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Microbiology

QUANTITATIVE AND QUALITATIVE CHANGES OF MICROFLORA
IN A SKIN SLIME OF COD-FISH STORED IN A STERILE ICE

ZMIANY ILOŚCIOWE I JAKOŚCIOWE MIKROFLORY
W ŚLUZIE SKÓRY DORSZA BAŁTYCKIEGO SKŁADOWANEGO
W JAŁOWYM LODZIE

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Subject of the investigations was a quantitative and qualitative composition of microflora in slime of a Baltic cod-fish directly after catching and during its storing in a sterile ice. During the 12-day storing, the quantitative and qualitative changes in the bacterial composition were noted. The total quantity of bacteria and the percentage participation of the rods of *Pseudomonas*-*Achromobacter* group increased during the investigations.

A qualitative and quantitative composition of bacterial flora on the surface of a freshly-caught fish reflects the conditions of catching waters.

According to the literature data collected by *S h e w a n* (1962), the slime of an alive fish may contain from 10^3 to 10^6 of the bacteria in 1 cm^2 of the skin surface. The data relating to a qualitative composition determined by some former investigators and collected by *Z o b e l l* (1946), and the later investigations (*L i s t o n*, 1957; *G e o r g a l a*, 1958; *L i s t o n*, 1960; *S h e w a n et al.* 1960; *S p e n c e r*, 1961; *S c h o l e s* and *S h e w a n*, 1964), prove that a bacterial flora of the seas and of some existing in them commercially important species of fish, is composed in a high percent of the psychrophilic gram-negative rods. Such flora was most frequently classified as belonging to *Pseudomonas*, *Achromobacter* and *Flavobacterium* group, which in the moderate zone represented about 60-70 per cent of the bacteria total quantity (*S h e w a n*, 1949; *T a r r*, 1954; *S c h o l e s* and *S h e w a n*, 1964). Some reports have also been made on the high-percent presence of the gram-positive bacteria in a slime of the sea-fishes. (*W o o d*, 1940 and *D y e r* 1941). Investigating the microflora of Atlantic fresh cod-fish, *Dyer* estimated the presence of 78.5 per cent of the *Micrococcus* bacteria in a cod-fish slime.

Ascertaining the seasonal changes in the microflora composition of a N. Sea haddock's slime (*Melanogrammus aeglefinus*), *R e a y* and *S h e w a n* (1949) presumed, that they reflect the changes of a bacteria population in the

sea waters. Liston (1957), in his bacteriologic investigations in slime on a skin surface of the skate and lemon sole (*Pleuronectes microcephalus*) caught in the same waters, also noted some changes in the quantity of bacteria in relation to a fishing season. The highest quantity of bacteria at lemon sole were noted in July and in early Autumn, and at the skin of skate - towards the end of a year. Liston considers, that the species of investigated fish, may bear on such differences.

The psychrophilic bacteria present in the slime of a caught fish, have good living conditions and can grow in it. They further penetrate through the skin and its mechanical damages into the fleshy tissue and cause its spoilage.

An ice is generally used to protect the fish against quick spoilage: it extends the time of one generation of the psychrophilic bacteria present on a fish. The investigations effected under the industrial conditions proved, that if a temperature of the iced cod-fish does not exceed 0°C , the quantity of bacteria, after one or two days - decrease in growth, shall after 10-12 days rise to the value of 10^7 - 10^8 per 1 cm^2 of skin (Shewan, 1962).

During the first 5 days, the percent share of cocci is decreasing in favour of *Pseudomonas*, *Achromobacter* and *Flavobacterium* group. Of the mentioned gram-negative bacteria, the *Pseudomonas* species are attaining the highest quantity after the sixth day, to rise-up to about 90 per cent of the total quantity of gram-negative bacteria on the 12th day (Shewan, 1962). After 12-days of storing in ice, the cod-fish is not edible for consumption due to the far-advanced spoiling process.

The bacterial flora of a fish may be defined quantitatively and qualitatively in the two aspects: biocoenotic, as: species of fish, season of year, environment, etc. and exterior: past-catching contamination, conditions of investigations, temperatures of incubation etc. (Shewan, 1962).

