ORGANISMS WHICH CAUSE
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By

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The problem of blackening is a serious one in the canning industry. Blackening is a common form of deterioration, in advanced stages of which the contents of the tin becomes inky black and disintegrated. There is an intense disagreeable odour in which a metallic quality is combined with the odour of putrefaction. Often there is a large amount of gas, causing the cans to be swelled as well as blackened.

The cause of blackening is usually accepted as bacterial action. It has long been studied from this point of view. In 1897, a paper on "Discolouration in Canned Lobsters" was published by Macphail and Bruére.[1] The authors had isolated from spoiled cans four micro-organisms which, upon inoculation into sterile cans, produced the blackened condition. Prescott and Underwood,[2] in the same year, isolated from swelled tins of clams and lobsters nine species of bacteria, which led to similar conditions if introduced into normal cans. These organisms were found to be very resistive to heat. The authors of both papers pointed out the necessity of accurately determined minimal periods of processing. This determination involves a study of the bacteria concerned. It is also important to know the source of the micro-organisms which appear in the cans, in order to ascertain the chances of preventing infection.

The investigation described in this paper was undertaken at the suggestion of Dr. A. G. Huntsman, Curator of the Atlantic Biological Station at St. Andrews, N. B. Its aim has been a bacteriological examination of freshly dug clams (Mya arenaria), such as are taken to the factories for canning, with a view to the isolation and description of any blackening organisms normally present in such clams.

PROCEDURE.

Isolations.

The clams from which isolations were made were of medium size. They were dug at low tide on the beach near the Biological Station and brought in at once. The exteriors of the shells were thoroughly washed in a stream of running tap water and dried in the air. The edges were then seared in a flame and the muscles holding the shell quickly severed with a knife*. One half of the shell was then removed, the clam lying in the other half. The mantle was slit and turned back and four small pieces cut* from the body of the clam were at once transferred with forceps* to separate tubes of broth. Cuttings were thus made at different times from all parts of the clam within the mantle. In several cases, a culture was made from a little of the shell liquor withdrawn through the pedal orifice by means of a sterile pipette. No attempt was made to associate

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* All knives, scissors and forceps used for these purposes were kept standing in a jar of alcohol and sterilized by flaming immediately before use.

an isolated organism with the part of the clam in which it was found.

To simulate factory conditions, a few clams were washed as described above and allowed to stand at room temperature (about 20°C) for 18 hours before further steps were taken. Others were treated similarly and left 48 hours at room temperature before being opened. In both cases, the clams were found to be still alive.

Clam peptone broth and beef peptone broth were both used for first cultures in every case. Good growth was obtained in both media. In many instances, it appeared earlier in the clam broth and was more luxuriant. After growth for twenty-four hours at room temperature, plate cultures were made from each tube, both in clam peptone agar and beef peptone gelatin. These plates were examined as soon as growth appeared usually after from 14 to 18 hours. Plating was done in the late afternoon, the plates as a rule showing growth the next morning. Most of the organisms liquified gelatin rapidly, hence it was necessary to deal with gelatin plates first. Colonies were carefully examined and from each one chosen as representative of a new type, an agar stab, an agar slant and a smear for microscopic examination were made. Seventy-six cultures were thus obtained during four weeks beginning July 24, 1920. Agar stab cultures of each were kept until the opening of the autumn session at McGill University, when laboratory facilities permitted further investigation.

MEDIA USED

1. Isolation Media

Clam peptone broth and Clam peptone agar.

These were made according to directions given by Sadler in his paper on "The Bacteriology of Swelled Canned Sardines."

Beef peptone broth.

Distilled water	
Liebig's meat extract	0.5 per cent.
Difco peptone	1.0 per cent.
Sodium chloride	0.5 per cent.

Beef peptone gelatin.

Beef peptone broth with 12 per cent of Difco gelatin.

