes involved in the production of enoyl-CoA hydratase (Figure 7. A.). The pathways for naphthalene and methylnaphthalene degradation were also found and were consistent with TPH and CANT-HYD results. *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T both possessed *ndoB* and *ndoC* which are both required for the metabolism of naphthalene, but *Flavobacterium* sp.R2B_3I only possessed *ndoC,* the catalytic subunit. *Rhodococcus* sp.R1B_2T had the most complete pathway with *adhP*, *yiaY,* and salicylate hydroxylase, followed by *Pseudarthrobacter* sp.R2D_1T which possessed *adhP* but

lacked salicylate hydroxylase and the gene *yiaY*, involved in the production of alcohol dehydrogenase. *Flavobacterium* sp.R2B_3I only possessed *ndoC*, and no other genes were found for the pathway in its genome (Figure 7. B.).

2.3.4 Assessing extent of hydrocarbon degradation

The shortest alkane fraction, F1 which contains alkane chains ranging from $C_{6\ to}\ C_{10}$ was almost completely degraded in the control and the inoculated flasks alike and thus the data was removed since it was not usable. The most decrease in both alkanes and PAH was observed in *Pseudarthrobacter* sp.R2D_1T and *Rhodococcus* sp.R1B_2T across the whole range of tested hydrocarbons, with *Rhodococcus* sp.R1B_2T outperforming *Pseudarthrobacter* sp.R2D_1T in most cases (Figure 8. A). *Rhodococcus* sp.R1B_2T was shown possessing the most complete pathways for alkane and methylnaphthalene and the most decrease in hydrocarbon concentration was detected for all 3 alkane fractions and for the three methylnaphthalene isomers. The one exception was for naphthalene, where roughly the same decrease was observed in all three strains. *Pseudarthrobacter* sp.R2D_1T and *Rhodococcus* sp.R1B_2T were both successful in degrading Naphthalene, 1-Methylnaphthalene, 2-Methylnaphthalene, and Methylnaphthalene, 2-(1-) as opposed to *Flavobacterium* sp.R2B_3I which barely showed any degradation of those compounds (Figure 8. B.). These results identified the presence of *ndoB* in all three isolates but showed that *Flavobacterium* sp.R2B_3I lacks the gene *ndoC*.

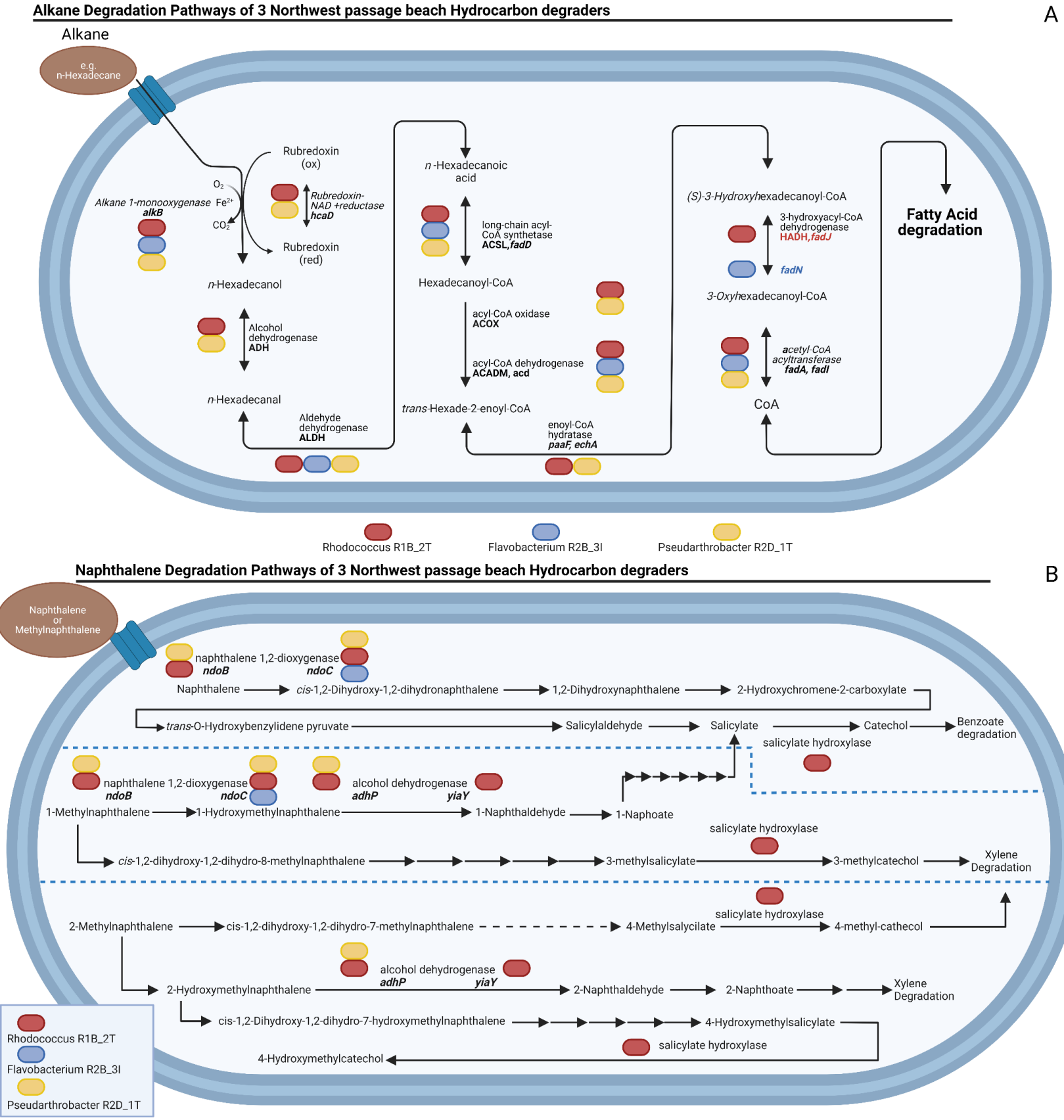
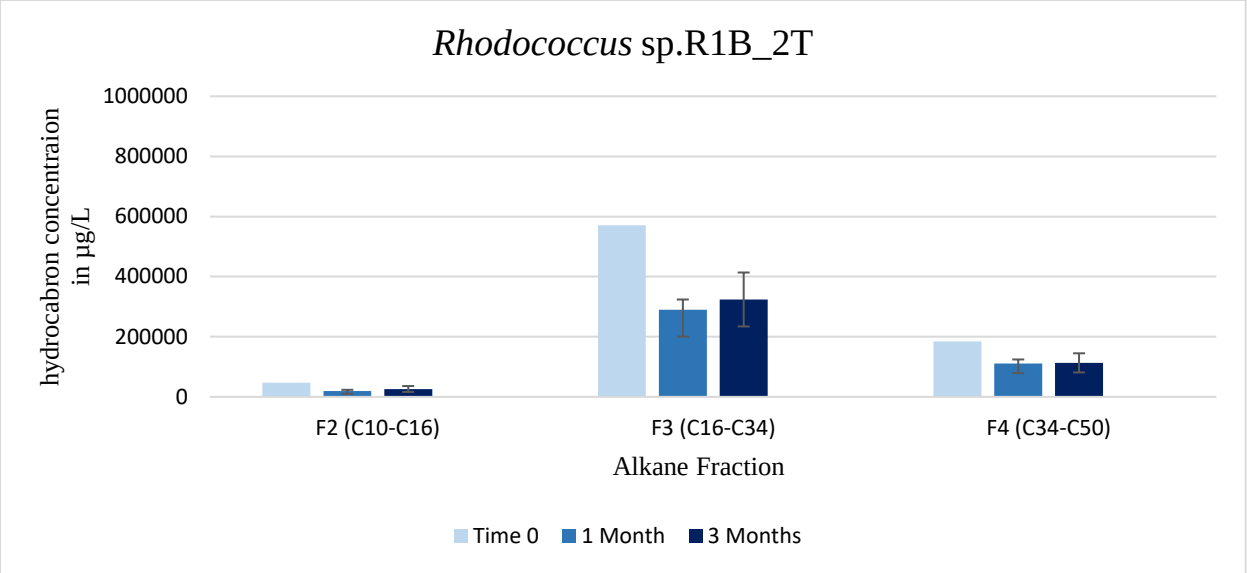

