# BACTERIAL SPOILAGE OF FRESH FISH

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Like all living things in order to grow bacteria require food, moisture and a suitable temperature. The main characteristics of the bacteria can be summarized as follows:

a. Bacteria are minute living organisms found almost everywhere in nature.

b. They possess enormous powers of reproduction when the right conditions prevail.

c. One of their chief activities in nature is to break down plant and animal tissues and other complex organic materials.

d. Their modes of actions are by means of enzymes that they secrete.

e. Bacteria differ in numbers and kinds of enzymes they secrete, and therefore in the kind and the extent of spoilage they produced.

f. Live fish swimming in the ocean already harbour the bacteria that will ultimately decompose them.

Living fish muscle is free from bacteria. Skin is the chief barrier to the bacteria. When the fish dies, the skin is no longer a protective means for the fish. This can be demonstrated by the fact that spoilage of gutted fish takes place first at the surface. In ungutted fish the bacterial spoilage also occurs inside the gut wall. The spoilage does not stop only at the skin or the gut wall because fish is not a solid thing but actually contains about 80% of water and it is able to absorb many soluble substances. If large numbers of bacteria are present in the surface slime and in the gut cavity, they not only start growing, but they begin producing the enzymes that gradually decompose the slime and the membrane of the gut cavity. Some of the soluble bad flavoured substances resulting from this surface spoilage will gradually diffuse right into the muscle itself. Also some of the normal soluble constituents of the muscle will diffuse from the sterile muscle out to the surface where the bacteria can act on them.

As mentioned before, live fish in nature are practically free from bacteria. The possible sources from which bacteria may come are 1. the sea or ocean where fish live and 2. contamination during handling and preparation processes.

The first thing should be said is about the bacteriology of the sea related to fish (live fish). Rowan (*12*) took samples from a quay in the fish harbour, Cape Town docks and did the bacterial counts. The samples were incubated at two different temperatures for five days. The number of bacteria per ml. of water were as follows:

| | at 21 C | at 27 C |
|---|---|---|
| surface | 13,500 | 7,000 |
| midwater | 1,800 | 3,000 |
| bottom | 2,300 | 3,000 |

She compared these figures to the samples taken 4 or more kilometers offshore where she found less than 250 bacteria per 1 ml. of water. This also suggests that the water close to the land is likely to be contaminated compared to the off-shore water. Even the water far away from land can be contaminated especially in the fishing area. The following ratios between numbers of bacteria in the open sea and in the fishing ground give the idea how much more the fishing area can get contaminated from the fishing gears.

Before nets were shot 1 : 7
After nets being shot 1 : 17

Next, an attempt was made to study the relationship of bacteria and live fish. Some of the studies lead to the belief that certain species of bacteria, e.g. luminous bacteria, are associated predominantly with a certain species of fish, e.g. haddock. There are many other bacteria which are associated with marine fish. In North Sea cod, for example, there are three predominant groups namely, *Pseudomonas*, *Micrococcus* and *Achromobacter*. The following data show the flora of Arctic cod and of North sea cod.

| | Arctic | North Sea |
|---|---|---|
| *Pseudomonas* | 40 | 10 |
| *Micrococcus* | 5 | 20 |
| *Achromobacter* | 50 | 50 |

Of cause there are many other organisms. Some can not grow on ordinary media and some require chitin-containing media. The bacterial population seems to be varied according to the season of the year. In Liston's studies (*10*) on flat fish, he made bacterial counts at intervals for a period of 27 months, on skin, gut, and gill samples of freshly caught skates and soles. He showed that the maximum count had tendency to occur about two months after spring and autumn plankton blooms. He also found that there was no correlationship between the numbers of bacteria and the temperature of the sea water. Counts for skin and gill samples were in general higher on an agar medium made with sea water than on a horse heart agar medium made with tap water. Incubation at 6 C usually gave somewhat lower counts than incubation at 20 C, but in a few instances the reverse was the case; counts at 37 C were never more than 3% of those at 20 C.