2. Media for determining the production of Hydrogen Sulphide.

(a) Lead carbonate gelatin (Beijerinck)

Beef peptone gelatin + lead carbonate 0.1 per cent.

(b) Peptone-lead acetate solution (Pake)

This medium was prepared according to the following directions:- Emulsify 30 g. peptone with 200 cc. tap water at 60°C. Wash into a litre flask with 80 cc. tap water. Add 5 g. sodium chloride and 3 g. sodium phosphate. Heat at 100° for half an hour. Filter through paper. Tube. To each tube of 10 cc, add 0.1 cc. of 1 per cent. solution of lead acetate. This produces a yellow precipitate which is blackened if H_2S is formed. The solutions should be

neutral.

Difco peptone was used.

(c) Clam media.

1 part clam meat + 2 parts sea water.

3. Media for differentiation.

Beef peptone agar.

Beef peptone broth + 1.5 per cent. of Japanese agar.

Beef peptone gelatin

Prepared as already described.

Beef peptone Broth. Two kinds were used.

(1) as described above.

(2) Difco nutriment broth 0.8 per cent.

Sodium chloride 0.8 per cent.

Distilled water.

Peptone water. (Dunham's)

Peptone 1 per cent.

Sodium chloride 0.5 per cent.

Distilled water.

Nitrate Broth.

Dunham's peptone water + 0.5 per cent. KNO_3

Potato.

Wedges were soaked 30 minutes in 1 per cent. sodium carbonate, rinsed thoroughly in distilled water and sterilised.

Litmus Milk.

Klim adjusted to +1 reaction and sterile litmus solution added.

Fermentation Media .

Dunham's peptone water was used as a foundation.

Dextrose Medium.

Peptone water + 2 per cent. dextrose.

Lactose Medium.

Peptone water + 2 per cent. lactose.

Saccharose Medium

Peptone water + 2 per cent. saccharose.

Glycerine Medium

Peptone water + 6 per cent glycerin.

To each of these 2 per cent Andrade indicator was added.

Mannite protein-free broth

Mannit	15.0	grams
$K_2 HPO_4$	0.2	"
$Mg SO_4$	0.2	"
Na Cl	0.2	"
$Ca SO_4$	0.1	"
$Ca CO_3$	5.0	"

10% $Fe_2 Cl_6$ solution 1.0 drop
Distilled water 1000 cc.
Do not filter.

- Sterilize at 120°(autoclave) for 10 minutes.

Mannit protein-free agar.

1.5 per cent. washed agar added to mannit solution prepared as described.

Reaction of Media

Gelatin, Agar, Broth and Milk were adjusted to +1 reaction.

Sugars and glycerin broth were neutralised.

Peptone water and Nitrate broth were left unadjusted (.4 per cent acid to phenol phthalein).

Sterilisation of Media.

All media except milk and gelatin were sterilised in the autoclave for 15 minutes under 15 pounds pressure.

Gelatin and milk were sterilised by the discontinuous method in the Arnold Steam Steriliser.

Separation of blackening organisms.

The first phase of the problem was the search for blackening orgamisms. Three methods were employed:

Method 1

Streak cultures were made upon plates of lead carbonate gelatin. The result was unsatisfactory. Complete liquefaction followed before definite conclusions could be drawn with regard to blackening. Cultures i.j.r.s.t.u. produced slight darkening of the lead carbonate after two days, then rapidly liquefied the plates.

Method 2

Cultures were transferred from 24 hour broth cultures to the strong peptone solution, recommended by Pake,

as a test for the production of Hydrogen sulphide.

Fifteen organisms - viz., 1. 3. 11. 12. 16. 17. 28. 29. 31. b. e. j. r. s. t., turned the precipitate black and were thus differentiated as H_2S formers. Some others darkened the precipitate to brown only. All cultures were kept under observation for more than four weeks.

The fifteen organisms were transferred from broth cultures to other media, a study being made of preparations in Dextrose broth, Gelatin stick, Litmus milk and potato.

