Microbiological Studies on the Spoilage of "Semi-Dried Fishes"¹⁾.

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From olden times, in Japan, "semi-dried fishes" such as chub mackerel, carangoid fish, synentognathous fish, etc. have been consumed widely, as food matter.

The preparation of "semi-dried fishes" is as follows:

Comparatively small fishes such as chub mackerel, sardine, carangoid fish, synentognathous fish, etc. are used generally as a raw material. The fish is cut open on the dorsal or ventral side (depending on the kind of fishes), eviscerated, washed in clean water, and immersed in dilute brine for a little time, and then dried in the sun during several hours up to a suitable degree, that is, until the water content reaches about 60-70 percent.

These "semi-dried fishes" can not be kept in an apparently edible condition for a long time, but they are spoiled after several days if stored at the room temperature. The skincovered-side of the fishes is almost unchanged, while the flesh-side is covered by a butyrous and viscid substance, and a strong smell is produced. The muscle tissue becomes brittle, in such a state, and has an astrigent taste. In order to keep them in palatable condition for a considerably long time, therefore, they must be stored in a cold storage.

Microbiological researches on the spoilage of fresh fishes, hitherto, have been reported by many investigators⁽¹⁻¹⁵⁾. According to these authors, the decomposing bacteria of fresh fishes are principally the water bacteria from marine or fresh water origin, which are actively motile and Gram-negative short rods belonging to *Pseudomonas*, *Achromobacter* and *Flavobacterium*. These bacteria are present in slime, gill and intestinal canal of living fishes, and immediately grow when the fishes die, causing decomposition. And also *Micrococcus* group is found, however, it does not play an important rôle on the spoilages of the fresh fishes. According to STEWART⁽¹²⁾, the groups of *Aerobacter*, *Escherichia*, *Proteus* and *Bacillus* are not found. SNOW and BEARD⁽¹⁵⁾ could not isolate the *coli-aerogenes* group and spore forming bacteria. Recently, MARKOFF⁽¹⁶⁾ found that *Bact. coli* and spore forming bacteria are not the normal flora on the fresh fish.

As for the spoilage of smoked or salted dried fishes (e.g. smoked codfish, boned codfish, etc.) which resemble the "semi-dried fishes", studies

1) The "semi-dried fishes" make dishes after they have been roasted to a suitable degree.

hitherto reported show that *B. vulgaris* was isolated from the spoilage of the smoked codfish by HARRISON⁽²⁾ and *Torula Wehmeri*, *Micrococcus albus-translucens*, *Micrococcus lutulentus* and *Bacterium Zopfii* were isolated from the reddened boned codfish by HANZAWA and TAKEDA⁽¹⁷⁾.

So far as the present author is aware, however, little is known of the microbiological studies on the spoilage of the "semi-dried fishes." The present study aims to ascertain how the microorganisms play upon the spoilage of the "semi-dried fishes."

Materials and method.

The materials used in the experiment were the "semi-dried fishes" of chub mackerel, *Scomber japonicus* (TEMMINCK et SCHLEGEL) and carangoid fish, *Trachurus japonicus* HOUTTUYN, which were got from a market store. After standing for several days at the room temperature, when the flesh-side of the materials was covered by the butyrous and tacky substances, the microbiological examination was carried out.

By the microscopic examination of the butyrous and viscid substances on the flesh-side, many kinds of microorganisms such as coccus forms and rod-shaped bacteria were detected. From the substance on the flesh-side, a platinum loopful was taken and put into a test tube filled with about 10 c.c. of the sterilized physiological salt solution, and the content well shaken. One platinum loopful was taken from the tube in agar media of about 10 c.c., which had previously been warmed and melted. Thus, by the dilution method, one loopful of the media was put, in regular order, into other melted agar media. And finally the plate culture was carried out on Petri dishes. All cultures were incubated, aerobically, at 28°C.

The microorganisms of all the colonies grown in such a way, were investigated from the bacteriological point of view.

The same experiment has been done three times in different season, that is, in April, in July and in October.

The moisture of the "semi-dried fishes" used in the experiment was as follows:

Season	April		July		October	
Kind of fish	chub mackerel	carangoid	chub mackerel	carangoid	chub mackerel	carangoid
Moisture %	69.45	60.18	63.23	65.45	62.15	70.58

Experimental results.

The number of the colonies of bacteria grown on Petri dishes, was

shown in the following table.

Season	April		July		October	
Kind of fish	chub mackerel	carangoid	chub mackerel	carangoid	chub mackerel	carangoid
Colonies	28	26	32	21	41	33

The morphological, cultural and physiological features of every one of these microorganisms were studied by the ordinary method. The classification and the identification were carried out according to BERGEY'S manual⁽¹⁸⁾, and moreover referred with the descriptions given by LEHMANN und NEUMANN⁽¹⁹⁾, MIGULA⁽²⁰⁾ and BUCHANAN⁽²¹⁾.

The kinds and characteristics of these isolated microorganisms were described below. Among them, there are no groups belonging to *coli-aerogenes* group and *Proteus* group.

Description of the isolated microorganisms.

1. *Micrococcus caseolyticus* EVANS.

Morphology.