Alkane Degradation Pathways of 3 Northwest passage beach Hydrocarbon degraders
A
Alkane
e.g.
n-Hexadecane
Alkane 1-monooxygenase
alkB
Rubredoxin
(ox)
Rubredoxin-
NAD +reductase
hcaD
Rubredoxin
(red)
n-Hexadecanol
Alcohol
dehydrogenase
ADH
n-Hexadecanal
Aldehyde
dehydrogenase
ALDH
n-Hexadecanoic
acid
long-chain acyl-
CoA synthetase
ACSL,fadD
Hexadecanoyl-CoA
acyl-CoA oxidase
ACOX
acyl-CoA dehydrogenase
ACADM, acd
trans-Hexade-2-enoyl-CoA
enoyl-CoA
hydratase
paaF, echA
(S)-3-Hydroxyhexadecanoyl-CoA
3-hydroxyacyl-CoA
dehydrogenase
HADH,fadJ
fadN
3-Oxyhexadecanoyl-CoA
acetyl-CoA
acyltransferase
fadA, fadI
CoA
Fatty Acid
degradation
Rhodococcus R1B_2T
Flavobacterium R2B_3I
Pseudarthrobacter R2D_1T
Naphthalene Degradation Pathways of 3 Northwest passage beach Hydrocarbon degraders
B
Naphthalene
or
Methylnaphthalene
naphthalene 1,2-dioxygenase
ndoB
ndoC
Naphthalene
cis-1,2-Dihydroxy-1,2-dihydronaphthalene
1,2-Dihydroxynaphthalene
2-Hydroxychromene-2-carboxylate
trans-O-Hydroxybenzylidene pyruvate
Salicylaldehyde
Salicylate
Catechol
Benzoate
degradation
salicylate hydroxylase
naphthalene 1,2-dioxygenase
ndoB
ndoC
alcohol dehydrogenase
adhP
yiaY
1-Methylnaphthalene
1-Hydroxymethylnaphthalene
1-Naphthaldehyde
1-Naphoate
salicylate hydroxylase
cis-1,2-dihydroxy-1,2-dihydro-8-methylnaphthalene
3-methylsalicylate
3-methylcatechol
Xylene
Degradation
salicylate hydroxylase
2-Methylnaphthalene
cis-1,2-dihydroxy-1,2-dihydro-7-methylnaphthalene
4-Methylsalycilate
4-methyl-cathecol
alcohol dehydrogenase
adhP
yiaY
2-Hydroxymethylnaphthalene
2-Naphthaldehyde
2-Naphthoate
Xylene
Degradation
cis-1,2-Dihydroxy-1,2-dihydro-7-hydroxymethylnaphthalene
4-Hydroxymethylsalicylate
salicylate hydroxylase
4-Hydroxymethylcatechol
Rhodococcus R1B_2T
Flavobacterium R2B_3I
Pseudarthrobacter R2D_1T

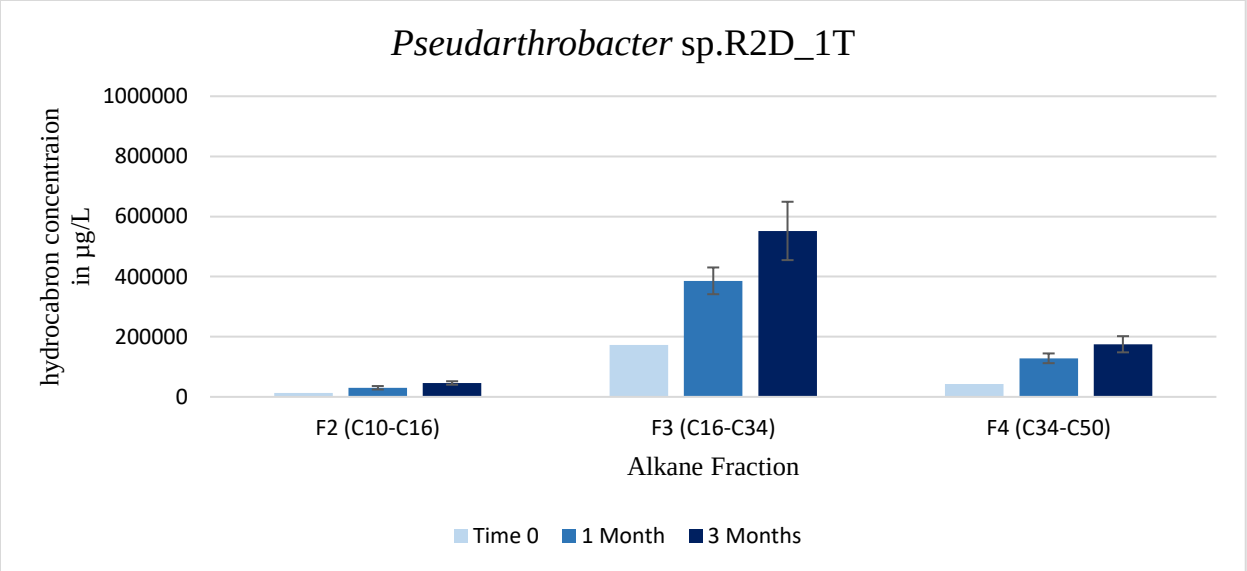
Figure 7. Alkane and PAH Degradation Pathways in 3 Isolates from Northwest Passage Beach Sediments

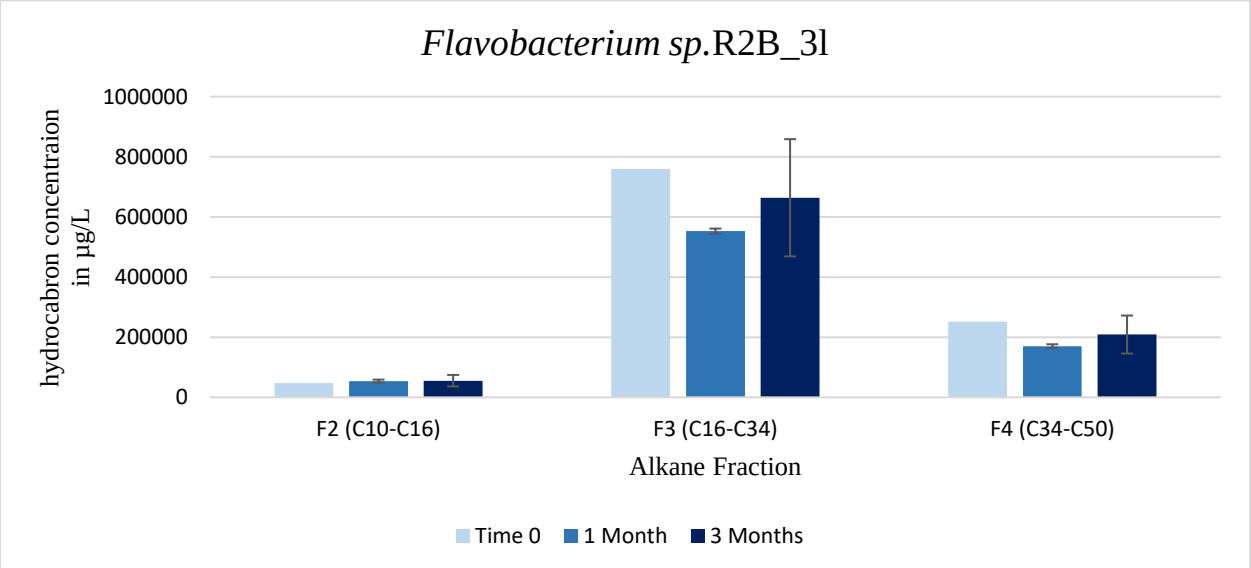
A. Alkane degradation pathway using *n*-hexadecane as an example for the chemical breakdown. For each step the name of the active enzyme and gene or genes responsible for the step are indicated. For each step it is indicated which bacterium possesses the specific enzyme. B. Naphthalene and Methylnaphthalene isomers degradation pathways, as above. The blue dotted line separates the 3 parallel pathways.

