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Title

THE PSYCHROPHILIC FLORA OF HADDOCK SLIME AND FECES.

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Report of Work Done at the Biological Station, St. Andrews, N.B.

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During the Summer of 1929.

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of the salmon. These, again, were mainly Pseudomonas and Achro-

bacters. Most of these Kingston, Ontario. at temperatures of 15°

to 37°C. and, as indicated, the ordinary water forms, Achromobacters,

Pseudomonas. The ability of organisms, isolated from fish, to grow at low

temperatures has been noted by various authors. In this investiga-

tion an attempt has been made to determine the nature of this 'psy-

chrophilic' flora in the haddock. The practical importance of the

subject lies in the presence of proteolytic forms.

In a study of the bacterial flora of haddock feces, Reed (Reed and Spence, 1929) noticed that many of the organisms isolated grew at low temperatures. This must be the case when we consider the temperature of the water in which the haddock lives (8° - 12°C). He found the predominating organism to be Proteus vulgaris. This has a practical bearing on fish spoilage as the digestive tract is an important source of infection of fish flesh. Harrison, Perry and Smith (1926) found Achromobacters and Pseudomonas to be the most common forms in sea fish and believe these types to be of importance

in the decomposition of fish. They record considerable increases in the bacterial content of fish kept on ice and at temperatures of 3°-5°C.

The slime is another recognised source of infection. Haddock slime has been studied by Spence (Reed and Spence, 1929). Achromobacters and Pseudomonas were found to predominate. Harrison (1929) lists organisms isolated from the slime of halibut which grow at 2°C.

Williamson (1929) gives an account of the bacteria isolated from fish and frogs in the course of an investigation of furunculosis of the salmon. These, again, were mainly Pseudomonas and Achromobacters. Most of these investigators worked at temperatures of 15° to 37°C. and, as indicated, the ordinary water forms, Achromobacters, Pseudomonas and Flavobacteria, predominate.

Harrison (1928) records an organism, *B. salmonis pestis*, causing a disease in salmon, which grows at 0°C. and is killed at 37°C. in six days. Other fish pathogens have been described which prefer low temperatures.

In this investigation samples were taken from living fish as they were being removed from the trawl lines. Care was taken to prevent the fish from coming in contact with any outside source of infection before the samples were taken. Slime was collected by means of a sterile swab. In the case of feces, the abdomen was pressed and the feces thus extruded (10 to 15c.c.) were caught in a sterile bottle. The samples were cultured as soon as possible after collection.

The medium used consisted of the following:-

Haddock muscle	500 gms.
Peptone	10 gms.
Agar	15 gms.
Sea water	200 cc.
Tap water	800 cc.

Fresh haddock were filleted, the fillets ground in a meat chopper, weighed and covered with the tap and sea water. This was allowed to stand overnight, then boiled 10 to 15 minutes and filtered through cheese cloth. The peptone and agar were added and the resulting mixture boiled ten minutes, adjusted pH 7.2, made up to volume and autoclaved 10 mins. at 15 pounds. This was then filtered through a thin layer of absorbent cotton to remove the precipitate formed and finally autoclaved.

The slime was plated out directly on agar plates. The feces were diluted with sterile saline and plated. All plates were incubated at 10°C. and examined at intervals until good growth was observed. Representative colonies from these primary cultures were planted on a second series of plates. Pure cultures thus obtained were studied in detail. Growth rates were studied at 5°, 10° and 25°C. and an attempt was made to identify the organisms growing at the lower temperatures. Bergey's Manual of Determinative Bacteriology was used but very few of the organisms could be run down to species.

Forty-six strains were isolated from some fourteen fish. These included nineteen Achromobacters, five Flavobacteria, nine cocci (5 gram positive, 5 gram negative), four bacilli, two Pseudomonas and three which do not fit into the classification. A detailed description of the organisms follows:

Slime Organisms.

From Haddock Slime.

Culture No.- S.A. & S.A.4.

Rods, short, thick, Gram negative. Motile.

Agar colony - Round, entire, smooth, glistening, convex, white.

Gelatin stab- no liquefaction.

Broth - sediment, turbid.

Litmus milk - acid.

Potato - moderate, filiform, raised, smooth, glistening, dirty-grey.

Indol is formed.

Nitrates reduced.