Spheres: 0.5-1.2 microns in diameter (variable in size), occurring singly and in irregular masses. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular with auriculate or lobate margin, smooth, faint orange, flat or raised, glistening, translucent, finely granular; Agar slant: Moderate growth, echinulate or arborescent, raised, glistening, smooth, opaque, light orange, moist; Gelatin colonies: Circular with lobate margin, smooth, crateriform liquefaction, faint yellow or orange, coarsely granular; Gelatin stab: Infundibuliform liquefaction with orange coloured sediment. Potato: Scanty filiform growth, orange. Certain strains may show a moderate growth; Broth: Turbid, with slight sediment. No growth on surface.

Physiology.

Litmus milk: Acid, coagulated, peptonized; Indol is not formed; Nitrates are reduced to nitrites; Acid is formed in dextrose, sucrose, lactose and maltose, but not in raffinose. Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°C.

This coccus form is closely allied to *Micrococcus caseolyticus* EVANS, although it slightly differs in respect of chromogenesis on agar and gelatine, and also all its characteristics can not be compared with the previous description which has comparatively few details. This coccus is presumed to be *Micrococcus caseolyticus*.

2. *Micrococcus mucofaciens* THÖNI and THAYSEN.

Morphology.

Spheres: 0.7-1.0 microns in diameter, occurring singly, in pairs and in irregular masses,

and occasionally in fours. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular (2-3 m.m. in dia.), smooth, flat or raised, yellowish brown, glistening, translucent, entire, finely granular or amorphous; Agar slant: Moderate, filiform, raised, glistening, smooth, opaque, yellowish brown, butyrous; Gelatin colonies: Small circular (ca. 1 m.m. in dia.), smooth, crateriform liquefaction, light yellow or orange, opaque, entire, granular; Gelatin stab: Napiform to saccate liquefaction; Potato: Scanty growth, light orange, filiform; Broth: Turbid, with a small amount of sediment. No growth on surface.

Physiology.

Litmus milk: Slightly acid, coagulated, becoming viscid and peptonized, with orange coloured ring; Indol is not formed; Nitrates are not reduced; Acid is not formed in dextrose, sucrose and lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 30°C.

It closely resembles *Micrococcus mucofaciens* THÖNI and THAYSEN, although all its characteristics can not be compared with the description in the literature which has been published previously, and in which the description has less details. This coccus, therefore, seems to be *Micrococcus mucofaciens*.

3. *Micrococcus conglomeratus* (FLÜGGE) MIGULA.

Morphology.

Spheres: 0.7-1.2 microns in diameter, occurring singly, in pairs and in fours, and occasionally in irregular masses. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular (2-3 m.m. in dia.), smooth, raised or convex, gray to pale orange, glistening, opaque, entire, granular; Agar slant: Moderate, filiform, becoming echinulate or plumose, raised center, smooth, becoming slightly rugose, opaque, light orange center and grayish margin, viscid; Gelatin colonies: Punctiform, smooth, convex, light orange, opaque, entire, granular; Gelatin stab: Filiform growth along the line of puncture. After 10-20 days crateriform liquefaction occurs; Potato: No growth. Some strains may show a very scanty growth with pale orange colour; Broth: Turbid, with light orange ring and little sediment.

Physiology.

Litmus milk: Unchanged; Indol is not formed; Nitrates are reduced to nitrites; Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

It possesses all the characteristics of *Micrococcus conglomeratus* (FLÜGGE) MIGULA, although it differs in its action on dextrose and sucrose. Therefore, it is recognized as a variety of *Micrococcus conglomeratus*.

4. *Micrococcus ochraceus* ROSENTHAL.

Morphology.

Spheres: 0.6-0.8 microns in diameter, occurring singly and in pairs. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Small circular (1-3 m.m. in dia.), smooth, convex, pale yellow, glistening, opaque, entire, granular; Agar slant: Moderate, filiform or echinulate with undulate margin, raised, smooth, opaque, yellow, moist; Gelatin colonies: Punctiform, smooth, convex, light orange, glistening, entire, amorphous. Gelatin stab: No liquefaction. Filiform, grayish-yellow growth along the line of puncture; Potato: Very scanty, filiform, yellowish growth; Broth: Slight turbid, with slight granular sediment.

Physiology.

Litmus milk: Unchanged; Indol is not formed; Nitrates are reduced to nitrites very slightly; Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

It seems to be *Micrococcus ochraceus* ROSENTHAL though its action on carbohydrates can not be known from the original description.

5. *Micrococcus himonoi* nov. sp.

Morphology.

Spheres: 0.6-0.9 microns in diameter, occurring singly, in pairs and in irregular masses. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular (ca. 5 m.m. in dia.), smooth, raised, yellowish-orange, glistening, opaque, entire, granular or amorphous; Agar slant: Abundant, filiform to spreading, raised, smooth, opaque, orange, moist; Gelatin colonies: Small circular (ca. 1 m.m. in dia.), crateriform liquefaction, yellowish-orange, entire, granular; Gelatin stab: Saccate to infundibuliform liquefaction, rapidly; Potato: Scanty, filiform, pale orangish growth; Broth: Turbid, with slight sediment and ring formation.

Physiology.

Litmus milk: Coagulated, peptonized. No acidification; Indol is not formed; Nitrates are reduced to nitrites; Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

Although this coccus resembles *Micrococcus caseolyticus* EVANS and *Micrococcus mucofaciens* THÖNI and THAYSEN, it differs from the former in its action on milk and on dextrose, and in some respects of the cultural characteristics, while it differs from the latter in its action on milk, on sugars and on nitrates and in some respects of the cultural features. As it does not appear to have been described previously, the name *Micrococcus himonoi* is proposed.