2.3.5 Cold and salt tolerance genes

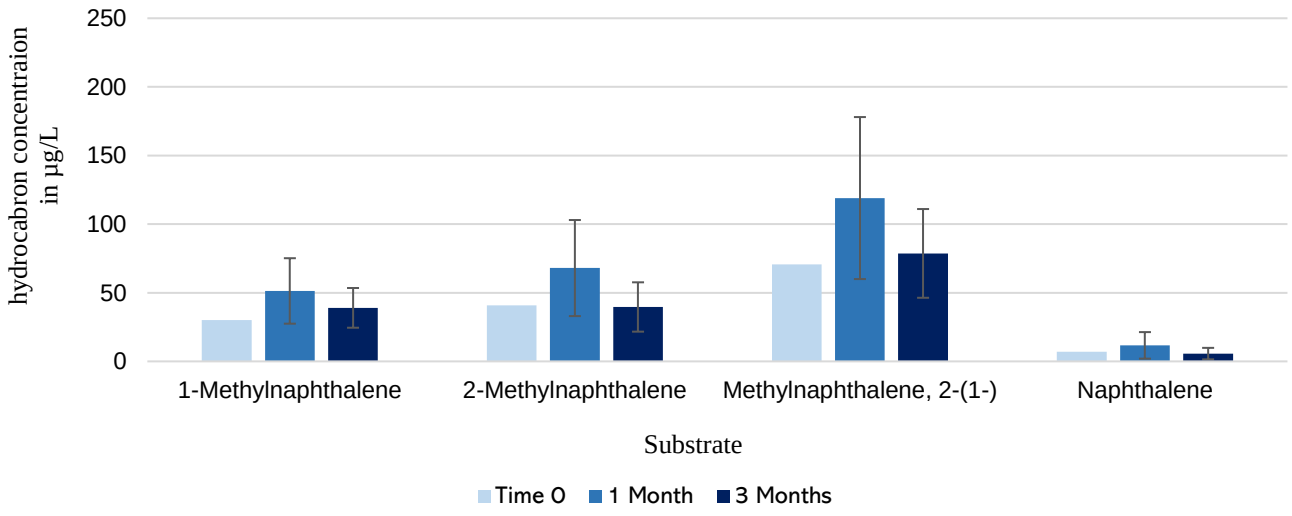
The proteins encoded by cold tolerance genes identified in each isolate were compiled in a similar fashion to the CANT-HYD data (Figure 9.). The results demonstrate the ubiquity of these proteins in the bacterial strains isolated from NWP beach sediments. Osmotic and oxidative stress response proteins were especially numerous which suggests these isolates are well adapted to the extreme cold and high salt concentrations found on NWP beaches. They also support the results of the growth analysis in low temperatures and on high salt concentration medium. All identified isolates could grow at concentration of 3% and 5% salt in the medium indicating halotolerance at the same level as sea water or above. The cold tolerance genes were ubiquitous among the isolated bacterial genomes which is consistent with the environment they were isolated from, and the findings of the growth trials where all isolates, except *Flavobacterium* sp.R1C_1I, were successfully growing at temperatures of 0 ℃. The isolates able to grow at -5℃ did not always possess more cold adaptation proteins, and none of the proteins identified in our study were consistently found in them but not in other strains.

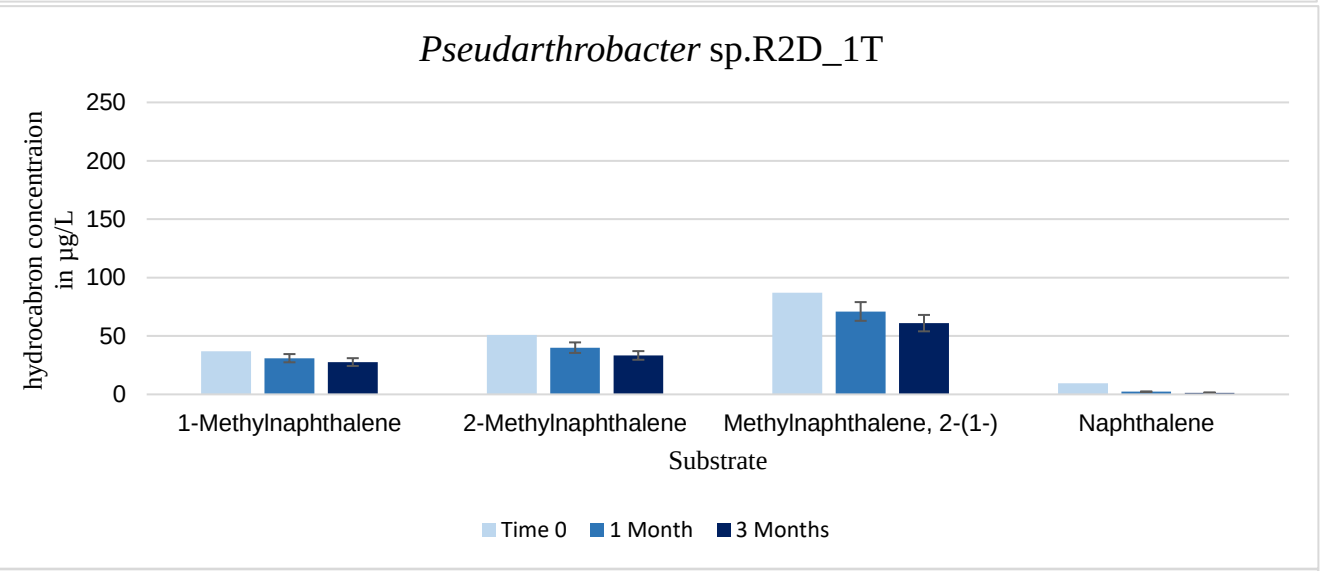

Rhodococcus sp.R1B_2T
hydrocabron concentraion in µg/L
1000000
800000
600000
400000
200000
0
F2 (C10-C16)
F3 (C16-C34)
F4 (C34-C50)
Alkane Fraction
Time 0
1 Month
3 Months

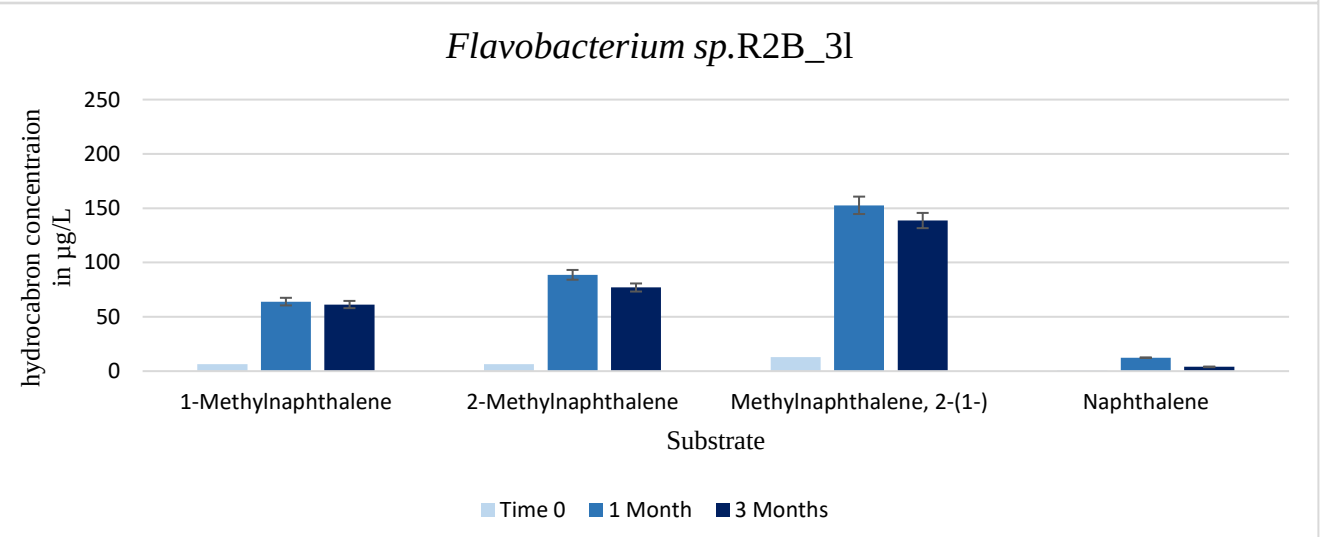

Pseudarthrobacter sp.R2D_1T
hydrocabron concentraion in µg/L
1000000
800000
600000
400000
200000
0
F2 (C10-C16)
F3 (C16-C34)
F4 (C34-C50)
Alkane Fraction
Time 0
1 Month
3 Months


*Flavobacterium sp.*R2B_3l
hydrocabron concentraion in µg/L
1000000
800000
600000
400000
200000
0
F2 (C10-C16)
F3 (C16-C34)
F4 (C34-C50)
Alkane Fraction
Time 0
1 Month
3 Months

B


Rhodococcus sp.R1B_2T
hydrocabron concentraion in µg/L
250
200
150
100
50
0
1-Methylnaphthalene
2-Methylnaphthalene
Methylnaphthalene, 2-(1-)
Naphthalene
Substrate
Time 0
1 Month
3 Months