Method 3

Myers[7] called attention to the fact that some organisms produce hydrogen sulphide on one brand of peptone and not on another. This suggested the advisability of testing all organisms in clam meat itself. Tubes were prepared according to Dr. Harrison's suggestion, using clam meat and sea water in the proportions of one to two. They were prepared in triplicate - about seven hundred in all - arranged in the following series:-

A. Clam meat + sea water + iron.

B. Clam meat + sea water + tin.

C. Clam meat + sea water + iron + tin.

Chemically pure iron wire was used in small pieces (1/8 in. to a tube) and chemically pure flaked tin. Sterilisation was done in the autoclave, 15 minutes at 15 pounds pressure.

Dr. Huntsman kindly arranged that fresh clams and sea water for this experiment be sent from St. Andrews, N.B. They arrived in perfect condition and were opened and prepared at once.

Transfers were made from all original stab cultures to beef peptone broth and plated after 24 hours to determine purity. This having been established, tubes of series A.B.C. were seeded in triplicate from broth cultures of all organisms. Three tubes of each series were kept uninoculated as controls.

The seeded clam tubes were left at room temperature until growth was evident - 24 to 48 hours - then kept in an unheated room from four to six weeks.

Blackening developed rapidly in many tubes, slowly in others. The final result is seen in the accompanying table.

(+) indicates very deep blackening of all clam meat

\+ " deep " of part of clam meat

± " dark greenish-brown colour - more characteristic of tin sulphide.

\- " No blackening

Twenty-eight of the seventy-six original cultures were thus seen to be capable of producing blackening when provided with the elements to be found in a can. Among these, with the exception of Number 29, are the organisms separated by methods 1 and 2, though Number 16 showed such a feeble result in the clam medium that it has been disregarded. Number 13, which showed a positive result by the third method only, was also rejected.

No.	Fe.	Fe.	Fe.	Sn.	Sn.	Sn.	Fe. Sn.	Fe. Sn.	Fe. Sn.
1	(+)	(+)		+	+	+ ±	(+)	−	−
3	(+)	+	+	−	−	−	(+)	(+)	+
6	+	(+)	(+)	−	−	−	(+)	+	+
10	(+)	(+)	(+)	±	+	+ ±	(+)	(+)	(+)
11	(+)	(+)	(+)	+	+	+	(+)	(+)	(+)
12	(+)	(+)	(+)	±	−	±	(+)(+)	(+)	(+) *
13	(+)	+	±	−	−	−	+	±	+
16	±	±	±	−	−	−	−	−	−
17	(+)	(+)	−	+	+	+	(+)	(+)	(+)
20	(+)	(+)	(+)	±	+	+	(+)	(+)	(+)
26	+	(+)	(+)	+	−	−	(+)	(+)	(+)
28	(+)	(+)	(+)	+	±	+	(+)	(+)	(+)
31	(+)	+	−	±	−	−	−	−	−
d	(+)	(+)	− Reddish	(+)	±	− Reddish	(+)	(+)	(+)
e	(+)	(+)	(+)	+	+	+ ±	(+)	(+)	(+)
f	(+)	(+)	(+)	+	+	±	+	+	+
g	(+)	(+)	(+)	±	±	±	(+)	(+)	(+)
h	(+)	(+)	(+)	+	+	+	(+)	(+)	−
1	(+)	(+)	(+)	+	±	−	(+)	(+)	(+)
j	(+)	(+)	(+)	+	+	+	(+)	(+)	(+)
R	(+)	(+)	(+)	+	+	(+)(+) *	(+)	(+)	(+)
S	(+)	(+)	(+)	(+)	(+)	−	(+)	(+)	(+)
t	(+)	(+)	(+)(+)	(+)	(+)	(+)	(+)(+)	(+)(+)	(+) *
u	(+)	(+)	(+)	+	+	+	(+)	(+)	(+)
w	(+)	(+)	(+)(+)	±	+	+	+	+	+
18	(+)	(+)	(+)	+	−	−	(+)	(+)	(+)
30	(+)	(+)	(+)	±	+	+	(+)	(+)	−
4	−	(+)	(+)	(+)	(+)	± Reddish	(+)	+	(+)

* Extra tubes.