Acid and gas in dextrose, maltose, sucrose, levulose, mannitol, adonitol, salicin, and dextrin. Acid in lactose.

Acetyl-methyl-carbinol not formed. Methyl red positive.

Maximum growth at 25°C. in 48 hours, at 10°C. in 72 hours, at 5°C. in 168 hours. Growth at 5°C. in 24 hours.

An atypical strain of the coli-aerogenes group, probably *Escherichia pseudocoloides*. Fits into this genus better than into *Proteus*.

Culture No.- S.A.1.

Rods, short, thick, Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony- round, entire, smooth, glistening, white.

Broth - turbid, sediment.

Litmus milk - acid, coagulated.

Potato - scanty, white, glistening.

Indol not formed.

Nitrates not reduced.

Maximum growth at 25°C. in 48 hours, at 10°C. in 72 hours, at 5°C. in 96 hours. Growth at 5°C. in 24 hours.

Belongs to genus *Achromobacter*.

Culture No.- S.A.2.

Rods, short. Gram positive. Motile.

Gelatin stab - liquefaction.

Agar colony- dry, spreading, yellow, opaque.

Broth - slight turbidity, sediment, pellicle.

Litmus milk - slightly acid.

Potato - yellow, glistening, spreading.

Indol not formed.

Nitrates reduced.

Maximum growth at 25°C. in 42 hours, at 11°C. in 90 hours. Growth at 5°C. in 24 hours.

Belongs to genus *Flavobacterium*.

Culture No.- S.A.6.

Small rods. Gram positive. Motile.

Gelatin stab - crateriform liquefaction.

Agar colony - punctate, entire, smooth, glistening, pulvinate, yellow.

Broth - turbid sediment.

Litmus milk - very slow peptonization.

Potato - scanty yellow growth.

Indol not formed.

Nitrates reduced.

Maximum growth at 25°C. in 66 hours, at 11°C. in 90 hours. Growth at 5°C. in 24 hours.

Belongs to genus *Flavobacterium*, probably *aquatilis*.

Culture No.- S.B.

Small rods. Gram negative. Motile.

Gelatin stab- no liquefaction.

Agar colony - round, undulate, smooth, glistening, umbonate, yellowish white, medium coloured green.

Broth - turbid, sediment, pellicle.

Litmus milk- no coagulation, alkaline.

Potato - scanty, yellowish brown, glistening. Medium browned.

Acid in dextrose.

Indol not formed.

Nitrates reduced.

Maximum growth at 25°C. in 48 hours, at 11°C. in 66 hours. Growth at 5°C. in 24 hours.

Pseudomonas, probably *Ps. putida*.

Culture No.- S.B.1.

Rods. Gram negative. Motile.

Gelatin stab- no liquefaction.

Agar colony- round, smooth, glistening, white.

Broth - turbid, sediment.

Litmus milk - peptonized.

Potato - no growth.

Indol not formed.

Nitrates not reduced.

Belongs to genus *Achromobacter*.

Culture No.- S.B.2.

Rods, very short, coccoid, singly and in pairs. Gram negative. Non-motile.

Gelatin stab- no liquefaction.

Agar colony - round, erosive, smooth, glistening, creamy.

Broth- turbid, sediment.

Litmus milk - acid.

Potato - scanty, white, glistening, becoming yellowish. Potato darkened.

Indol not formed.

Nitrates not reduced.

Acid in dextrose.

Maximum growth at 25°C. in 48 hours, at 11°C. in 72 hours. Growth at 5°C. in 24 hours.

Probably *Achromobacter acidum*.

Culture No.- S.B.3.

Rods, Gram negative. Motile.

Gelatin stab- no liquefaction.

Agar colony- round, small, hard, greyish.

Broth - turbid, sediment, slight pellicle.

Litmus milk - acid, coagulated.

Potato - yellow, mucoid, glistening, becoming dry, wrinkled, dirty-grey.

Indol not formed.

Nitrates reduced.

Acid in dextrose, maltose, lactose, and mannitol.

Maximum growth at 25°C. in 48 hours, at 11°C. in 72 hours. Growth
at 5°C. in 24 hours.Belongs to genus *Achromobacter*, agrees most closely with *A. reticularum*.

Culture No.- S.C.1.

Rods, medium length. Gram positive. Non-motile.

Gelatin stab - stratiform liquefaction.

Agar colony- dirty white, confluent colonies.