6. *Micrococcus candicans* FLÜGGE.

Morphology.

Spheres: 0.8-1.0 microns in diameter, occurring singly and in irregular masses. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, white, glistening, opaque, entire, granular; Agar slant: Moderate, filiform, raised, glistening, smooth, opaque, with translucent margin, white, viscid; Gelatin colonies: Small circular, raised, smooth, white, glistening, entire; Gelatin stab: No liquefaction. Filiform growth along the line of puncture. Growth on surface, white, very irregular lobed; Potato: Very scanty growth; Broth: Turbid, with a small amount of sediment, becoming clear.

Physiology.

Litmus milk: Slightly acid. No coagulation; Indol is not formed; Nitrates are not reduced, but reduced to nitrites by some strains; Acid is not formed in sucrose and lactose, but is formed very slightly in dextrose after a long time; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

As mentioned above, it possesses all the characteristics of *Micrococcus candicans* FLÜGGE. It seems, therefore, to be *Micrococcus candicans*.

7. *Micrococcus perflavus* BERGEY et al.

Morphology.

Spheres: 0.7-0.9 microns in diameter, occurring singly and in pairs, and occasionally in fours. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular with lobate margin, smooth, flat, light orange, opaque, granular; Agar slant: Moderate, echinulate to spreading, flat, dull, smooth, opaque, light orange, undulate margin; Gelatin colonies: Small circular, crateriform liquefaction, yellow, lobate, granular; Gelatin stab: Crateriform or napiform liquefaction, becoming stratiform (about 20 days); Potato: Filiform, light orange, dull, flat or raised; Broth: Turbid, with slight sediment and yellowish-gray pellicle.

Physiology.

Litmus milk: Slightly acid, coagulated, peptonized. Litmus is reduced; Indol is not formed; Nitrates are reduced to nitrites and ammonia; Acid is formed in dextrose and sucrose, but slightly in lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

It is closely allied to *Micrococcus perflavus* BERGEY et al. though it can not be compared with the action on carbohydrates, owing to the lack of previous descriptions on this point. It is presumed to be *Micrococcus perflavus*.

8. *Micrococcus subflavescens* BERGEY et al.

Morphology.

Spheres: 0.9-1.1 microns in diameter, occurring singly, in pairs and in fours. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular (3-4 mm. in dia.), smooth, raised, yellowish-orange, glistening, opaque, entire, granular; Agar slant: Moderate, filiform, raised, glistening, smooth, opaque,

yellow, undulate margin, moist or viscid; Gelatin colonies: Small circular, crateriform liquefaction, orange, undulate margin, opaque, granular; Gelatin stab: Napiform to infundibuliform liquefaction; Potato: No growth. Some strains may show a orangish filiform growth very scanty; Broth: Turbid, with slight sediment and ring formation.

Physiology.

Litmus milk: Alkaline, peptonized. Litmus is reduced; Nitrates are reduced to nitrites; Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°C.

Its characteristics closely resemble those of *Micrococcus subflavescens* BERGEY et al., although it differs in its action on nitrates and also it can not be compared in respect of the action on carbohydrates with the previously description, in which, that action is not reported. It may be the variety of *Micrococcus subflavescens*.

9. *Rhodococcus cinnebareus* (FLÜGGE) HOLLAND.

Morphology.

Spheres: 0.8-1.1 microns in diameter, occurring singly and in pairs. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, bright red, opaque, entire, finely granular; Agar slant: Filiform, raised, glistening, smooth, opaque, moist, becoming carmine red; Gelatin colonies: Small circular, raised, bright red, smooth, opaque, entire, amorphous; Gelatin stab: No liquefaction. Filiform growth along the line of puncture. Thick, raised, red growth on the surface; Potato: Scanty growth, vermilion red streak; Broth: Turbid, with reddish-gray thin pellicle and sediment.

Physiology.

Litmus milk: Slightly acid. No coagulation and peptonization; Indol is not formed; Nitrates are reduced to nitrites; Starch is not hydrolyzed; Aerobic; Optimum temperature: 25°-30°C.

This organism corresponds to the description of *Rhodococcus cinnebareus* (FLÜGGE) HOLLAND, although the characteristics of the latter have not been fully described previously. It seems to be *Rhodococcus cinnebareus*.

10. *Rhodococcus roseus* (FLÜGGE) WINSLOW and WINSLOW.

Morphology.

Spheres: 1.0-1.3 microns in diameter, occurring singly and in pairs. Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: Circular, smooth, convex, rose-red, glistening, opaque, entire, granular; Agar slant: Thick, filiform, glistening, smooth, rose-red, opaque, viscid; Gelatin colonies: Small circular, slow crateriform liquefaction, rose-red, granular; Gelatin stab: Crateriform to stratiform liquefaction; Potato: Thick, smooth, rose-red, glistening, moist; Broth: Slightly turbid, with rose-red coloured sediment.

Physiology.

Litmus milk: Becoming alkaline; Indol is not formed; Nitrates are not reduced; Aerobic; Optimum temperature: 25°-30°C.

It possesses all the characteristics of *Rhodococcus roseus* (FLÜGGE) WINSLOW and WINSLOW.

11. *Achromobacter middletownii* (CHESTER) BERGEY et al.

Morphology.