Most of the material in this paper refers to the marine type bacteria. Marine bacteria may be defined as those which can not grow at all in media containing no salt or sea water. Marine bacteria have the following outstanding characteristics :

1. They are extremly versatile and adapt themselves readily to considerable changes in environment.

2. They act mainly, if not entirely, when adsorbed on surfaces.

3. There is evidence that at least some of their reactions are due to a symbiosis or metabiosis in which two or more organisms are involved.

As with many other things, marine bacteria have both good and bad effects to human interests. The bad effects are:

1. spoilage of fish,
2. rotting of fishing nets and gear,
3. fouling of ships,
4. pollution of water and beaches, and
5. corrosion of metals.

The benefical effects of marine bacteria are:

1. serving as food for molluscs and plant–eating fish,
2. releasing nutrients from plant and animal sources,
3. changing hydrological conditions,
4. fermenting marine foods to make them utilizable as food for man and animals,
5. removing the iron and colloidal calcium sulphate by precipitation of salt from the sea.

With reference to spoilage, the condition of fish may be classified into 4 stages.

Stage I. Real fresh with no sign of spoilage.

Stage II. Good quality fish but freshness going.

Stage III. Spoiled fish but still edible.

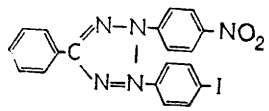
Stage IV. Condemned fish.

Attempts have been made to develop procedures to determine stages of spoilage. It is generally accepted that bacterial population on the fish is one of the best methods used nowadays. Bacterial population can be determined by either direct or indirect methods. The direct method for determining the bacterial population is to plate out samples, incubate, and count the colonies. There are several indirect methods among which the catalase test, the resazurin test and the tetrazolium test are well known.

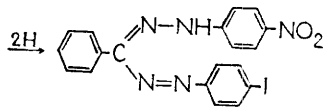
The enzyme catalase is produced by bacteria as fish spoil. The higher amount of oxygen gas released from hydrogen peroxide means more catalase produced; and the more catalase produced means high degree of spoilage.

Straka (*13*) pointed out that the susceptiblity of many prepared foods to microbiological contamination has made the development of rapid methods for determining bacterial count imperative. The new method developed by U.S.D.A. requires only 3–5 hrs. The new technique is a modification of the resazurin reduction method originally advised by the dairy industry to assay possible contamination of dairy products. Basically, the assay work is as follows. The test sample is mixed with distilled water and added to the blue dye, resazurin, and incubated. The bacteria reduce the resazurin changing it from blue to colorless. The length of time required for the dye to lose its color is an indication of the number of microbes present. Best results are obtained with this test when the contamination is great and a large number of bacteria are present. When less than 100,000 bacteria per gram are present the reduction take 5 hrs. For count of 100,000 to 1,000,000 per gram 5–3 hrs. are required, while less than 3 hrs. is necessary to complete the color change for bactreia counts over 1,000,000 per gram.

The peculiar property of tetrazolium is that it does not have a color in the oxidized state but it has red or blue color in the reduced state. Bacteriologists use this compound to measure the amount of dehydrogenase enzyme activity. Dehydrogenase activity is presumably related to the number of bacteria growing. This method is very fast and needs no incubation The reaction when tetrazolium is reduced is,