Classification

Nothing had yet been done with a view to eliminating repetition of cultures, except the preliminary observation in four media of the fifteen H_2S formers found by method 2. Of these, all had liquefied gelatin rapidly and fermented dextrose without the production of gas. Variation had been noted in the growth upon potato and litmus milk.

With the aim of separating and determining different forms, it was decided to grow subcultures of all organisms simultaneously. Transfers were, therefore, made from the most recent stab cultures of all blackening organisms to peptone water for invigoration. After 24 hours, they were thence transferred in duplicate to Gelatin stick, sugars, potato, litmus milk, nutrient broth, agar slants and nitrate broth. Fresh peptone water cultures were made daily until inoculations were completed. Slides for microscopic examination were also prepared. Growth characteristics were noted and comparisons made from day to day, all cultures being kept for six weeks. Plate cultures on agar, gelatin, and starch agar were also prepared and observed.

The most recent descriptive chart of the Society of American Bacteriologists was used as a guide in choosing media and recording results.

For the indol test, cultures were grown in Dunham's peptone water for five days and the Nitroso-indol-nitrate test made at the end of this period.

For nitrate reduction, sulphanilic acid and naphthylamine hydrochloride were added in equal quantities (3 drops of each)

to nitrate-broth cultures 48 hours old.

Controls were used in both these cases.

Reduction was unmistakable in all cultures, so that it was not necessary to repeat the tests after longer periods of growth.

Six forms were finally separated, repeated tests being made in many cases. Potato cultures, for instance, were tried both at room temperature and at 37.5°C.

Characteristics common to all the Isolated Organisms.

Preparation stained for flagella revealed the fact that all belonged to the genus Pseudomonas, one variety possessing a tuft of polar flagella, the others having a single flagellum attached to one pole. Endospores were not observed in any of the species. All liquefied gelatin rapidly and digested casein, though in one instance the latter process was very slow. All formed hydrogen sulphide and reduced nitrates to nitrites. All fermented dextrose, saccharose and glycerin. At first they gave strong acid reaction, which began to change about the fourth day, the contents of the tube gradually becoming alkaline throughout. In one case only, lactose was fermented, though all cultures grew well, and produced turbidity in lactose broth. The common characteristics here noted will not be mentioned in the detailed descriptions below, which rather aim to point out distinguishing traits.

Organisms Identified

1. Pseudomonas fluorescens (Flügge) Migula.

The organism was easily stained and appeared as a rod of medium size with rounded ends, which usually occurred in pairs. Six flagella were observed in the polar bundle. On gelatin plates the colonies appeared after two days as round centres of liquefaction. A white mass of bacteria occupied the centre of the depression which was cup-shaped. Deep colonies were white, with shadowy margins which, as the microscope revealed, were made up of radiating hairs.

In the gelatin stab cultures, the line of inoculation showed a trace of liquefaction after 24 hours. In 36 hours its margin was clouded by numerous discrete, white, punctate colonies. At the same time, a crateriform liquefaction had developed on the surface, with a white film lining the depression. The liquefaction soon became infundibuliform and the medium acquired a distinct fluorescence.

On potato, a dirty-greyish growth appeared after 24 hours. Though dull at first, it became moist, spreading and shining, deepening in colour to fawn. The potato was at the same time darkened to brown.

Broth was rendered turbid with a strong yellow green fluorescence and a membranous shining pellicle.

Diastatic action on starch was positive.

On agar slants the growth was of a yellowish-grey colour, moist, shining and spreading, the subtratum becoming

distinctly fluorescent.