Pseudarthrobacter sp.R2D_1T
hydrocabron concentraion in µg/L
250
200
150
100
50
0
1-Methylnaphthalene
2-Methylnaphthalene
Methylnaphthalene, 2-(1-)
Naphthalene
Substrate
Time 0
1 Month
3 Months


*Flavobacterium sp.*R2B_3l
hydrocabron concentraion in µg/L
250
200
150
100
50
0
1-Methylnaphthalene
2-Methylnaphthalene
Methylnaphthalene, 2-(1-)
Naphthalene
Substrate
Time 0
1 Month
3 Months

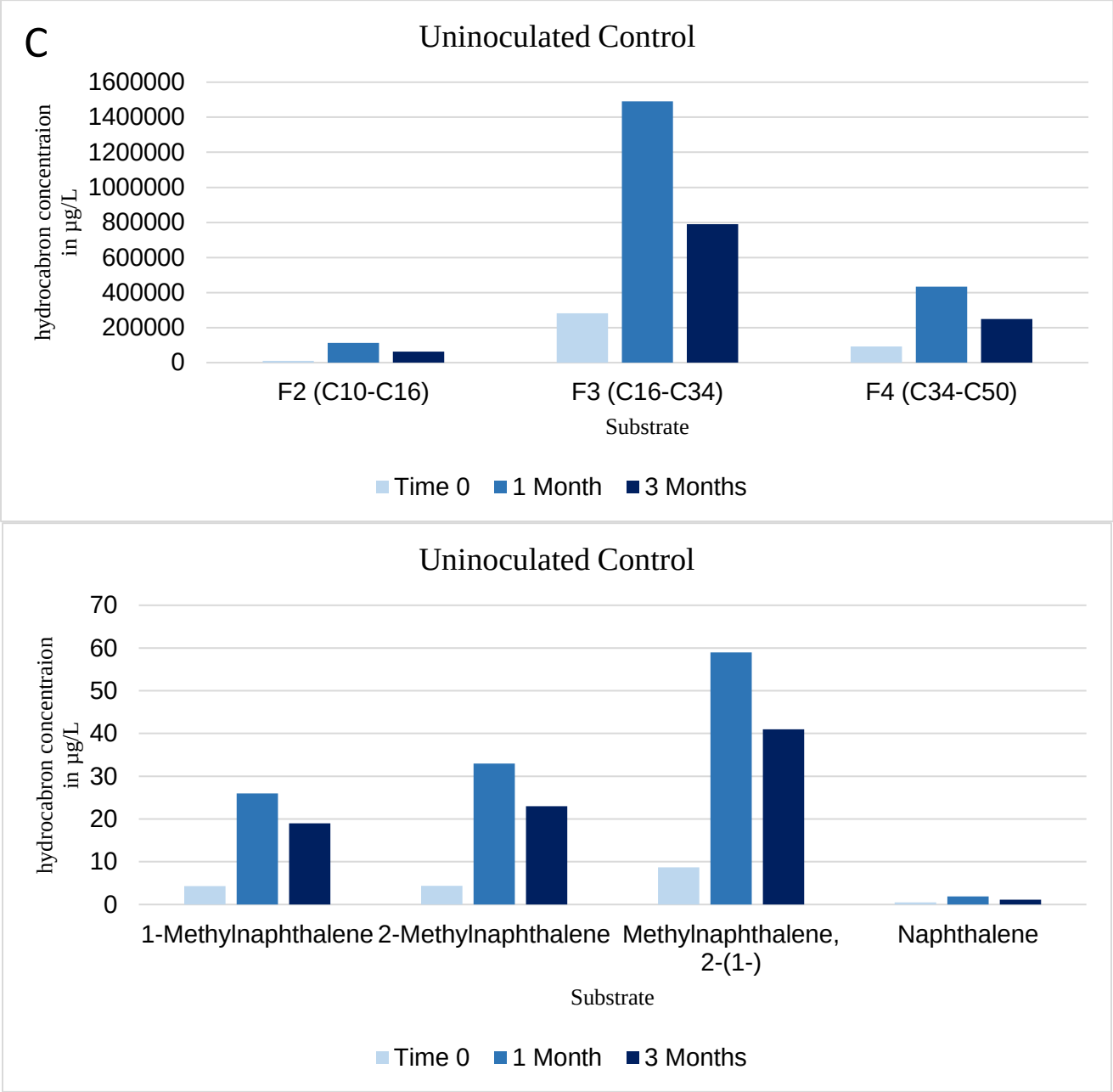
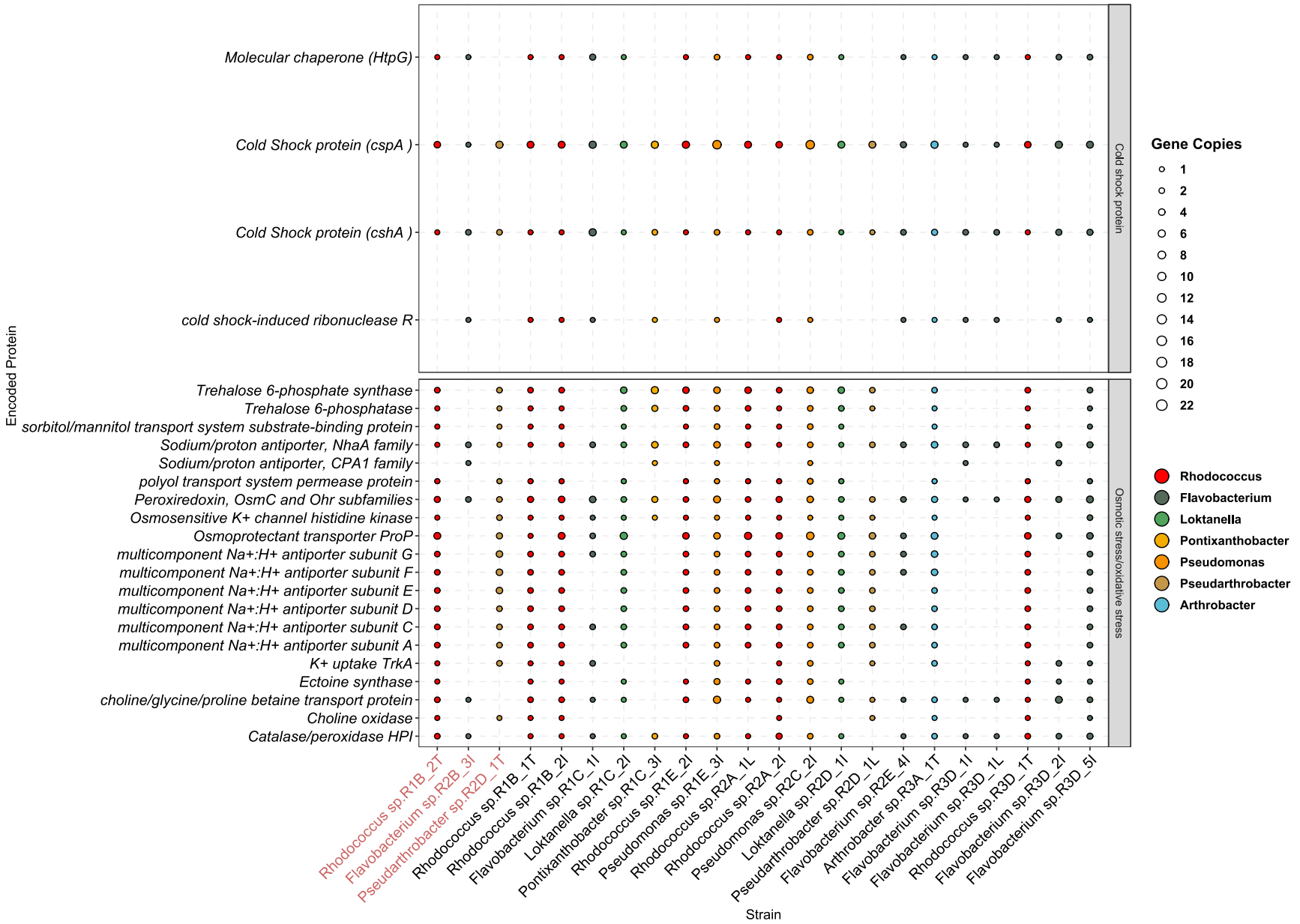


Figure 8. Results of TPH Analysis Performed on Bacterial Cultures with ULSFO as Sole Carbon Source over a 3 Month Period.

Result of hydrocarbon degradation assays conducted by SGS. A. Degradation of Alkane fraction by isolate type. B. Degradation of PAHs by Isolate types. C. Degradation of Alkane and PAHs in the inoculated controls.