Broth - turbid with pellicle.

Litmus milk - no change.

Indol not formed.

Nitrates reduced.

Acid in dextrose and maltose.

Does not fit into the classification.

Culture No.- S.C.2.

Rods, varying from 1.5 to 6.0. Gram positive. Motile.

Gelatin stab - saccate liquefaction. Complete in 4 days.

Agar colony - small, entire, bluish white, translucent.

Broth - turbid, light pellicle.

Litmus milk - no change.

Indol not formed.

Nitrates reduced.

Acid in dextrose, and maltose.

Does not fit the classification.

Culture No.- S.C.3.

Rods, vary in length. Gram positive. Non-motile.

Gelatin stab- saccate liquefaction.

Agar colony- slightly raised, wrinkled, brownish white.

Broth - turbid, pellicle.

Litmus milk - no change.

Indol not formed.

Nitrates reduced.

Acid in dextrose and maltose.

Genus -

Culture No.- S.F.1. & S.F.5.

Rods, small. Gram positive. Spores, central, rods slightly swollen.
Motile.

Gelatin stab- liquefaction, capiform to saccate.

Agar colony - irregular, entire, raised, smooth, glistening, watery,
and mucoid with chalky scum, becoming flat, dry and rough.

Broth - slightly turbid, granular sediment, thin pellicle.

Litmus milk - peptonized.

Potato - dull, white, folded, gummy, spreading.

Indol not formed.

Nitrates reduced.

Acid in dextrose, sucrose and mannitol.

Bacillus subtilis, var. viscosus. (Chester, 1903).

Culture No.- S.F.2.

Rods. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony - round, smooth, glistening, erose, white; later conc.
ringed.

Broth - turbid, sediment.

Litmus milk - acid.

Potato - dry, spreading, white becoming creamy.

Indol not formed.

Nitrates reduced.

No acid or gas in carbohydrates used.

Maximum growth at 25°C. in 48 hours, at 11°C. in 72 hours, at 5°C. in
96 hours. Growth at 5°C. in 24 hours.

Belongs to genus Achromobacter, resembles A. fermentationis.

Culture No.- S.F.3.

Rods, small, chains. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony - round, erose, smooth, glistening, white.

Broth - turbid, sediment.

Litmus milk - peptonized.

Potato - scanty, dirty white.

Indol not formed.

Nitrates. reduced.

No acid or gas in carbohydrates used.

Maximum growth at 25°C. in 48 hours, at 11°C. in 90 hours, at 5°C. in
168 hours. Growth at 5°C. in 24 hours.

Belongs to genus Achromobacter, probably A. ubiquitum.

Culture No.- S.L.4. & S. L.1.

Coccus, small. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony,- round, smooth, glistening, entire, white.

Broth - turbid, sediment.

Litmus milk - no change.

Potato - no growth.

Indol not formed.

Nitrates reduced.

Acid in dextrose.

Genus Micrococcus, M. epidemiae according to Hacker (1920).

Culture No.- S.L.

Rods, slender. Gram negative. Non-motile.

Gelatin stab -

Agar colony - round, smooth, glistening, creamy.

Broth - turbid, slight pellicle.

Litmus milk - some peptonization.

Potato no growth.

Indol not formed.

Nitrates reduced.

Acid in dextrose, maltose and sucrose.

Belongs to genus *Achromobacter*.

Culture No.- S.L.5. & S.M.1.

Coccus. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony.- round, entire, smooth, glistening, white.

Broth - turbid, sediment.

Litmus milk - no change.

Potato - no growth.

Indol not formed.

Nitrates not reduced.

Acid in dextrose.

Probably *Micrococcus candidus*. (Cohn).

Culture No.- S.M.4.

Rods, short, thick. Gram negative. Non-motile.

Gelatin stab -

Agar colony- small, round, entire, smooth, glistening, raised, white.

Broth - turbid, sediment.

Litmus milk - no change.

Potato - no growth.

Indol not formed.

Nitrates reduced.

Slight acid in dextrose.

Genus *Achromobacter*.

Culture No.- Ps. 2.

Rods, medium. Gram negative. Motile.

Gelatin stab - no liquefaction, medium coloured green.

Agar colony - round, entire, smooth, glistening, raised, white,

Medium coloured yellow-green.

Broth- turbid, sediment, pellicle.