In litmus milk, the reaction was distinctly alkaline after 24 hours, the blue colour deepening for a week, then remaining constant; even at the end of 6 weeks no digestion was observable. A white shining pellicle was produced on all liquid media.

2. Pseudomonas Jaegeri(Migula).

This organism was somewhat variable in form. As a rule, it was a short, thick rod with rounded ends. Flagella were difficult to count; in many cases they were turned back and formed loops along the sides of the organism. In one instance, two were plainly observed, attached at one pole.

On gelatin plates, round, creamy-white, zoned colonies developed within two days. They produced a crateriform liquefaction with a dense white mass in the centre. Outside this, lay white turbid zones, concentrically arranged and becoming gradually thinner toward the margin. Under the microscope, the small dew-drop-like colonies appeared round, finely granular and light brown. The larger colonies had a dense, dark brown central nucleus surrounded by a coarsely-granular zone, outside of which was a finely-granular area with a ciliated margin.

Gelatin stab cultures rapidly became saccate and uniformly turbid. In 96 hours, liquefaction was complete. On the surface of the liquefied gelatin, a light flocculent pellicle was formed and a heavy creamy sediment lay in the bottom of the test tube.

Growth on agar plates was characteristic of the old genus, Proteus. The moist, cream-coloured colonies developed a variety of projections, some confluent and arborescent, others circular, with irregular, curved, radiating arms. A yellow-green fluorescence was produced in the medium.

On starch plates, there was diastatic action and marked green fluorescence in the medium.

On potato, a thick cream-coloured, raised, luxuriant growth developed rapidly and soon covered the entire surface of the medium.

Milk was coagulated quickly with production of acid. Digestion followed, a dull, sage-green layer appearing at the surface and leaving a green ring on the tube. A strong pungent and cabbage-like odour was produced on this as on other media.

Litmus milk gave parallel results.

This organism formed acid and gas in dextrose, saccharose and glycerin. The reaction changed after one week and gas formation ceased, except in glycerin. After five weeks, glycerin cultures were strongly acid and were still producing gas.

In broth, fluorescence was noted, as well as turbidity, a dense sediment and a light pellicle.

4. Pseudomonas serica (Mig.) fluorescens.

This organism was a short, thick, gram-negative rod with a single, delicate, polar flagellum.

Gelatin colonies were at first punctate, bluish-white, later becoming definitely round. Under the microscope they were coarsely granular with grumose centres and a clearly defined margin. Some of the larger colonies had a few club-shaped processes.

On agar, the surface colonies were round and concentric with slightly irregular margins. Beneath the surface, small, dense, granular, pyramidal colonies were numerous. Under the microscope all appeared to have a grumose structure.

On agar slants a white, lustrous, spreading layer was formed. Growth first appeared as discrete, pearly-white colonies (diameter about 1 mm) which later became confluent. The water of condensation was turbid, with a white deposit.

In Gelatin stab cultures, a bowl-shaped depression was first produced, a white film lining the cavity. This became broadened until the sides of the tube were reached and proceeded in a horizontal layer to the bottom.

Broth cultures were moderately turbid with a flocculent pellicle and a sediment. A negative result was obtained by the indol test.

Potato cultures had at first a dull slightly granular surface which later became smooth, shining and waxy. The colour varied from pink to buff. In old cultures the buff tone was constant.