Encoded Protein
Molecular chaperone (HtpG)
Cold Shock protein (cspA)
Cold Shock protein (cshA)
cold shock-induced ribonuclease R
Cold shock protein
Trehalose 6-phosphate synthase
Trehalose 6-phosphatase
sorbitol/mannitol transport system substrate-binding protein
Sodium/proton antiporter, NhaA family
Sodium/proton antiporter, CPA1 family
polyol transport system permease protein
Peroxiredoxin, OsmC and Ohr subfamilies
Osmosensitive K+ channel histidine kinase
Osmoprotectant transporter ProP
multicomponent Na+:H+ antiporter subunit G
multicomponent Na+:H+ antiporter subunit F
multicomponent Na+:H+ antiporter subunit E
multicomponent Na+:H+ antiporter subunit D
multicomponent Na+:H+ antiporter subunit C
multicomponent Na+:H+ antiporter subunit A
K+ uptake TrkA
Ectoine synthase
choline/glycine/proline betaine transport protein
Choline oxidase
Catalase/peroxidase HPI
Osmotic stress/oxidative stress
Rhodococcus sp.R1B_2T
Flavobacterium sp.R2B_3I
Pseudarthrobacter sp.R2D_1T
Rhodococcus sp.R1B_1T
Rhodococcus sp.R1B_2I
Flavobacterium sp.R1C_1I
Loktanella sp.R1C_2I
Pontixanthobacter sp.R1C_3I
Rhodococcus sp.R1E_2I
Pseudomonas sp.R1E_3I
Rhodococcus sp.R2A_1L
Rhodococcus sp.R2A_2I
Pseudomonas sp.R2C_2I
Loktanella sp.R2D_1I
Pseudarthrobacter sp.R2D_1L
Flavobacterium sp.R2E_4I
Arthrobacter sp.R3A_1T
Flavobacterium sp.R3D_1I
Flavobacterium sp.R3D_1L
Rhodococcus sp.R3D_1T
Flavobacterium sp.R3D_2I
Flavobacterium sp.R3D_5I
Strain
Gene Copies
1
2
4
6
8
10
12
14
16
18
20
22
Rhodococcus
Flavobacterium
Loktanella
Pontixanthobacter
Pseudomonas
Pseudarthrobacter
Arthrobacter

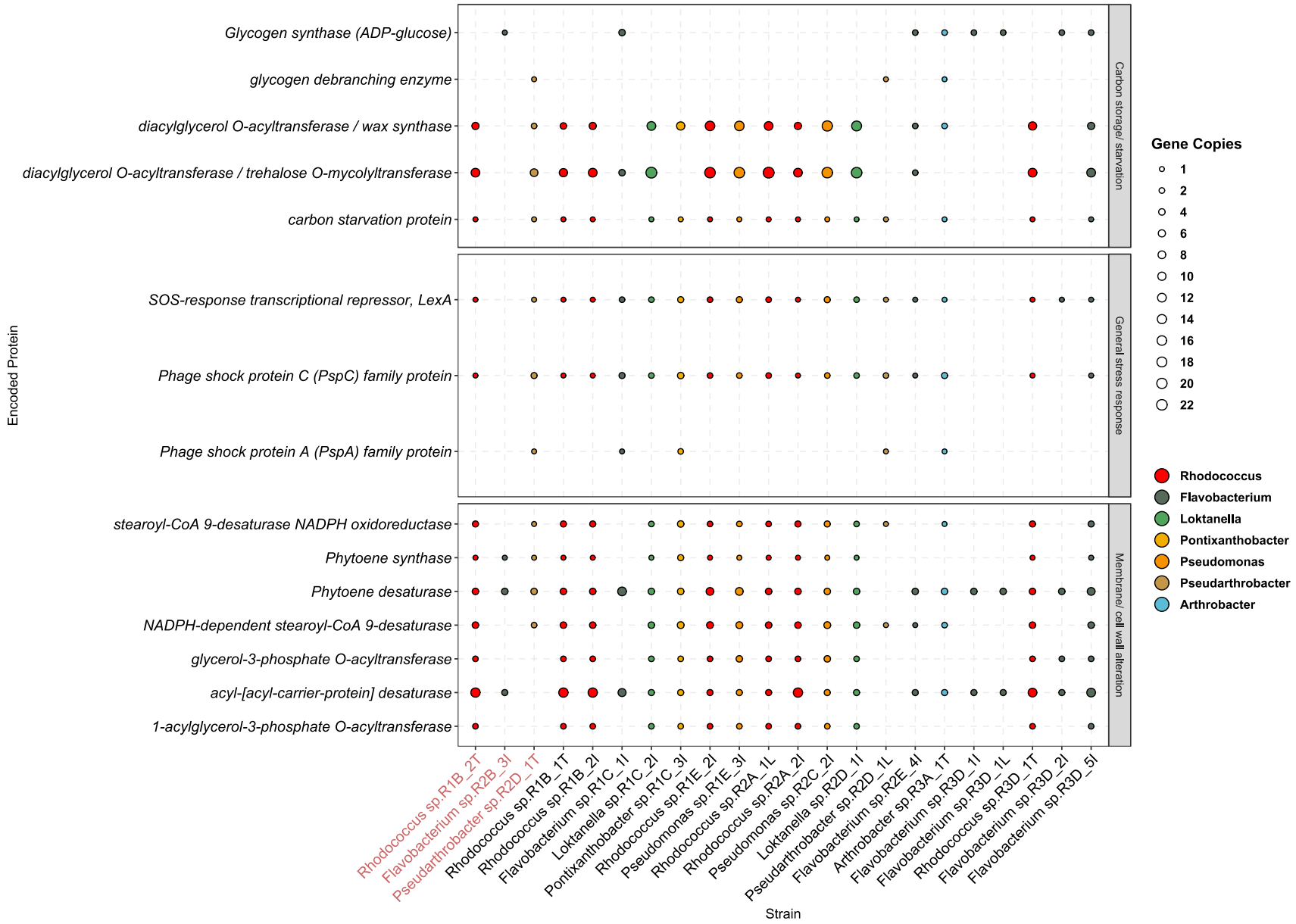

Encoded Protein
Glycogen synthase (ADP-glucose)
glycogen debranching enzyme
diacylglycerol O-acyltransferase / wax synthase
diacylglycerol O-acyltransferase / trehalose O-mycolyltransferase
carbon starvation protein
SOS-response transcriptional repressor, LexA
Phage shock protein C (PspC) family protein
Phage shock protein A (PspA) family protein
stearoyl-CoA 9-desaturase NADPH oxidoreductase
Phytoene synthase
Phytoene desaturase
NADPH-dependent stearoyl-CoA 9-desaturase
glycerol-3-phosphate O-acyltransferase
acyl-[acyl-carrier-protein] desaturase
1-acylglycerol-3-phosphate O-acyltransferase
Carbon storage/ starvation
General stress response
Membrane/ cell wall alteration
Rhodococcus sp.R1B_2T
Flavobacterium sp.R2B_3I
Pseudarthrobacter sp.R2D_1T
Rhodococcus sp.R1B_1T
Rhodococcus sp.R1B_2I
Flavobacterium sp.R1C_1I
Loktanella sp.R1C_2I
Pontixanthobacter sp.R1C_3I
Rhodococcus sp.R1E_2I
Pseudomonas sp.R1E_3I
Rhodococcus sp.R2A_1L
Rhodococcus sp.R2A_2I
Pseudomonas sp.R2C_2I
Loktanella sp.R2D_1I
Pseudarthrobacter sp.R2D_1L
Flavobacterium sp.R2E_4I
Arthrobacter sp.R3A_1T
Flavobacterium sp.R3D_1I
Flavobacterium sp.R3D_1L
Rhodococcus sp.R3D_1T
Flavobacterium sp.R3D_2I
Flavobacterium sp.R3D_5I
Strain
Gene Copies
1
2
4
6
8
10
12
14
16
18
20
22
Rhodococcus
Flavobacterium
Loktanella
Pontixanthobacter
Pseudomonas
Pseudarthrobacter
Arthrobacter

Figure 9. Presence of Cold, Salt, and Stress Tolerance Proteins Identified in Whole Genome Sequences of Three Bacterial Strains of Interest Isolated from Northwest Passage Beach Sediments Used in a Column Experiment

Cold adaptation genes observed in the 22 bacterial isolates from Tupirvik beach sediments, the expressed proteins are separated in 4 categories based on their function either for cold shock, osmotic or oxidative stress tolerance, carbon storage, membrane alteration, or other general stress response proteins.