Potato - moderate, spreading, flat, smooth, dull, tan. Medium greyed.

Litmus milk - no change.

Indol not formed.

Nitrates slightly reduced.

Acid in dextrose.

Maximum growth at 25°C. in 24 hours, at 11°C. in 72 hours, at 5°C. in

96 hours. Growth at 5°C. in 24 hours.

Genus *Pseudomonas*, resembles *Ps. ovalis*.

From Cod Slime.

Culture No.- S.H.3.

Rods, short, plump. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony.- creamy-white, raised, translucent.

Broth - clear, sediment. Slimy.

Litmus milk - no change.

Potato -

Indol not formed.

Nitrates reduced.

No acid or gas in carbohydrates used.

Probably of genus *Achromobacter*.

Culture No.- S.H.4.

Rods, short, plump. Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony.- round, raised, smooth, glistening, white.

Broth- slightly turbid, sediment. Slimy.

Potato -

Litmus milk - no change.

Indol not formed.

Nitrates reduced.

Slight acid in dextrose.

Of the genus *Achromobacter*.

Culture No.- S.H.5.

Rods. Gram positive. Spores. Motile.

Gelatin stab- saccate liquefaction.

Agar colony.- flat, spreading, dry, slightly wrinkled, brownish white.

Broth- turbid, slight pellicle.

Litmus milk - no reaction.

Potato -

Indol not formed.

Nitrates reduced.

Acid in dextrose, maltose, sucrose and lactose.

Belongs to genus *Bacillus*.

From Haddock Feces.

Culture No.- F.C.

Rods, short, thick, coccoid, singly and in pairs. Gram negative.
Non-motile.

Gelatin stab - no liquefaction.

Agar colony - round, entire, smooth, glistening, white.

Broth - turbid, sediment.

Potato - moist, dirty white, spreading.

Litmus milk - acid.

Indol not formed.

Nitrates not reduced.

No acid in carbohydrates used.

Maximum growth at 25°C. in 42 hours, at 11°C. in 66 hours.

Belongs to genus *Achromobacter*, resembles *A. acidum*.

Culture No.- F.D.1.

Rods, pleomorphic. Gram negative. Motile.

Gelatin stab - no liquefaction.

Agar colony - round, smooth, glistening, white.

Broth - slightly turbid, slimy sediment.

Potato - no growth.

Litmus milk - no change.

Indol formed, slight.

Nitrates not reduced.

Acid in dextrose, lactose, maltose and mannitol.

Maximum growth at 25°C. in 42 hours, at 11°C. in 114 hours.

Apparently an *Eberthella* but does not agree with any described species.

Culture No. - F.D.2.

Rods, small slender, Gram negative. Non-motile.

Gelatin stab - no liquefaction.

Agar colony - punctate, entire, smooth, glistening, yellow.

Broth - slight even turbidity.

Potato - slight yellow glistening.

Litmus milk - acid.

Indol not formed.

Nitrates not reduced.

Maximum growth at 25°C. in 72 hours.

Genus *Flavobacteria*, probably *Flav. brevis*.

Culture No.- F.E.1.

Rods, short, rounded ends. Gram negative. Motile.

Gelatin stab - no liquefaction.

Agar colony - round, smooth, glistening, greyish white.

Broth - turbid sediment.

Potato - slightly spreading, yellow.

Litmus milk - peptonised.

Indol not formed.

Nitrates reduced.

Acid in dextrose.

Belongs to genus *Achromobacter*.

Culture No.- F.E.2.

Rods, form chains. Gram negative. Non-motile.
 Gelatin stab- no liquefaction.
 Agar colony- round, entire, smooth, glistening, white.
 Broth- slight turbidity, sediment.
 Potato- no growth.
 Litmus milk - no change.
 Indol not formed.
 Nitrates not reduced.
 Acid in dextrose and maltose.
 Maximum growth at 25°C. in 42 hours, at 11°C. in 66 hours, at 5°C. in 168 hours.
 Belongs to genus *Achromobacter*.

Culture No.- F.H.2.

Rods, short, thick. Gram negative. Non-motile.
 Gelatin stab- crateriform liquefaction.
 Agar colony- round, undulate, smooth, glistening, creamy becoming flat, conc. ringed.
 Broth- turbid, sediment.
 Potato- white, glistening spreading.
 Litmus milk- acid curd.
 Indol not formed.
 Nitrates not reduced.
 Acid in dextrose, maltose, sucrose and mannitol.
 Of genus *Achromobacter*.