No data relating to the quantitative and qualitative composition of bacteria in slime of the Baltic cod-fish, which to a certain extent is free of exterior impurities, have been traced in the so far obtainable literature.

MATERIALS

The skin surface slime of *Gadus morrhua* L. caught on the Baltic fishing grounds R-10, R-11, R-12, S-11 and S-12 during February, March, April, May, June and July 1967 and during February and March 1968, was used as the material for the investigations.

METHODS

At sea, the fishes (*Gadus morrhua* L.) were sterily picked-up from the bottom trawl-net wings; sterily iced and stored in a sterile thermos. The fish had been handled in the sterile conditions from catching through all the time of investigations. As the investigations lasted for 12 days and the ice was

melting away, the fish had to be protected against the contact with water by a partition-plate located at the thermos bottom. Every second day, the water was removed from thermos and the fish charged with ice.

The first quantitative and qualitative records of the microbes present in the slime of skin of cod-fish, were taken after 2 days from catching; further - after 4, 6, 8, 10 and 12 days.

a) **Q u a n t i t a t i v e e s t i m a t i o n s.** The 2.5 per cent agar prepared on a sea-water was used for the count plates. The slime was collected with a sterile tampon from 4 cm^2 of skin, after thorough rinsing in a test-tube with 10 ml of physiological solution, further dilutions were prepared and inoculated on the surface of plates, each of 0.1 ml. The inoculations had been incubated for 48 hrs at the temperature of 22°C .

b) **Q u a l i t a t i v e e s t i m a t i o n s.** About 100 of colonies of the quantitative inoculations were taken from surface of the cut plates; the ratio of gram-negative to gram-positive bacteria was marked. For this purpose, the specimen were stained by Gram method modified by K o p e l o f f and B e e r m a n. Finally the gram-negative rods and vibrio were identified according to pattern of S h e w a n et al. (1960), as prepared by B u r z y ń s k a (1964) for identification of the bacteria *Pseudomonas-Achromobacter* and of the relative groups.

As per pattern, a motility of bacteria was noted and a type of respiration defined. Noted: an ability of oxidation and of fermentation of the glucose on Hugh-Leifson medium and alkalization ability of such medium. Tested: an ability of decomposition of the arginine under oxygenic and anaerobic conditions and an oxidase generating ability for detection by Kovac's method. The bacteria of *Pseudomonas* group were subjected to the fluoresceine generation test by an inoculation on the King's B medium. Composition of the applied mediums and the methods of identification were complying with data supplied by B u r z y ń s k a (1964), acc. to S h e w a n et al. (1960).

RESULTS

Results of the total quantity of bacteria and of the gram-positive and gram-negative bacteria present in slime of 1 cm^2 of the cod-fish skin after two-days storing in sterile ice and during the further storing, are shown on Fig.1.

The first tests effected after two days from catching proved the presence of 3 400 to 21 700 of bacteria in the slime of 1 cm^2 of cod-fish skin. The average value for all tests amounted to 10 930 of bacteria in slime of 1 cm^2 of cod-fish skin. During the further storing upto 12 days, the quantity of bacteria in slime was successively increasing in average of upto 18 240 000 per 1 cm^2 of skin.

The quantity of gram-negative bacteria in slime was increasing in average from $4\,500/\text{cm}^2$ on second day from catching to $14\,585\,000/\text{cm}^2$ on the 12th day. The quantity of gram-positive bacteria in slime was increasing in average from $6\,430/\text{cm}^2$ of skin to $3\,655\,000/\text{cm}^2$ on the last day of test.