2.3.6 Statistical analysis

Data from the hydrocarbon degradation assays was first subjected to one-way ANOVA and Kruskal-Wallis test for equal medians, however much of the data failed ($p > 0.05$) the assumptions of normality (Shapiro-Wilkins, Anderson Darling, Lilliefors, Jarque-Bera) required for ANOVA and thus non-parametric statistical tools were selected to determine significance of hydrocarbon degradation in our isolates. Analysis of similarities (ANOSIM) and One-Way PERMANOVA were used, and neither could determine significant ($p<0.05$) degradation between time points using all 3 strains or using individual strains alone for each hydrocarbon. T tests for equal means, F test for equal variance, and Mann-Whitney test for equal medians were also performed for all hydrocarbons using either all strains or each strain individually between time points and all failed to show significant decrease ($p>0.05$). All data and data analysis result has been compiled in one table which will be accessible in the supplementary data (Table S1).

2.4 Discussion

Properly investigating the ability of endemic microbial communities of Arctic shorelines in the NWP for hydrocarbon biodegradation is a necessary first step to implement informed and efficient remediation strategies in the event of an oil spill. Recent studies of biodegradation have often focused primarily on high throughput sequencing and omics analyses (Góngora et al. 2022). While those results are of great importance to the study of hydrocarbon biodegradation by revealing the genetic potential of microbial communities for hydrocarbon metabolism, they do not provide evidence of hydrocarbon metabolization by members of the community. The goal of the present study was thus to enhance current understanding of bacterial hydrocarbon biodegradation in the context of beaches of the Canadian NWP by using a culture-based approach to demonstrate metabolism of hydrocarbons is happening in members of the community in addition to performing genetic and chemical analyses.

2.4.1 Hydrocarbon degrading bacteria can be isolated from oil-enriched Arctic Sediments

All 22 isolates which were characterized in this study successfully grew on plates containing ULSFO as sole carbon source at both 5°C and 0°C which demonstrates their ability for metabolism of hydrocarbons at cold temperatures. Since aromatics are complex molecules that are more difficult to degrade, in part due to their low bioavailability (Johnsen et al. 2005) this result does not come in as a surprise. Half of the identified strains, or 11 in total, were also able to metabolize hydrocarbon at sub-zero temperatures. These results suggest bacterial strains naturally occurring in NWP beach sediments possess the ability for biodegradation, and thus are potentially useful for bioremediation in the cold Arctic environment. Furthermore, all identified isolates grew at salinities as low as 0%v/v sea salt, and up to 5%v/v sea salt, with most isolates also growing at

7%v/v, and a few more at 10%v/v which suggest their biodegradative potential broad range of usability in Arctic conditions. Arctic beaches are prone to temperature shifts due to tides; in the summer season the air can reach temperatures upward of 10 ℃ (Environment and Climate Change Canada), but the water never goes much above 0 ℃ meaning the microbes living in their sediments must be able to tolerate such temperature differences. Similarly, the salinity of the water around the beaches can increase or decrease from the usual 3% sea water salinity due to freshwater runoffs from melts, or in the presence of highly saline brine channel. A wide range of tolerance for temperatures and salinities is thus an asset for bacterial strains growing under these conditions which also makes them a potentially versatile tool in remediation.

2.4.2 Genetic Potential for Hydrocarbon Biodegradation

After identification by 16S rRNA sequencing, the whole genomes of the isolates were sequenced to be queried for genes conferring them an ability for hydrocarbon biodegradation in an effort to estimate their genetic potential as hydrocarbon biodegraders. Moreover, these same assembled genomes were queried for genes pertaining to cold and salt tolerance to offer genetic support to the growth trial results highlighted above. The 22 assembled genomes were queried using CANT-HYD software for key hydrocarbon genes, mostly those encoding enzymes, and the results demonstrated the ubiquity of those genes in the isolated strain's genomes. All isolates possessed genes for both alkane and PAH degradation, most were for aerobic degradation, but all possessed at least one anaerobic alkane degradation gene, and one anaerobic PAH degradation gene. Those with the highest potential for hydrocarbon biodegradation possessed up to 11 genes for aerobic alkane degradation, and up to 13 genes for aerobic aromatic biodegradation. The gene found in most copies across all genomes was *dszC*. This prevalence of *dszC* might be a biproduct of the utilization of sediment previously used in the column experiment for this study, which may have

led to the selection of certain genes. ULSFO was used as a contaminant in the column experiment and although the sulfur quantity in ship fuels is reduced as much as possible and is generally low as the name implies, there is still a certain quantity of it that remains after the cleaning process. Much of the sulfur that exists within ship fuels such as ULSFO exist under the form of DBT, and *dszC* is responsible for the conversion of DBT to DBT sulfone. This transformation is the first step in the 4S pathway responsible for biodesulfurization of DBT (Gray et al. 1996) and thus isolates which possessed the gene responsible for it were able to degrade DBT in the column experiment and might have possessed an advantage. This advantage would explain the prevalence of bacteria possessing *dszC* in the isolated strains. These results reflect the ability of isolated bacterial strain for hydrocarbon degradation at the gene level, but not at the expression level and the fact they have the gene does not necessarily mean it will be expressed or in what quantity it is expressed providing there is expression and further testing should be completed before they are used in bioremediation. Naphthalene 1,2-dioxygenase is a hexameric protein consisting of 3 alpha subunits and 3 beta subunits (Parales. 2003). The alpha subunits of the enzyme, which are encoded by *ndoB*, form the catalytic domain responsible for degradation of Naphthalene or Methylnaphthalene isomers (Parales. 2003, Mohapatra & Phale. 2021). The role of the beta subunit encoded by *ndoC* is still undetermined, but catabolic activity is not detected in its absence; furthermore, evidence suggests it is not involved in substrate specificity in the way the beta subunit of related enzymes such as toluene dioxygenase are (Parales. 2003). The presence of both alpha and beta subunits in *Rhodococcus* sp. R1B_2T and *Pseudarthrobacter* sp.R2D_1T, and their high degrees of degradation of naphthalene and methylnaphthalene when compared with *Flavobacterium* sp.R2B_3I, which is missing the beta subunit of Naphthalene 1,2-dioxygenase, are thus in line with the findings of Parales. 2003.

2.4.3 Potential novelty

Of the 22 identified isolates, 5 possessed a relatively low percentage identity to their closest 16S hit, verified with average nucleotide identity (ANI) through software GTDB-TK. These strains could be identified to the genus but not species level with ANI percentage of 90.16% for *Flavobacterium* sp. R3D_1L, 90% for *Flavobacterium* sp. R3D_1I, 88.72% for *Flavobacterium* sp.R2B_3I, and 94.12% for *Rhodococcus* sp.R1B_1T which confirmed these isolates were not close enough to another known bacterial species to classify together taxonomically. *Pseudarthrobacter* sp.R2D_1L also had a low 16S percent identity to its closest relative, and the closest hit was an unnamed strain named *Pseudarthrobacter* sp014217545 to which it clustered at 97.69%. Further characterization must be accomplished before novelty is established.

2.4.4 Extent of hydrocarbon biodegradation

The 3 isolates selected chosen for TPH analysis were picked following 16S analysis and a first round of growth trials. *Rhodococcus* sp.R1B_2T was chosen because *Rhodococcus* species are well established hydrocarbon degraders (Whyte et al. 1998, Van Hamme & Ward. 2001, Veselý et al. 2003) and recent results by our team detected large quantities of this genera on high Arctic beaches (Ellis et al. 2022, Góngora et al. unpublished). Confirming their ability to metabolise hydrocarbons on NWP beaches was thus of particular interest to us. Similarly, *Flavobacteria* are well known hydrocarbon degraders ubiquitous to Arctic regions of Canada (Greer et al. 2014), and the selected isolate was identified as potentially novel based on 16S rRNA amplicon sequencing results (97.76% similarity to its closest known relative). Although a few *Pseudarthrobacter* species, namely *P. polychromogenes* and *P. sulfonivorans,* have recently been identified as