tetrazolium



formazan

*Bacteria Causing Fish Spollage*

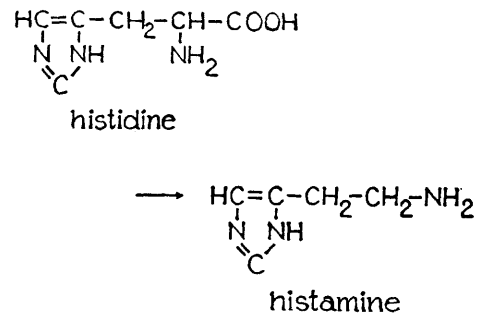
Fish spoilage may be caused by 1) non–pathogenic bacteria, 2) parasitic bacteria e.g. *Streptococcus* and *Staphylococus*, 3) pathogenic bacteria e.g. *Clostridium botulinum*, 4) yeasts and molds, 5) enzymes e.g. *rigor mortis* and autolysis, and 6) viruses. Bacteria causing fish spoilage are mostly of the non–pathogenic type. Fortunately fish does not carry bacteria pathogenic or parasitic in nature. Moreover, parasitic bacteria cannot grow in low temperature at which fish is usually kept. *Clostridium botulinum* poisoning is only accidental disease and is not directly related with spoilage of fresh fish.

There are attempts to classify or to group spoilage bacteria. Some investigators have suggested grouping of spoilage bacteria into those producing histamine, those producing ammonia, and those producing neither ammonia nor histamine.

*Bacteria producing histamine.* It was found that in fish spoilage at 20 C, 5–30 % of the total bacterial population were histamine formers. A species identified was named *Achromobacter histaminicum*. Further study on this particular type of bacteria revealed that *A. histaminicum* includes two types of organisms, giving colonies of somewhat different appearence on agar media and having optimum growth temperatures of 20–25 C for the type I and 30–35 C for the type II; optimum pH values for both types were 6–7.

The rate of growth of *A. histaminicum* (both type I and type II) during the logarithmic phase and the total count of the cells at maximum growth increased with the increase of concentration of fish muscle extract in the medium. Growth was not effected by the presence or absence of added histidine in the medium or by the species of fish from which the muscle extract was obtained. Production of histamine and percentage of histidine converted to histamine increased with the concentration of the fish muscle extract and histidine. The rate of production of histamine varied with the species of fish used in the medium, even in the medium with the same concentration of solids and histidine.

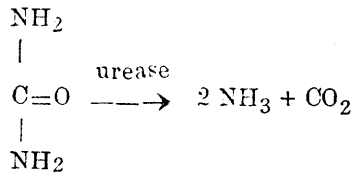
Histidine changes into histamine according to the following decarboxylation reaction:




*Bacteria producing ammonia.* There are many kinds of bacteria which cause fish spoilage and produce ammonia as the end product. The most known species are those that utilize the simple substrate urea. Kemata and Hata (*8*) reported that urea splitting bacteria formed about 5% of the bacterial populations of several species of fish uudergoing spoilage. Further investigation showed that fish containing high concentration of urea, such as the elasmobranchs, did not show larger population of urea–splitting bacteria than the other fish species.

Urea–splitting bacteria can be divided into two groups. The first group grow well with urea as sole source of nitrogen; *Achromobacter thalassius*, *Pseudomonas geniculata* and *Flavobacterium fucatum* are examples of this group. The second group require some other source of nitregen; examples of this group are *Achromobacter butyri*, *Achromobacter delicatulum*, *Pseudomonas putrifaciens*, and *Micrococcus ureas*.

The reaction below shows the production of ammonia from urea in the presence of the enzyme urease.




*Bacteria producing trimethylamine.* Trimethylamine is a substance commonly occurs in fish spoilage. Many methods have been proposed to measure the amount of trimethylamine hoping that it will be a very good index to determine different stages of fish spoilage.

The important precurser of trimethylamine is trimethylamine oxide. The ability to produce trimethylamine from trimethylamine oxide is charactistic to the family Enterobacteriaceae. Baltic herring (*Clupea harengus*) contains no trimethylamine oxide, but it was found that trimethylamine and ammonia were formed during the spoilage of the fish for a short time. After about 8 days at 0 C or 4 days at 18 C, the ammonia content of the fish continued to increase but the formation of trimethylamine decreased and finally ceased. It was explained that probably trimethylamine occured through methylation of ammonia and the methylated ammonia was then oxidized to methylamine oxide and that the oxide may again be oxidized.