Culture No.- F.H.3.

Rods, short, occurring singly and in pairs. Gram negative. Motile.
 Gelatin stab- no liquefaction.
 Agar colony- flat, smooth, white transparent.
 Broth- slightly turbid.
 Litmus milk - no change.
 Indol not formed.
 Nitrates not reduced.
 Potato- no growth.
 Acid in dextrose and maltose.
 Genus *Achromobacter*, resembles *A. inunctum*.

Culture No.- F.K.

Rods, short, thick. Gram positive. Non-motile.
 Gelatin stab- liquefaction, napiform to stratiform.
 Agar colony- round, entire, smooth, glistening, tan.
 Litmus milk - peptonized.
 Broth- turbid, sediment.
 Potato- moderate, flat, smooth, glistening, orange-yellow.
 Indol not formed.
 Nitrates reduced.
 Acid in dextrose, maltose, sucrose and mannitol.
 Genus *Flavobacterium*. Resembles *Flav. fucatum* (Harrison) except for sugars. Resembles Williamson's No. 13 except for motility and T.C.d280.H.8.2.

Culture No.- F.K.2.

Rods, short, thick, short chains. Gram positive. Central spores, rods swollen. Motile.

Gelatin stab- saccate liquefaction.

Agar colony- irregular, rough, dull, raised, undulate, greyish, Media turning brown in old cultures.

Broth - clear, pellicle.

Potato - white, spreading, dull becoming wrinkled.

Litmus milk - peptonized.

Indol not formed.

Nitrates reduced.

Acid in dextrose and maltose.

Bacillus subtilis.

Culture No.- F.R.

Rods, -short, thick. Gram negative. Non-motile.

Gelatin stab- no liquefaction.

Agar colony- round, smooth, glistening, white.

Broth- turbid, sediment.

Potato - scanty growth, if any.

Litmus milk- acid.

Indol not formed.

Nitrates reduced.

No acid or gas in carbohydrates used.

Belongs to genus *Achromobacter*.

From Haddock Muscle.

Culture No.- H., H.1., & H.2.

Rods, short, thick, coccoid. Gram negative. Non-motile.

Gelatin stab- no liquefaction.

Agar colony- round, smooth, glistening, entire, white.

Broth- turbid, sediment.

Litmus milk- acid, reduced.

Potato - glistening, creamy. Potato greyed.

Indol not formed.

Nitrates not reduced.

No acid in carbohydrates used.

Maximum growth at 25°C. in 24 hours, at 11°C. in 48 hours, at 5°C. in 96 hours. Growth at 5°C. in 24 hours.

Resembles *Achromobacter acidum*.

Culture No.- H.C.2.

Rods. Gram negative. Actively motile.

Gelatin stab- Liquefaction.

Agar colony- round, smooth, yellow.

Broth- turbid, sediment, yellowish white pellicle.

Potato- deep golden yellow growth.

Litmus milk - slightly alkaline.

Indol not formed.

Nitrates reduced.

Acid in dextrose.

Maximum growth at 25°C. in 42 hours, at 11°C. in 66 hours, at 5°C. in 120 hours. Growth at 5°C. in 24 hours.

Genus *Flavobacterium*. Either *Flav. squamula* or *condatum*.

The Achromobacters are the predominating organisms in both slime and feces. This is significant if these are factors in decomposition, as Harrison points out. No organisms of the Proteus group were found in feces, contrary to the findings of Reed. However, the different temperatures of incubation may account for this, as Reed incubated the primary cultures at 25°C., while the primary cultures in this investigation, as previously noted, were incubated at 16°C. The two strains which most nearly approach Proteus are believed to be atypical members of the coli-aerogenes group and it is interesting to note that both of these were from slime. Hunter (1922) found several members of this group in decomposing salmon.

About the same distribution of Achromobacters and Flavobacteria were found in both slime and feces, 10 Achromobacters and 2 Flavobacteria in slime and 6 Achromobacters and 2 Flavobacteria in feces. Pseudomonas was found only in the slime. It seems odd that so few types were found.

All the strains described grow well at low temperatures, some showing good growth in 24 hours at 5°C., others much slower growth.

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