Rods: 0.7-0.8 by 1.1-1.8 microns, with rounded ends, occurring singly and in pairs. Non-motile. It does not produce endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, grayish-white, glistening, opaque, entire, finely granular; Agar slant: Moderate, filiform, raised, glistening, smooth, opaque, grayish-white, moist; Gelatin colonies: Small circular (1-2 m.m. in dia.), raised, grayish-white, glistening, smooth, opaque, entire, granular; Gelatin stab: No liquefaction. Filiform growth along the line of puncture. Grayish-white, slightly spreading, raised growth on surface; Potato: Moderate growth, dirty brown, moist. Medium assumes grayish colour; Broth: Turbid, with grayish pellicle and sediment.

Physiology.

Litmus milk: Slightly acid. No coagulation; Indol is not formed; Nitrates are not reduced; Acid is not formed in sucrose and lactose, but is formed slightly after a long time in dextrose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

This bacterium mostly resembles *Achromobacter middletownii* (CHESTER) BERGEY et al. though the latter wants of the description in many respects of the cultural and physiological features. It seems to be identified to *Achromobacter middletownii*.

12. *Achromobacter refractans* (WRIGHT) BERGEY et al.

Morphology.

Rods: 0.5-0.7 by 0.7-1.5 microns, short rods, with rounded ends, occurring singly and in pairs. Non-motile. It does not produce endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular (2-3 m.m. in dia.), smooth, flat, grayish-white, glistening, translucent, somewhat iridescent, entire, finely granular; Agar slant: Narrow, filiform, flat, glistening, smooth, translucent, whitish-gray, somewhat undulate margin, moist; Gelatin colonies: Small circular (ca. 1 m.m. in dia.), raised, white, glistening, smooth, opaque, somewhat lobate margin, coarsely granular; Gelatin stab: Filiform growth along the line of puncture. White wrinkled growth on surface. No liquefaction; Potato: No growth; Broth: Very slightly turbid, with slight pellicle and sediment.

Physiology.

Litmus milk: Coagulated, peptonized, becoming alkaline. Litmus is reduced; Indol is not formed; Nitrates are not reduced; Acid is formed in dextrose, sucrose and lactose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

It is closely allied to *Achromobacter refractans* (WRIGHT) BERGEY et al. within the description of the latter, although the latter has not been described in many respects of the cultural and physiological characteristics. Therefore, it may be identified to *Achromobacter refractans*.

13. *Achromobacter luminosum* BERGEY et al.

Morphology.

Rods: 0.6-0.7 by 0.7-2.0 microns, with rounded ends, occurring singly, in pairs and in short chains. Non-motile. It forms no endospore. It readily stains all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, gray, glistening, translucent, entire, finely granular; Agar slant: Moderate, filiform, flat or raised, glistening, smooth, translucent to opaque, gray, moist; Gelatin colonies: Small circular, convex, yellowish-gray, glistening, smooth, entire, becoming serrate margin, amorphous; Gelatin stab: No liquefaction. Filiform growth along the line of puncture; Potato: No growth; Broth: Very slightly turbid, with slight sediment.

Physiology.

Litmus milk: Unchanged; Indol is not formed; Nitrates are not reduced; Acid is not formed in dextrose, sucrose and lactose; Starch is not hydrolyzed; Luminescence: Greenish; Aerobic, facultative; Optimum temperature: 25°C.

In the literature, the description of *Achromobacter luminosum* BERGEY et al. is simpler than that of the bacterium isolated by the present author, so that we can not compare with all the characteristics of both. However, so far as the details of the description are available both are mostly identical with each other. Therefore, this bacterium seems to be *Achromobacter luminosum*.

14. *Achromobacter ambiguum* (WRIGHT) BERGEY et al.

Morphology.

Rods: 0.5-0.8 by 0.5-2.0 microns, with rounded ends, occurring singly, in pairs and in short chains. Motile, possessing a polar flagellum. It forms no endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Irregular, smooth, flat or effuse, gray, glistening, transparent, undulate or lobate, finely granular; Agar slant: Moderate, echinulate, flat, glistening, smooth, translucent, gray, moist; Gelatin colonies: Irregular, umbonate, light yellow, glistening, smooth, translucent, granular; Gelatin stab: No liquefaction. Filiform growth along the line of puncture;

Potato: Abundant, spreading, gray to creamy, viscid, glistening; Broth: Strongly turbid, with thin pellicle and gray sediment.

Physiology.

Litmus milk: Acid, slowly coagulated. No peptonization; Indol is not formed, or very slightly; Nitrates are not reduced; Acid is formed in dextrose, but not in sucrose and lactose; Starch is not hydrolyzed; Optimum temperature: 30°C.

This bacterium mostly resembles *Achromobacter ambiguum* (WRIGHT) BERGEY et al., although it slightly differs in respect of the indol formation and although in the description of the latter there is lack in respects of the culture on agar plate and of the action on carbohydrates. This bacterium may be identified to *Achromobacter ambiguum*.

15. *Pseudomonas putida* MIGULA.

Morphology.

Rods: 0.5-0.8 by 1.0-2.5 microns, with rounded ends, occurring singly and in pairs. Motile with three to four polar flagella. It does not produce endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, grayish-green, glistening, translucent, entire amorphous, with fluorescent zone around the periphery; Agar slant: Moderate, filiform, raised, glistening, smooth, yellowish-green, becoming fluorescent; Gelatin colonies: Small circular, raised, gray, glistening, smooth, translucent, undulate, finely granular, with dark center. Medium assumes greenish fluorescent; Gelatin stab: No liquefaction. Filiform growth along the line of puncture. Grayish-white growth on surface, becoming greenish fluorescent from upper layer; Potato: Gray to dirty brown, moist. Medium assumes dirty; Broth: Strongly turbid, with thin pellicle and sediment, becoming fluorescent from upper layer.