hydrocarbon degraders (Naeimi et al. 2021, Zhang et al. 2016), the closest relative of *Pseudarthrobacter sp.*R2D_1T, *Pseudarthrobacter psychrotolerans*, has yet to be shown possessing any hydrocarbon degradation properties and was thus of interest for the purpose of this study. Moreover, this strains' relatively low 16S percent identity (98.47%) to its closest relative further motivated our decision. This was later confirmed by whole genome Average Nucleotide Identity (ANI) analysis through whole genomes sequencing results processed through software GTDB-TK v2.1.0 (Chaumeil et al. 2020). Throughout all the TPH analysis results, more decrease in hydrocarbon concentration was observed in *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T than in *Flavobacterium* sp. R2B_3I, except in the case of naphthalene. We believe this might be due to the starting concentration for naphthalene which was at least 4 times lower than the starting concentration of any other PAH with only about 10 µg/L at time 0. Nearly none of it was left by the end of the first month, and the results we received did not distinguish measures below 0.5 µg/L. Therefore, it is difficult to conclude whether or not *Flavobacterium* sp. R2B_3I performs as much naphthalene biodegradation as *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T. Though there is an observed reduction in the concentration hydrocarbon fractions over time when compared to Time zero, statistical analysis failed to identify significant degradation. We believe this to be due to the experimental design and physiochemical nature of the ship fuel we used. Wide variations in hydrocarbon fraction concentration were seen between flasks at each time point, both within and between cultures, and including the controls. Since we decided to sacrifice a triplicate of each culture at each time point instead of collecting a sample of the medium, we believe the starting concentration of each hydrocarbon was different in each flask making it difficult to compare the shifts in concentration. ULSFO is part of a new generation of fuels which also include hybrid fuel oils, ECA fuels, ULSFO, and Very Low Sulphur Fuel Oils (VLSFO) (Lepers & Legrand, 2022). A recent study about the weathering of such fuels obtained similar high

variability in their measurements (Lepers & Legrand, 2022). They believe this could be due to the heterogeneity of the fuel due to air bubbles or water getting trapped in the fuel during pouring, or that the "crust" formed by this oil could have much higher evaporation rates than the rest of it, causing an heterogenous distribution of hydrocarbon compounds within the fuel (Lepers & Legrand, 2022). Furthermore, current rules and regulation regarding the use of this type of fuel by regulatory bodies such as the American Bureau of Shipping (ABS) recommend the use of a homogenizing device to maintain fuel quality and prevent damage to the equipment (American Bureau of Shipping, 2021). Further experimentation is thus required to determine rates of biodegradation of ULSFO where the fuel is properly homogenized prior to introduction in the medium. While we acknowledge that the variation between samples were large, with certain hydrocarbons concentration within a single flask being both larger for some hydrocarbons and lower for others than the concentration of most other microcosms, we think these results are still relevant. Firstly, the degradation results are supported by the reconstructed metabolic pathways obtained through genetic survey, which showed complete or nearly complete alkane degradation pathways in the case of *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T, but a pathway lacking elements for *Flavobacterium* sp. R2B_3I. Similarly for the naphthalene and methylnaphthalene degradation pathways, the only gene present in *Flavobacterium* sp. R2B_3I, in which the lowest decrease in PAHs was observed, was *ndoB.* NdoB is the catabolic subunit of the protein involved in the first step of naphthalene biodegradation (Parales. 2003), but *Flavobacterium* sp. R2B_3I was missing *ndoC* which is required for activity; therefore, the lower extent of degradation observed in this strain when compared to the two others is supported by genetic evidence. Although the exact extent of degradation might not be established with the current results, all isolated bacterial strain used in this experiment were grown on medium containing ULSFO as a sole carbon source. This demonstrates their ability to grow on, and thus metabolise, ship fuel and the various fractions of hydrocarbons therein.

2.4.5 Presence of cold and salt tolerance genes

The genetic results showing cold and salt tolerance of isolated strains supported the results found in our prior growth essay. In the case of the few isolates unable to grow at 7.5% salt, they did not seem to necessarily possess fewer salt tolerance genes than those who could, except for *Flavobacterium* sp. R2B_3I, which was also used for the TPH degradation assays and possessed relatively fewer osmotic stress response genes than the other isolates. On the other hand, the few isolates that showed growth at 10%, were all among the strains with the larger number of salt tolerance proteins encoded by their genomes. On the other hand, in the case of the isolates which were able to grow at -5°C, neither the number of proteins present nor the selected proteins were a consistent predictor of their ability to grow at sub-zero temperatures; however, as the list built and used in this study is in no way exhaustive, this result does not suggest there are no reliable predictor for the ability to grow at -5°C found in these strains, but only that these proteins were not identified in this study.

2.4.6 Challenges and limitations

The results of this research corroborate prior findings from works focused on bioremediation of hydrocarbons in the Arctic; especially those which focused of meta data analysis alone. Though bacterial isolates were identified and demonstrated an ability to utilize and thus degrade hydrocarbons, whether or not this ability can be translated into an efficient *in situ* remediation strategy remains to be proven. During the chemical analysis assay, microcosms were sacrificed after only 1- and 3-month incubation periods, therefore their ability to degrade hydrocarbons over a longer period of time could not be established. Furthermore, the isolated bacterial strains were

shown to grow on a fairly common shipping fuel, namely ULSFO; however, several other such fuels are still widely used such as marine diesel and bunker C, and further studies involving those products would highly enhance our ability to predict the usability of endemic microbes as a remediation strategy.

Due to the events of the COVID-19 pandemic, the 2020 field season was cancelled and thus fresh sediments samples could not be obtained for the completion of this experiment. The used sediments collected from Tupirvik beach in 2019 were used in a column experiment study which were treated them with ULSFO and inorganic and oleophilic fertilizers over a period of 32 days. While the isolated bacteria are likely native from Tupirvik, the prior exposure to these chemicals may have affected the community structure and favoured the survival of certain strains over that of others, and thus further experimentation using pristine sediment might allow for the isolation of previously unculture strains that were selected out during the column study.

2.5 Conclusion

In this study, we used a culture-based approach to determine the ability of bacterial isolates from NWP sediments to metabolise hydrocarbons from ULSFO, and a combination of genomic and chemical approaches to support our findings and characterize the biodegradation potential of individual from the sediment's microbial communities. The CANT-HYD and TPH analysis results were consistent with each other, showing that the isolated *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T, which possessed complete degradation pathways, performed better in degrading both alkanes and PAHs from ULSFO when compared to *Flavobacterium*. *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T also possessed numerous cold and salt adaptations genes making them fit to tolerate the rough Arctic conditions. Furthermore,

*Pseudarthrobacter sp.*R2D_1T was shown growing on minimal plates containing ship fuel as sole carbon source at a temperature of -5℃, identifying it as a biodegrader of hydrocarbons at very low temperatures. The results of WGS for key hydrocarbon degradation and cold adaptation genes on 22 isolates from NWP beach sediments, and growth trials on ULSFO as sole carbon source suggest that several bacterial species found on those beaches possess the potential for biodegradation of ship fuels, and could thus potentially be used in the cleanup efforts in the events of an oil spill in the NWP. The results of our study demonstrate that isolates *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T have both possess the ability for hydrocarbon biodegradation, and could thus potentially be used for bioremediation in the events of an fuel spill reaching the shores of the NWP. Furthermore, the genetic and growth results of the experiments on 22 isolates from Tupirvik beach demonstrate that a large diversity of naturally occurring microbes found on those beaches also possess the potential for hydrocarbon biodegradation, which could offer an effective response if such a spill was to reach the sediments of NWP beaches.

2.6 Conflict of Interest

The authors declare no conflict of interest.

2.7 Acknowledgements

The funding support for this research was provided by the Department of Fisheries (DFO) to whom we would like to offer our utmost gratitude. Thank you to Northern Scientific Training Program (NSTP) for funding field work and to Canadian Polar Continental Shelf Program (PCSP) and Devon Manik for providing logistical support and help during field work.

2.8 Data Availability

All genomic samples are in the DOE JGI Genome Portal under Sequencing Project IDs Ga0646953 and Ga0648373 to Ga0648393 and Analysis Project IDs Ga0555976 and Ga0557208 to Ga0557228. Raw sequencing reads are available at NCBI under BioProject ID PRJNA945214.

Chapter 3. Discussion

Properly investigating the ability of endemic microbial communities of Arctic shorelines in the NWP to degrade hydrocarbons is paramount to inform and implement efficient remediation strategies in the near future. Recent studies of biodegradation have focused primarily on high throughput sequencing and analysis of metagenomes. While those result are of great importance to the field, they do not generally allow to understand and conclusively demonstrate the role microorganisms play in the biodegradation of hydrocarbons, which is only really achievable through isolation and characterization of bacterial strains. The aim of this work was thus to enhance the understanding of hydrocarbon biodegradation in the context of the Canadian NWP through the usage of isolation techniques and assessment of chemical and genetic factors involved in biodegradation.