At the frozen stage one can use trimethylamine to determine the quality of flesh at the time it was frozen. Good and Stern (*7*) worked with Englisn sole

which were held in iced storage for periods ranging from 0 to 15 days. At intervals during this period, samples were removed from the ice and filleted. Fillets were packed frozen, and held in frozen storage at 0 C for periods up to 24 weeks. They found that the trimethylemine content of the fillets did not change during the frozen storage.

*Baeteria producing nitrite from hydroxylamine.* In the experiment of Castell and Mappleback (*4*), it was found that cod muscle dipped in a solution of hydroxylamine developed small amount of nitrite when the muscle was held in storage (hydroxylamine was used as a preservative). Fresh muscle untreated with hydroxylamine however did not develop nitrite in the storage. The experiment proved that there were bacteria which utilized hydroxylamine and gave rise to nitrite. Later on it was found that many cultures of *Pseudomonas* species were able to convert hydroxylamine to nitrite.

This discovery is interesting because both hydroxylamine and nitrite are being used as bactericidal agents in some countries such as Canada (it was permitted in Great Britain during the war). Yet hydroxzlamine seems to be a growth factor for some bacteria.

*Bacteria producing odors.* The organisms of this type belong to the *Pseudomonas* and the majority are not proteolytic and do not breakdown trimethylamine oxide. They are chiefly achromogenic, although a few green pigment–forming species are included. Odor production by these organisms did not appear to be inhibited by sodium nitrite. It was found out also that similar odors were produced by similar type of organisms which had been observed on the dairy products, eggs, poultry and other protein foods held in cold storage.

Experiments have been performed by using *Pseudomonas perlens* (Turner). This kind of bacteria produce a sort of musty and potato-like odor. This particular type of odor has been observed before on chilled fillets of cod, haddock and flounder as well as on halibut steaks. Only rarely does it occur as the predominating odor on normally contaminated fish. Usually it is accompanied by sour, fruity or onion-like odors. It has not been observed on fresh fish or spoiling round fish or gutted fish, although the organisms have been isolated from the surface slime of iced cod and haddock. The odor is most frequently encountered from colonies growing on nutrient agar plates that have been prepared for making bacteria count from iced fillets. *Ps. perlens* is, in general, inert. The cells are neither proteolytic nor lipolytic. They do not ferment sugars and they do not reduce trimethylamine oxide. They produce little or non-observable changes in milk and they produce neither nitrite nor nitrate. They produce ammonia from peptone and produce various amino acids from urea. They produce hydrogen sulphide from methionine and cystine, but none or only traces from thiosulphate. They are very definitely psychrophilic, growing at all temperature between 0 and 25 C but not at 37 C or above.

*Coliform Bacteria.* Bacteriologists usually use the coliform bacteria to indicate human contamination because they are closely associated with human. In the large intestine one finds that coliform bacteria are the most common organisms. The bacteria are resistant to frozen storage. From the experiments in which fish were stored in the freezer for two weeks to two months, positive results in some instances were obtained. Coliform bacteria were isolated form frozen fish and cultured in laboratory media until heavy growth had developed; the culture survived 7 days at 0 C; sub-cultures on agar media held at −20 C for 36 hr. before incubation, showed growth slong the cross streaks of inoculation, but the growth was less extensive on the remainder of the surface than it was in control cultures which were not frozen before inoculation.

"*Milkiness of Lemon Sole*"

This particular type of spoilage does not occur very often. It was reported to occured in Union Bay, B.C., in 1951. Twenty per cent of the fish caught were rejected because of their milkiness appearance. It was reported that milkiness was associated with an abundance of a myxosporidium of the genus Chiormyxum. This protozoan may be the cause of the condition which manifests itself as softening and liquifaction of muscle tissues and is rarely evident in fresh fish but develops rapidly after death. The spores require no intermediate hosts. In a stock of fish, the incidence would be highest under the condition of high density.

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