Physiology.

Litmus milk: Unchanged; Indol is not formed; Nitrates are reduced to nitrites; Acid is not formed in dextrose, sucrose and lactose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

As mentioned above, it possesses all the characteristics of *Pseudomonas putida* MIGULA.

16. *Flavobacterium helvolum* (ZIMMERMANN) BERGEY et al.

Morphology.

Rods: 0.5-0.6 by 0.7-1.5 microns, with rounded ends, occurring singly and in pairs. Non-motile. It forms no endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, pale yellow, becoming greenish yellow, opaque, entire, finely granular; Agar slant: Abundant, spreading, raised, glistening, smooth, gray to pale yellow, becoming greenish-yellow, undulate, moist; Gelatin colonies: Small circular, raised,

yellowish-gray, smooth, granular; Gelatin stab: Napiform to stratiform liquefaction; Potato: Abundant, plumose to spreading, thick, dirty brown, becoming yellowish-green, glistening, becoming rough and dull; Broth: Turbid, with gray ring and sediment.

Physiology.

Litmus milk: Slightly acid, with soft coagulum, becoming alkaline with peptonization; Indol is not formed; Nitrates are not reduced; Starch is hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

All the characteristics of this bacterium are mostly identical with those of *Flavobacterium helvolum* (ZIMMERMANN) BERGEY et al. Therefore, it seems to be *Flavobacterium helvolum*.

17. *Flavobacterium aquatile* (FRANKLAND and FRANKLAND) BERGEY et al.

Morphology.

Rods: 0.5-0.6 by 1.5-2.0 microns, with rounded ends, occurring singly, in pairs and in short chains. Motile, possessing four to five peritrichous flagella. It forms no endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular with radiate margin, smooth, effuse or flat, grayish-yellow, translucent, lobate, finely granular, with dark center; Agar slant: Moderate, echinulate, flat, glistening, smooth, grayish-yellow, moist; Gelatin colonies: Small circular (ca. 1 m.m. in dia.), yellowish-orange, smooth, opaque, entire, granular, with dark center; Gelatin stab: Filiform growth along the line of puncture. Small amount of growth on surface, irregular, yellowish-gray. Saccate very slow liquefaction; Potato: Yellowish-orange streak, moist; Broth: Turbid, with grayish-yellow sediment.

Physiology.

Litmus milk: Unchanged; Indol is not formed; Nitrates are reduced to nitrites; Acid is formed in dextrose, but not in sucrose and lactose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

This organism corresponds to the description of *Flavobacterium aquatile* (FRANKLAND and FRANKLAND) BERGEY et al.

18. *Flavobacterium tabidum* nov. sp.

Morphology.

Rods: 0.4-0.6 by 0.7-1.5 microns, short rods, with rounded ends, occurring singly and in pairs. Motile, possessing a polar flagellum. It does not produce endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: Circular with auriculate margin, smooth, flat, greenish-orange, translucent, entire, granular; Agar slant: Moderate, echinulate, flat, glistening, smooth, greenish-orange, translucent, moist; Gelatin colonies: Small circular (ca. 1 m.m. in dia.), raised, orange, smooth, opaque, entire, finely granular; Gelatin stab: Napiform liquefaction with granular sediment; Potato: Abundant, orange, glistening, moist; Broth: Turbid, with gray pellicle and sediment.

Physiology.

Litmus milk: Slightly acid, coagulated, peptonized; Indol is not formed; Nitrates are not reduced; Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 25°-30°C.

It possesses all the characteristics of the genus *Flavobacterium* as given by BERGEY. But, as it appears that there are no bacteria which are analogous to this bacterium, the name *Flavobacterium tabidum* is proposed.

19. *Bacillus tumescens* ZOPF.

Morphology.

Rods: 1.3-1.4 by 3.0-3.5 microns, with rounded ends, occurring singly and in short chains. Motile, possessing peritrichous flagella. Gram-positive. It produces endospore in central or excentric; Endospores: Elliptical, 0.8-1.0 by 1.5-1.8 microns in size.

Cultural characteristics.

Agar colonies: Circular, smooth, raised, gray, somewhat dull, opaque, entire, granular; Agar slant: Thin, echinulate, dull, smooth, whitish-gray, opaque, slimy, becoming yellowish and glistening; Gelatin colonies: Circular, with auriculate margin, whitish-gray, homogeneous, liquefying; Gelatin stab: Crateriform to infundibuliform liquefaction; Potato: Raised, smooth, glistening, whitish, moist; Broth: Turbid, with grayish pellicle and sediment.

Physiology.

Litmus milk: Slightly acid, coagulated, peptonized; Indol is not formed; Nitrates are not reduced; Acid is formed in dextrose, sucrose and lactose; Starch is hydrolyzed; Aerobic, facultative; Optimum temperature: 30°-35°C.

It closely resembles *Bacillus tumescens* ZOPF, although it seems to be slightly different in some respects of the cultural features. This organism, therefore, may be *Bacillus tumescens*.