Litmus was completely reduced in 18 hours and a white pellicle was formed on the surface of milk cultures of that age. Digestion began at the surface of the medium on the fifth day and was complete in about two weeks. No clotting took place. The reaction was strongly alkaline. The medium became slimy and gradually deepened in colour to a golden-yellow. At the surface, a layer containing fluorescent pigment was formed. This layer appeared dark-purplish by transmitted light and sage-green by reflected light. Shaking produced this effect throughout the tube. A very strong pungent odour suggesting an amine was characteristic of these cultures. Tanner has described a similar effect produced upon litmus milk by one group of his green fluorescent water bacteria. His organisms, however, seem to have differed in other particulars from those described here. They differed, for instance, in their action upon sucrose. Migula has described a form known as Pseudomonas sericea as producing alkali and reducing litmus - though he has classified this organism among non-fluorescent forms. In the group under consideration, fluorescence, observed chiefly in milk but occasionally upon agar and broth, seemed the only important variation from the type described by Migula. Therefore, the tentative name of Pseudomonas sericea fluorescens has been given.

4. Pseudomonas liquefaciens (Tataroff. Migula) var. marina.

Stained with Loeffler's methylene blue, this organism appeared as a short rod, very often occurring in pairs. In a hanging drop it had a dodging and darting rapid movement, in a narrow field. It possessed one polar flagellum, two or three times the length of the organism. It was gram-negative.

On Gelatin Plates,

Colonies appeared in three days, first as round white points with a somewhat cloudy margin. Under the microscope, surface colonies were circular, granular, dense in the centre with radiating, cochleate filaments from the margin. The deep-set colonies had a sunburst appearance, the processes, which were very numerous and tangled, radiating in all directions.

On Agar Plates,

Growth was evident in 24 hours. Surface colonies were round, moist, raised, cream-coloured, 1 to 2 mm. in diameter, later widening to 15 mm. Microscopically, they were grumose, concentric, dense in the centre with successively thinner rings outside. Deep-set colonies were dense, disc-shaped, tilted, 0.5 to 1.0 mm. in diameter. Magnified, they appeared dark brown, grumose, with rough edges.

In gelatin stab cultures, liquefaction was at first crateriform. A cloudy appearance was produced along the line of inoculation by numerous, minute, discrete colonies. The margin of the liquefied ana was slightly dentate. The liquefaction

became infundibuliform about the third day and was invariably complete in ten days. The sediment was cream-coloured, viscid and abundant.

Nutrient broth was rendered uniformly turbid with a frosty membranous pellicle and a sediment.

Litmus milk became alkaline with reduction of litmus and digestion of casein. In this medium, growth was very slow in all cases. Tested soon after isolation and grown in the dark, the organism produced a change in the milk on the sixth day. In later cultures, grown at room temperature without protection from light, no change was apparent for four weeks, after which alkalinity, reduction of litmus, and digestion of casein were observed. Preparations in plain milk gave parallel results. A strong ammoniacal odour was characteristic of old cultures.

On potato, no growth was obtained, though repeated cultures were made. One vigorous strain, however, which grew more rapidly and luxuriantly than this type on all media, invariably produced a whitish spreading growth upon potato in 24 hours.

Tataroff's organism having been isolated from fresh water and this closely allied form from a salt water clam, it seems possible that the variation noted - namely, slower growth in litmus milk and the absence of growth on potato, were due to lack of salt in these media.

It is hoped that this will be confirmed at a later date.

5. Pseudomonas myae.

Though this form had well-defined characteristics it has not been discovered in the available classifications and descriptions. In many particulars it is suggestive of the Pseudomonas oogenes of Migula, which has been found in eggs.

In form, it was a short rod with one polar flagellum. It was gram-negative.

On gelatin plates, white colonies developed in between 2 and 3 days. Liquefaction was bowl-shaped, a dense white deposit occupying the central point in the depression. Around this, a less dense, uniform turbidity gave the remainder of the bowl a frosty-white appearance. Observed microscopically, the small deep-set colonies were found to be very dense, spherical, granular masses. The larger colonies seemed often to be built concentrically about such a colony as a central nucleus. Other large colonies were merely grumose in the centre, a loosely granulated zone occupying a comparatively wide area between the central portion and the margin.

On agar plates, small, deep-set, pyramidal colonies were numerous. Surface colonies were of the round, moist, cream-coloured type. Microscopically, they were granular without projections, and dense in the centre. Occasionally, branching and budding of the colonies was observed.