All 22 bacterial isolates which were characterized in this study successfully grew on growth minimal medium containing ULSFO as sole carbon source at both 5°C and 0°C which demonstrates their ability to metabolize hydrocarbons at cold temperatures. Half of the identified strains, or 11 in total, were also able to metabolize hydrocarbon at sub-zero temperatures (-5ºC). These results demonstrate bacterial strains naturally occurring in NWP beach sediments possess the ability for biodegradation at sub-zero temperatures, and thus are potentially useful for bioremediation in the events of oils spills in the cold Arctic environment. Furthermore, all identified isolates grew at salinities as low as 0%v/v sea salt, and up to 5%v/v sea salt, with most isolates also growing at 7%v/v, and a few also growing at 10%v/v. This suggest their biodegradative potential possesses a broad range of usability in Arctic conditions which is known to have variable salinities which can be lower than regular seawater due to freshwater runoffs and melts, or reaching much higher salt concentrations in brine channels. The isolates which

metabolized hydrocarbons did so on plates containing 5% v/v salt to avoid the freezing of the medium, further demonstrating the isolates' ability for metabolism of hydrocarbons in a combination of sub-zero temperatures in saline microhabitats.

The 22 assembled genomes were queried using CANT-HYD software for key hydrocarbon genes, mostly those encoding enzymes, and the results demonstrated the ubiquity of those genes in the isolated strain's genomes. All isolates possessed genes for both alkane and PAH degradation, most of which were for aerobic degradation, but all possessed at least one anaerobic alkane degradation gene and one anaerobic PAH degradation gene. Those with the highest potential for hydrocarbon biodegradation possessed up to 11 genes for aerobic alkane degradation, and up to 13 genes for aerobic aromatic biodegradation. These results reflect the ability of isolated bacterial strain for hydrocarbon degradation at the gene level, but not at the expression level and the presence of the gene in their genome does not necessarily mean it will be expressed or in what quantity it is expressed providing there is expression. Therefore, further testing should be conducted before they are considered for bioremediation. Biomass was extracted for the 3 strains used in the TPH experiment study after each timepoint and kept on RNA shield at -20 ºC. A follow up study using transcriptomics will be performed to confirm the expression of hydrocarbon degradation genes observed in our isolates' genomes.

Throughout all the TPH analysis results, a greater decrease in hydrocarbon concentration was observed in *Rhodococcus* sp.R1B_2T and *Pseudarthrobacter* sp.R2D_1T than in *Flavobacterium* sp. R2B_3I with the exception of naphthalene biodegradation. Though there is an observed reduction in the concentration of various hydrocarbon fractions over time, statistical analyses failed to identify significant degradation. While we acknowledge that the variation between samples were large, with certain hydrocarbons concentration within a single flask being both larger

for some hydrocarbon fractions and lower for others than the concentration of most other microcosms, we think these results are still relevant. We believe the lack of significance of the results may be a biproduct of the heterogeneity of the fuel rather than the absence of degradation. Firstly, the degradation results are supported by the reconstructed metabolic pathways obtained through genetic survey, which showed complete or nearly complete alkane degradation pathways in the case of *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T. That pathway however was lacking genes for rubredoxin recycling and alcohol dehydrogenase in *Flavobacterium* sp. R2B_3I. Similarly for the naphthalene and methylnaphthalene degradation pathways, the only gene present in *Flavobacterium* sp. R2B_3I, in which the lowest decrease in PAHs was observed, was *ndoB.* NdoB is the catabolic subunit of the protein involved in the first step of naphthalene biodegradation (Parales. 2003), but *Flavobacterium* sp. R2B_3I was missing *ndoC* which is required for activity; therefore, the lower extent of degradation observed in this strain when compared to the two others is supported by genetic evidence. The genetic results which demonstrated the absence or incompleteness of degradation pathways in *Flavobacterium* sp. R2B_3I thus supports the degradation assay in which *Flavobacterium* sp. R2B_3I showed the lower level of degradation, whereas *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T which possessed complete or nearly complete pathways had showed higher degradation. Moreover, although the exact extent of degradation might not be established with the current results, all isolated bacterial strain used in this experiment were grown on medium containing ULSFO as a sole carbon source. This demonstrates their ability to grow on, and thus metabolise, ship fuel and the various fractions of hydrocarbons therein.

Genomic analysis showing cold and salt tolerance of isolates supported the results found in our prior growth assays. In the case of the few isolates unable to grow at 7.5% salt, they did not seem

to consistently possess fewer salt tolerance genes than those who could grow under these conditions, with the exception of *Flavobacterium* sp. R2B_3I, also used for the TPH degradation assays, which possessed relatively fewer osmotic stress response genes than other isolates which successfully grew under 7.5% salinity. On the other hand, the few isolates that showed growth at 10%, were all among the strains with the larger number of salt tolerance proteins encoded by their genomes. This difference was not reflected in the case of the isolates which were able to grow at -5°C. Neither the number nor the presence of specific proteins were a consistent predictor of their ability to grow at sub-zero temperatures; however, the list of cold and salt resistance genes built and used in this study is in no way exhaustive, and thus these result do not suggest there are no reliable predictor for the ability to grow at -5°C found in these strains, but only that the genes responsible for this difference may not have been identified in this study.

While these findings help improve our grasp of microbial biodegradation in the Arctic, future research would highly improve our understanding. In this study, only one type of ship fuel, ULSFO, was used whereas other such as marine diesel and bunker C are still commonplace, and our results might not be translatable to those other fuels. Furthermore, due to the impact of the COVID-19 pandemic and the cancellation of the 2020 field season, sediment samples collected from Tupirvik beach in 2019 and which had been used, albeit carefully, in the column study detailed above were selected. Certain isolates of interest could thus have been missed if they did not survive until our isolation experiment or were removed due to the hydrocarbons or fertilizers used in the column study. Future studies using pristine sediments collected shortly prior to culture might allow the isolation of bacteria which reflect the naturally occurring population better. Moreover, it was discovered recently that the type of low sulphur fuel used in this study requires homogenisation, or otherwise its chemical composition may vary across the medium. We believe this impacted the

result of our TPH analysis negatively. Thus, with these two factors in mind, conducting a new study where TPH of fuel degraded by more bacterial species from NWP beaches using homogenizing fuel could offer greater insight on the extent of degradation offered by cultured organisms from NWP beaches. Additionally, *in situ* experiments using isolates identified as possessing hydrocarbon biodegradation abilities in the lab could be conducted on NWP beaches mesocosms to determine their ability to be deployed for remediation in the field in the events of an oil spill through a bioaugmentation treatment or through increasing their hydrocarbon biodegradation rates through biostimulation treatment options such as N and P or surfactant amendments. Additionally, other isolation methods including enrichment broth could have allowed for the screening of bacteria that were currently injured or dormant and were missed by our methods and a trial using such a method might offer better understanding of the hydrocarbon degraders found in the community.

Overall, the results of this study highlight the presence of hydrocarbons degraders in the Canadian high Arctic which possess the potential for hydrocarbon biodegradation in very cold and highly saline environments, extreme conditions which are frequently found in the Arctic. However, for these results to be translated into a response to oil spills on NWP beaches, much work remains. Firstly, the beaches are covered by ice for most of the year which may affect the metabolic activity of naturally occurring microbes severely. Additionally, possessing the genetic ability to degrade hydrocarbons, and being able to do so to an extent that is useful for their cleanup are separate results, and thus more research should be conducted about the implementation of microbial hydrocarbon degraders as a response to oil spills on Arctic beaches before it is considered. These results clearly identify the potential for bioremediation, whereas this potential can be used in practice, however, remains to be shown.

Chapter 4. Conclusion and summary

Despite these challenges and limitations, the findings of this study complement the findings of earlier studies of hydrocarbon biodegradation in Arctic conditions, such as those of Vergeynst et al. 2018, which relied on omics and chemical analyses alone, by isolating and characterizing naturally occurring bacterial strains from Arctic beach sediments and demonstrating their ability to successfully metabolize ship fuel. Furthermore, the growth and chemical analyses conducted demonstrate that degradation of specific hydrocarbon fractions present in ULSFO, and the genetic results showed that the potential for biodegradation is indeed present in NWP beach communities. Overall, this study contributes to a growing effort to understand microbial communities of the Canadian Arctic which aims at protecting our ecosystems from harm and helping them recover using bioremediation in the events of an oil and fuel spill. Our results suggest that strains *Rhodococcus sp.*R1B_2T and *Pseudarthrobacter sp.*R2D_1T have potential for bioremediation in the events of a fuel spill reaching the shores of the NWP, and that many more naturally occurring and diverse microbes could also offer an effective response if such a spill was to reach the sediments of NWP beaches.

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