20. *Bacillus subtilis* (EHRENBERG) COHN.

Morphology.

Rods: 0.7 by 1.5-3.5 microns, with rounded ends, occurring singly and in short chains. Motile, with peritrichous flagella. Gram-positive. It forms endospore in center or excentric and is slightly smaller in diameter than the endospore, thus causing a distinct bulging; Endospores: Elliptical, 0.5-0.8 by 0.7-0.9 microns in size.

Cultural characteristics.

Agar colonies: Amoeboid with crenate margin, grayish, raised, opaque, granular or gyrose; Agar slant: Spreading, dull, becoming glistening, adherent, grayish-white, opaque, butyrous; Gelatin colonies: Circular, thin, whitish, entire, granular, becoming creamy white, liquefying; Gelatin stab: Filiform growth along the line of puncture. Crateriform to stratiform liquefaction; Potato: Luxuriant, warty, gray, becoming pink to reddish-brown, dry, becoming moist and sticky; Broth: Turbid, with fragile pellicle and grayish sediment, becoming clear. Scum is precipitated as a whole in about a week.

Physiology.

Litmus milk: Alkaline, peptonized, never coagulated; Indol is not formed; Nitrates are

reduced to nitrites; Acid is formed in dextrose and sucrose, but not in lactose; Starch is hydrolyzed; Aerobic, facultative; Optimum temperature: 30°-35°C.

As the above mentioned description, this microorganism clearly seems to be *Bacillus subtilis* (EHRENBERG) COHN.

21. *Bacillus mesentericus* TREVISAN.

Morphology.

Rods: 0.6-0.8 by 1.5-4.0 microns, with rounded ends, occurring singly and in short chains. Motile, with peritrichous flagella. Gram-positive. It produces endospore in central or in sub-terminal; Endospores: Slightly more oval, 0.5 by 0.7-1.0 microns in size.

Cultural characteristics.

Agar colonies: Circular, becoming amoeboid, refractive, spreading, creamy-white, entire to auriculate, crenate; Agar slant: Thick, abundant, white to cream-white, spreading, non-adherent, serrate margin; Gelatin colonies: Circular, gray, crateriform liquefaction with granular ring at the edge; Gelatin stab: Liquefaction crateriform, becoming stratiform; Potato: Abundant, brown, rugose, becoming smooth, moist; Broth: Turbid, with fragile scum, becoming clear.

Physiology.

Litmus milk: Peptonization. No coagulation. No acidification; Indol is not formed; Nitrates are not reduced; Acid is formed in dextrose, but not in lactose; Starch is hydrolyzed; Hydrogen sulphide is slightly produced, or not; Aerobic, facultative; Optimum temperature: 30°-35°C.

It mostly resembles *Bacillus mesentericus* TREVISAN though the latter lack the description of its action on starch.

22. *Bacillus cereus* FRANKLAND and FRANKLAND.

Morphology.

Rods: 0.8-1.0 by 2.5-4.0 microns, with rounded ends, occurring singly and in long chains. Many cells of the organism show peculiar refractive bodies of various size as the culture get older. Motile, possessing peritrichous flagella. Gram-positive. It forms endospore in center or in excentric. Rods swollen slightly at the sporulation; Endospores: Elliptical, 0.8-1.0 by 1.1-1.5 microns in size.

Cultural characteristics.

Agar colonies: Circular, raised, highly refractive, dense, grayish-white, entire or undulate, curled structure. Pellucid dots are formed on the surface of colonies after several days; Agar slant: Abundant, thick, spreading, grayish-white, becoming yellowish-white, mealy. Pellucid dots are formed on the surface after several days; Gelatin colonies: Loosely filamentous, grayish, spreading with irregular margin, liquefying rapidly; Gelatin stab: Liquefaction crateriform to saccate; Potato: Thick, white, mealy, becoming yellowish to brown. The brownish growth may become very moist and slimy; Broth: Strongly turbid, with ring formation. Then a heavy fragile pellicle is produced, which is entirely precipitated within a short time. Becoming clear gradually. Flocculent sediment is deposited.

Physiology.

Litmus milk: Rapid peptonization. No coagulation; Indol is not formed; Nitrates are reduced to nitrites; Acid is formed in dextrose and sucrose, but not in lactose; Aerobic facultative; Optimum temperature: 30°-35°C.

As the above mentioned description, this bacillus possesses all the characteristics of *Bacillus cereus* FRANKLAND and FRANKLAND.

23. *Bacillus laterosporus* LAUBACH.

Morphology.

Rods: 0.5-0.6 by 1.5-4.0 microns, with rounded ends, occurring singly, in pairs and in short chains. Motile, possessing peritrichous flagella. Gram-positive. It forms endospore in central or in sub-terminal. Rods swollen slightly at sporulation; Endospores: Elliptical, 0.6-0.8 by 1.0-1.2 microns in size.

Cultural characteristics.

Agar colonies: Irregular, smooth, flat or raised, grayish, translucent to transparent, glistening, auriculate, granular; Agar slant: Abundant, spreading, flat, glistening, smooth, grayish, moist, with metallic lustre; Gelatin colonies: Circular, thin, gray, transparent, entire, finely granular; Gelatin stab: Crateriform liquefaction. Filiform growth; Potato: Dull, gray, finely wrinkled, becoming smooth and glistening; Broth: Slightly turbid, with flocculent pellicle which is precipitated within a short time.

Physiology.