In litmus milk, the litmus was quickly reduced. Clotting and digestion followed rapidly. The whey was perfectly clear without a pellicle, while the clot became deep pink in colour.

P. myae was the only one of these blackening organisms which fermented lactose.

In gelatin stab, a crateriform-filiform liquefaction was well-developed at the end of 24 hours. The liquefied area gradually widened, reaching the sides of the tube in 3 or 4 days. A stratiform area thus appeared above an ever-widening central turbid column. In ten days the gelatin was completely liquefied, with a heavy, creamy, viscid sediment.

In nutrient broth, there was a light pellicle, marked turbidity and a viscid sediment.

The organism grew delicately upon potato, the growth appearing as a narrow white film along the line of inoculation. It was at first mucoid but soon became flattened, dry and shining, remaining unchanged at this stage for weeks.

The indol test gave a negative result.

6. Pseudomonas sp?

Another form, having all of the features mentioned in the general description, was observed. Though it was very motile, the number of flagella was not determined.

This was a small rod which had a tendency to bipolar staining. It frequently formed short chains. It was gram-negative.

On gelatin plates, it developed round, bowl-shaped, liquefying colonies, in which there was a uniform white turbidity. A dense white deposit occupied the centre of the colony, and smaller, opaque, white masses of bacteria lay at many points mid-way between the centre and the circumference.

Under the microscope, the whole colony was found to be granular. The dense centre appeared dark brown, the outer zones lighter in colour. Approximately midway between the centre and

circumference, a denser line, broken at intervals, corresponded with the deposits noted microscopically.

On agar plates, colonies were round, moist and cream-coloured. Microscopically, they appeared concentrically zoned, with a granular structure. Dense masses like a tilted-disc were often embedded in the colony near the centre.

In gelatin stab cultures, liquefaction was fairly rapid. It was at first crateriform but rapidly became infundibuliform. In 6 days liquefaction was complete. A whitish sediment and a uniform white turbidity were characteristic.

On potato, luxuriant growth was produced. It was at first yellow and shining with an even contour; later, it became deeper in colour, spreading and of a painty consistency. The potato itself was darkened.

On litmus milk, a light pellicle was formed within 24 hours, followed by reduction of litmus, and the clotting and digestion of casein, proceeding from the surface downward. The clear liquid became pinkish in colour. The clot, also, which was at first white, later became pink.

On agar slants, the growth was luxuriant, spreading evenly along the line of inoculation. It was raised, cream-coloured, moist and shining, spreading gradually over the entire slant. There was a creamy-white deposit in the water of condensation. Indol was formed.

SUMMARY

1. Many forms of bacteria occur normally in Mya arenaria, the long-necked clam.

2. Six of these, belonging to the genus Pseudomonas, are capable of causing blackening, if supplied with a favorable medium, containing iron or tin. This number includes Pseudomonas fluorescens (Flügge) Migula. Pseudomonas Jaegeri. Mig. Pseudomonas liquefaciens (Tataroff. Mig.) var. marina., and two forms which, for reasons stated above, have been given the tentative names, Pseudomonas sericea fluorescens and Pseudomonas myae.

3. All these blackening organisms were facultative anaerobes.
All liquified gelatin.
All reduced nitrates to nitrites.
All formed acid from dextrose, saccharose and glycerine.
A change of reaction from acid to alkaline was characteristic in sugar media, and usually began on the third or fourth day.
All were motile, gram-negative rods.
No spores were observed.

4. Fluorescence was characteristically produced by three of these organisms, namely, Ps. fluorescens (Flügge) Mig,, Ps. Jaegeri, and Ps. sericea (Mig.) var. fluorescens. Ps. myae and Ps. sp? formed an acid curd, followed by digestion of casein, in milk.
Ps. Jaegeri was the only gas-former among the blackening organisms isolated.

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