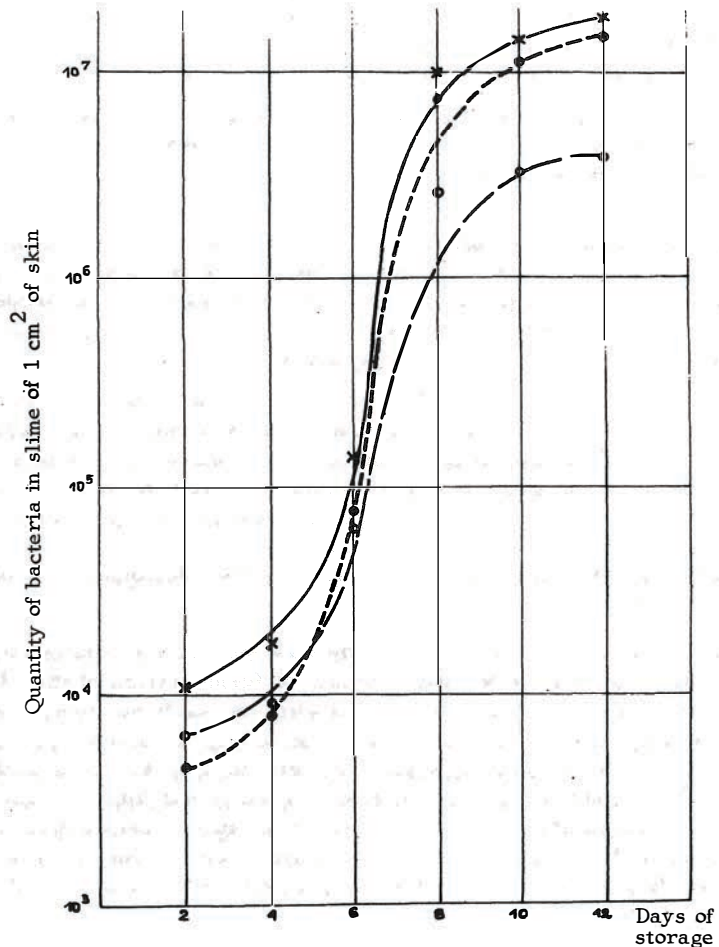


Fig.1

×—× Total quantity of bacteria. o—o Quantity of the gram-positive bacteria. ●—● Quantity of the gram-negative bacteria.

Total quantity of bacteria and the quantity of gram-positive and gram-negative bacteria in slime of 1 cm² of cod-fish skin during the storage in sterile ice.

The results expressed in per cent increase of the gram-negative bacteria are shown in Fig.2. After the second day of storing, the quantity of gram-negative bacteria was 38.5 per cent in average. This was successively changing during the further storing of cod-fish in sterile ice, to attain the mean value of 78.8 per cent.

Assuming the quantity of the tested gram-negative bacteria to be 100 per cent, the percentage relations within the extent of the results are also illustrated on Fig.2. It appears from the curves, that the changes are also here taking place. After two days of storing, the gram-negative microflora contains mainly the bacteria of *Pseudomonas* (43.1 per cent), *Achromobacter* (31.5 per cent), and *Flavobacterium* (17 per cent). The remaining bacteria

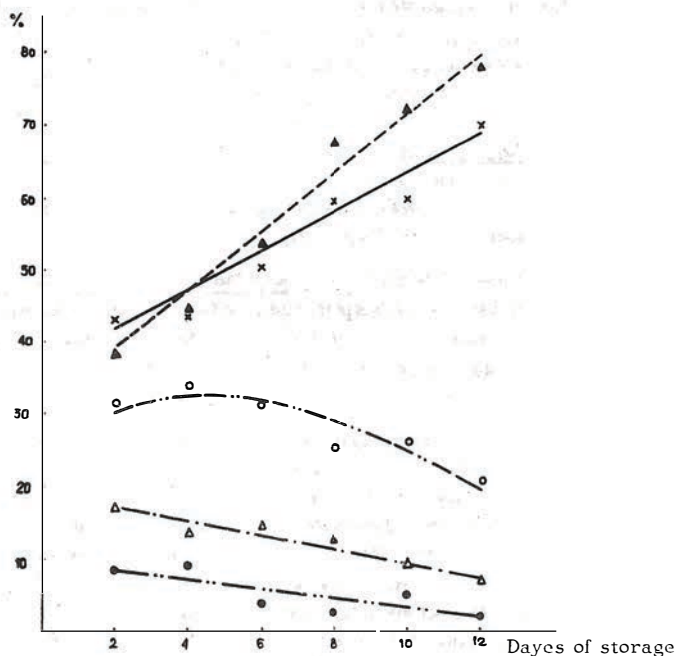


Fig. 2

x — x Pseudomonas species, o — o Achromobacter species,
 Δ — Δ Flavobacterium species, ● — ● Other remaining gram-negative rods, ▲ — ▲ Per-cent of gram-negative bacteria in total quantity of bacteria