Litmus milk: Slightly acid. Soft coagulum. Peptonization. Becoming slightly alkaline. Litmus is reduced; Indol is not formed; Nitrates are not reduced. Acid is formed in dextrose and sucrose, but not in lactose; Starch is not hydrolyzed; Aerobic, facultative; Optimum temperature: 30°-35°C.

It is mostly identical with *Bacillus laterosporus* LAUBACH, excepting some respects of the morphological features. Therefore, it is presumed to be *Bacillus laterosporus*.

Relation between the number of strains of and the kinds of the isolated microorganisms.

There were isolated in total, 181 strains of microorganisms from two kinds of the spoiled "semi-dried fishes" using common agar media during three series of experiments, that is, 54 strains (28 strains from chub mackerel and 26 strains from carangoid fish) in April, 53 strains (32 strains from chub mackerel and 21 strains from carangoid fish) in July and 74 strains (41 strains from chub mackerel and 33 strains from carangoid fish) in October. These strains were classified into 23 kinds, that is, 8 kinds of *Micrococcus*, 2 kinds of *Rhodococcus*, 4 kinds of *Achromobacter*, 1 kind of *Pseudomonas*, 3 kinds of *Flavobacterium* and 5 kinds of *Bacillus*. These results obtained were shown in the following tables.

Microorganisms	April		July		October	
	Chub mackerel	Carangoid fish	Chub mackerel	Carangoid fish	Chub mackerel	Carangoid fish
1. <i>Micrococcus caseolyticus</i> EVANS	3	2		1		3
2. <i>Micrococcus mucofaciens</i> THÖNI and THAYSEN		2	3	2	4	3
3. <i>Micrococcus conglomeratus</i> (FLÜGGE) MIGULA	4	2	2		6	
4. <i>Micrococcus ochraceus</i> ROSNTAL		1	2	2		2
5. <i>Micrococcus himonoi</i> nov. sp.	2	3			5	
6. <i>Micrococcus candicans</i> FLÜGGE		2	3	1	3	
7. <i>Micrococcus perflavus</i> BERGEY et al.	2		4	1		1
8. <i>Micrococcus subflavescens</i> BERGEY et al.		2		2		3
9. <i>Rhodococcus cinnebareus</i> (FLÜGGE) HOLLAND		1		1	1	
10. <i>Rhodococcus roseus</i> (FLÜGGE) WINSLOW and WINSLOW	2		2	1	3	3
11. <i>Achromobacter midthletounii</i> (CHESTER) BERGEY et al.	2	2			3	4
12. <i>Achromobacter refractans</i> (WRIGHT) BERGEY et al.		2	2	1	2	
13. <i>Achromobacter luminosum</i> BERGEY et al.	2		3	1	3	1
14. <i>Achromobacter ambiguum</i> (WRIGHT) BERGEY et al.	1					
15. <i>Pseudomonas putida</i> MIGULA	1					
16. <i>Flavobacterium heliolum</i> (ZIMMERMANN) BERGEY et al.	3	4	3	3	5	3
17. <i>Flavobacterium aquatile</i> (FRANKLAND and FRANKLAND) BERGEY et al.						1
18. <i>Flavobacterium tabidum</i> nov. sp.						2
19. <i>Bacillus tumescens</i> ZOPF	3		2	1	2	1
20. <i>Bacillus subtilis</i> (EHRENBERG) COHN		2	1		2	
21. <i>Bacillus mesentericus</i> TREVISAN	1		2	3	1	3
22. <i>Bacillus cereus</i> FRANKLAND and FRANKLAND	2	1	1		1	
23. <i>Bacillus litorosporus</i> LAUBACH			2	1		3
Total	28	26	32	21	41	33

The microorganisms isolated in April.

Organisms	Chub mackerel		Carangoid fish		Total	
	Strains	%	Strains	%	Strains	%
Cocci	13	46.5	15	58	28	52
Non-spore forming rods	9	32	8	31	17	31
Spore forming rods	6	21.5	3	11	9	17
Total	28	100	26	100	54	100

The microorganisms isolated in July.

Organisms	Chub mackerel		Carangoid fish		Total	
	Strains	%	Strains	%	Strains	%
Cocci	16	50	11	52	27	51
Non-spore forming rods	8	25	5	24	13	24.5
Spore forming rods	8	25	5	24	13	24.5
Total	32	100	21	100	53	100

The microorganisms isolated in October.

Organisms	Chub mackerel		Carangoid fish		Total	
	Strains	%	Strains	%	Strains	%
Cocci	22	53.5	15	45.5	37	50
Non-spore forming rods	13	32	11	33	24	32.5
Spore forming rods	6	14.5	7	21.5	13	17.5
Total	41	100	33	100	74	100

Total of three cases.

Organisms	Chub mackerel		Carangoid fish		Total	
	Strains	%	Strains	%	Strains	%
Cocci	51	51	41	51	92	51
Non-spore forming rods	30	30	24	30	54	30
Spore forming rods	20	20	15	19	35	19
Total	101	100	80	100	181	100

1. *Cocci*.

It was found that all of the isolated coccus forms are belonging to *Micrococcus* and *Rhodococcus*. Ninety-two strains among 181 of the isolated microorganisms in three cases were coccus forms, that is, 28 strains (13 strains from chub mackerel and 15 strains from carangoid fish) in April, 27 strains (16 strains from chub mackerel and 11 strains from carangoid fish) in July and 37 strains (22 strains from chub mackerel and 15 strains from carangoid fish) in October. The strains of the cocci were found in about 50% of those of the isolated microorganisms even from the different spoiled "semi-dried fishes" in the different time. And also the number of the kinds were the greatest.