Per cent increase of the gram-negative bacteria and the per cent relation between the gram-negative bacteria in slime of cod-fish skin during the storage in sterile ice

of relative groups represent 8.4 per cent and comprise mainly the species of Alcaligenes, Xanthomonas, Aeromonas and Anitratum. During the further storing of cod-fish in sterile ice, the participation of Pseudomonas bacteria is distinctly increasing, to attain on the 12th day the value of 70.2 per cent of the total quantity of the gram-negative bacteria. The percentage of Achromobacter, after the slight increase on the 4th day of storing, is decreasing to 20.9 per cent after 12 days, while of Flavobacterium - to 7.0 per cent. The quantity of remaining gram-negative bacteria is gradually decreasing to 1.9 per cent. The level of Pseudomonas fluorescens strain in relation to the total quantity of Pseudomonas species was oscillating within about 18 per cent to 29 per cent with no uniformity noted.

DISCUSSION

A part of the gram-negative bacteria in spoilage process of the food products is well known. Such spoilage of the sea-fish is also due to an activity

of the bacteria. The investigations on spoilage of a fish tissue under industrial conditions proved, that at the final stage of spoilage the strains of *Pseudomonas* group play the very vital part (S h e w a n et al., 1960 and S p e n c e r, 1961). This had been conformed by application of the pure-cultured strains on a sterile meat of fish (S h e w a n and J o n e s 1957). In a process of fish spoilage, the *Pseudomonas* are accompanied by the *Achromobacter* strains. After some negligent increasing upto the 5th day, they maintain somehow on an even level (D e S i l v a, 1960), causing some minor changes in appearance of meat and an emanation of a mild spoilage odour (S h e w a n et al., 1960).

The highest participation of the *Pseudomonas* and *Achromobacter* species towards the final process of a fish spoilage, was also proved by S h e w a n and L i s t o n (1956) and d e S i l v a (1960) in their investigations on the quantitative changes of microflora in a skin slime of the cod-fish and of herrings iced on the ship and in the processing plants.

The examination in question performed with use of the sterile ice, had to prove whether the gram-negative microorganisms as the additional post-catching contaminations, play any part in a process of fish spoilage. Should the spoilage process of a fish stored in sterile ice be consequent to the bacterial flora of a land origine, the results of the presented investigations would principally differ with the results of other scientists. It is ascertained in our investigations, that the gram-negative rods participated in a smaller extent during the initial period; however, 5 days after catching, the percentage relation was equal and after 12 days, the gram-negative rods reached the value of 78.8 per cent of the total quantity of bacteria. At that period, the per cent participation of *Pseudomonas* sp. increased to about 70 per cent of the total gram-negative bacteria. The *Achromobacter* and *Flavobacterium* species participated in a smaller extent.

It may be worth to note, that the higher was the initial quantity of bacteria in slime of a cod-fish on the second day after catching, the higher were the absolute quantitative values of bacteria on the last day of investigations. This indicates towards the necessity of the optimal conditions when hauling the fish from sea onto deck. The present methods may, thus, rise some doubts from the sanitary point of view, and make difficult to maintain the fish within a good quality class. The last aspect is still more aggravated by the fact, that during the net-hauling on deck, the fish, which already possess in slime certain bacterial charge, is additionally contaminated with the bacteria extracted together with a content of the intestines.

CONCLUSIONS

1. Basing on the results of this investigations, it may be ascertained, that during the storing of cod-fish in a sterile ice, the total quantity of bacteria present in slime of 1 cm² of skin is increasing from some few thousands to reach several milions on the twelfth day after catching.

2. During the storing of a cod-fish in sterile ice, the gram-negative psychrophilic bacteria increase from about 38 per cent present on the second

day after catching, to about 79 per cent on the 12th day after catching.

3. At the final stage of a cod-fish storing in sterile ice, the gram-negative microflora contains mainly the *Pseudomonas* sp. which participate in about 70 per cent of total quantity of these bacteria.

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ZMIANY IŁOŚCIOWE I JAKOŚCIOWE MIKROFLORY W ŚLUSZIE SKÓRY DORSZA BAŁTYCKIEGO SKŁADOWANEGO W JAŁOWYM LODZIE

S t r e s z c z e n i e

Badano bakteriologicznie śluz dorsza (*Gadus morrhua* L.) poławianego na łowiskach bałtyckich w ciągu 8 miesięcy i przechowywanego w jałowym lodzie przez okres 12 dni. Pierwsze badania wykonano po upływie dwóch dni od chwili połowu, a ostatnie w dwunastym dniu.

Ogólna ilość bakterii w śluzie z powierzchni 1 cm² skóry dorsza w drugim dniu od chwili połowu wynosiła średnio 10 930 i wzrosła średnio do 18 240 000 bakterii w dwunastym dniu. Po drugim dniu przechowywania ilość bakterii gram-ujemnych wynosiła średnio 4 500/cm², a po dwunastym - 14 585 000/cm² skóry. Ilość bakterii gram-dodatnich wzrastała średnio w śluzie dorsza z 6 430/cm² skóry do 3 655 000/cm² skóry w ostatnim dniu badania. Po drugim dniu przechowywania bakterie gram-ujemne stanowiły średnio 38,5%. Pozostałość stanowiły bakterie gram-dodatnie, w szczególności ziarniaki. Stan ten ulegał zmianie i w ostatnim dniu badania bakterie gram-ujemne osiągnęły średni poziom 78,8%.

Określono również układy procentowe w obrębie bakterii gram-ujemnych. W pierwszym badaniu stwierdzono, że w skład mikroflory gram-ujemnej wchodzi głównie bakterie z rodzajów *Pseudomonas* (43,1%), *Achromobacter* (31,5%) i *Flavobacterium* (17,0%). Pozostałe bakterie z grup pokrewnych stanowiły 8,4%. W trakcie przetrzymywania dorsza w sterylnym lodzie wzrastał znacznie udział *Pseudomonas* sp., który w dwunastym dniu składowania wynosił 70,2% ogólnej ilości bakterii gram-ujemnych. Udział pozostałych bakterii gram-ujemnych malał: *Achromobacter* sp. do 20,9%, *Flavobacterium* sp. do 7,0% i innych pałeczek z grup pokrewnych do 1,9% ogólnej ilości bakterii gram-ujemnych.

КОЛИЧЕСТВЕННЫЕ И КАЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ МИКРОФЛОРЫ В СЛИЗИ КОЖИ БАЛТИЙСКОЙ ТРЕСКИ ХРАНИМОЙ В СТЕРИЛЬНОМ ЛЬДУ

Р е з ю м е

Изучалась бактериологически слизь трески ловленной в Балтике в течение 8 месяцев и затем хранённой в стерильном льду в течение 12 дней. Пер-

вые опыты проводились со второго дня ото дня лова и заканчивались в 12 день.

Общее количество бактерий в слизи с поверхности 1 см^2 кожи трески спустя 2 дня со времени лова составляло в среднем 10 930 и росло в среднем до 18 240 000 бактерий на 12 день. После 2 дней хранения количество г.-отрицательных бактерий составляло в среднем 4500 см^2 , а после 12-14 585 000 см^2 кожи. Количество г.-положительных бактерий росло в среднем в слизи трески с $6\ 430/\text{см}^2$ до $3\ 655\ 000/\text{см}^2$ кожи в последний день опыта. В два дня после лова г.-отрицательные бактерии составляли в среднем 38,5%. Остальные это г.-положительные а особенно земляные. Однако эта картина изменялась и в последний день опыта г.-отрицательные бактерии достигали в среднем уровня в 78,8%.

Определялись также процентные соотношения г.-отрицательных бактерий. В начальных опытах установлено, что в состав г.-отрицательной микрофлоры входят преимущественно бактерии родов *Pseudomonas* (43,1%), *Achromobacter* (31,5%) и *Flavobacterium* (17,0%). Остальные бактерии из родственных групп составляли 8,4%. Во время хранения трески в стерильном льду росло относительное количество *Pseudomonas* sp. который на 12 день хранения составлял 70,2% общего числа г.-отрицательных бактерий, в то время как доля остальных г.-отрицательных бактерий уменьшалась; *Achromobacter* sp. до 20,9%, *Flavobacterium* sp. до 7,0% а других палочек из родственных групп до 1,9% общего количества г.-отрицательных бактерий.

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