From the facts, therefore, it is presumed that the cocci have an important agency on the spoilage of the "semi-dried fishes". Some species of these cocci, however, does not seem to be predominant especially on the spoilage, and every one of these flora seems to grow on as well to nearly the same extent.

2. *Non-spore forming rods*.

All of the non-spore forming rods which were isolated from the spoiled "semi-dried fishes" were belonged to *Achromobacter*, *Pseudomonas* and *Flavobacterium*, and those belonging to the *coli-aerogenes* group and the *Proteus*

group were not found among them. Among the non-spore forming rods which were isolated from the spoiled "semi-dried fishes", the motile rods were 4 kinds only—*Achromobacter ambiguum*, *Pseudomonas putida*, *Flavobacterium aquatile* and *Flavobacterium tabidum*. The first two were isolated only from chub mackerel in April while the last two from carangoid fish in October, with rather small number of the strains isolated. Any one of the motile rods was never found in the other cases. Chub mackerel in April was contained 69.45% of moisture, and carangoid fish in October was contained 70.58% of moisture, that is, both were contained more than the others in moisture. The motile rods, therefore, seems to be able to cause the spoilage of the "semi-dried fishes" which contain a considerable amount of the moisture, but does not for those containing smaller amount of the moisture.

Non-motile rods were isolated from the spoiled "semi-dried fishes" in all of the cases. And, from the above mentioned fact it is clear that the majority of the isolated non-spore forming rods are the non-motile rods. As shown in the above mentioned tables, the number of the strains of the non-spore forming rods was about 30% of those of the isolated microorganisms in all of the cases excepting the case in July, in which the number of the non-spore forming rods was comparatively smaller. Of the non-motile rods, *Flavobacterium helvolum* was found in the comparatively large number in all of the cases. Therefore, it probably can grow well on the "semi-dried fishes," causing the spoilage.

3. Spore forming rods.

All of the spore forming rods which were isolated from the spoiled "semi-dried fishes" belonged to *Bacillus* and 5 kinds were found in them. The number of the strains of the spore forming rods was about 15-20% of the total strains of the isolated microorganisms. But in the case of July, it was about 25%. Certain species among the 5 kinds of these bacteria does not seem to be predominant, but they seem to have almost the same tendency on the spoilage.

Discussion.

From the above mentioned results, it is clear that the spoiling bacteria for the "semi-dried fishes" are very varied in kinds and in characteristics from those for the fresh fishes. Namely, it is well known that the fresh fishes are spoiled principally by the action of the water bacteria which are Gram-negative, actively motile and non-spore forming rods belonging to *Pseudomonas*, *Achromobacter* and *Flavobacterium*. On the contrary, it seems

that the "semi-dried fishes are spoiled principally by the action of cocci, non-spore forming and non-motile rods, and spore forming rods belonging to various genus. These microorganisms are, in soil, water and air, ordinary present. But, in the case of the "semi-dried fishes" containing a considerable amount of moisture, the spoilage may be caused to some extent by the typical water bacteria which are actively motile and non-spore forming rods, as recognized from the results obtained in the experiments of chub mackerel in April and carangoid fish in October.

It seems that among the above mentioned three bacterial forms the cocci are the most important, the next are the non-spore forming and non-motile rods, and the least are the spore forming rods. From the results of the experiment in July, however, it may be thought that the spore forming rods can have their action to some extent on the spoilage of the "semi-dried fishes" in a hot season such as in summer days.

The microflora on the spoilage does not seem to be very varied in kinds and in characteristics, by the different two kinds of fishes used.

Summary.

1. The spoilage of the "semi-dried fishes" such as chub mackerel and carangoid fish was studied from the bacteriological standpoint in 3 cases, that is, in April, in July and in October.

2. One hundred and eighty one strains of bacteria were isolated from the spoiled "semi-dried fishes". These microorganisms were divided into 3 groups, that is, cocci (92 stains, viz. 51%), non-spore forming rods (54 stains, viz. 30%) and spore forming rods (35 strains, viz. 19%). In all of the cases excepting the case of July, percentage of each of the isolated microorganisms belonging to these groups was as follows: The cocci were about 50%, the non-spore forming rods were about 30% and the spore forming rods were about 20%.

3. The cocci isolated were classified into 8 kinds of *Micrococcus* and 2 kinds of *Rhodococcus*. The non-spore forming rods isolated were classified into 4 kinds of *Achromobacter*, 1 kind of *Pseudomonas* and 3 kinds of *Flavobacterium*. The spore forming rods isolated were classified into 5 kinds of *Bacillus*.

4. Among the non-spore forming rods isolated, the motile rods were found only in the spoiled "semi-dried fishes" containing a considerable amount of moisture.

5. In a hot season (in July) the strains of the spore forming rods were isolated comparatively much (25%) from the spoiled "semi-dried".

6. The microorganisms on the spoilage were not very varied in kinds and in characteristics for the different two kinds of fishes (viz. chub mackerel

and carangoid fish) used.

7. The typical water bacteria which were actively motile, non-spore forming and Gram-negative short rods were not the normal flora on the spoilage of the "semi-dried fishes".

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