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A New System for Studying the Properties of Microorganisms Isolated From the Gastrointestinal Tract of Fish

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Abstract: The world's resources of seafood and freshwater fish are not unlimited, therefore, over the past century, interest in aquaculture has increased so much, that is, the breeding and cultivation of fish, algae, mollusks, and crustaceans. A positive factor is non-waste, which makes it possible to produce products for feeding farm animals, for example, fishmeal, from the waste of the fishing industry.

Keywords: pathogenic microbes, environmentally friendly, temperature, meat-peptone broth.

In Uzbekistan, as a result of constant attention to the introduction of modern technologies, stable development of all branches of agriculture is achieved. In particular, the expansion of fish farming in the country contributes to providing the population with environmentally friendly and healthy products.

The study of the ecological characteristics of pathogenic microbes is important in determining the places of their reservation in the natural environment and the conditions of cultivation in artificial environments.

Buchanan, Fulmer (1980), L.G. Loginova et al. (1966) noted the ability to grow many typically mesophilic bacteria of various species (Bacillus, Olostridium Pseudomonas) at temperatures from 0 ^{0}C to +40 ^{o}C.

Most microorganisms are best develops at a concentration of hydrogen ions close to pH 7.0, which is typical for many natural media (2).

The possibility of isolating Yersinia cultures from the soil and their growth at temperatures from -2 ^{0} C to +400 C is noted by D. Kashner (1982), E. A. Kiryanov (1990), G. P. Somov (7) and others.

In order to study the ecological plasticity of microorganisms, we determined the dynamics of the growth of microorganisms in a broth medium at various temperatures, pH of the medium, and salt concentration.

The results of our experiments showed that Listeria are very sensitive to a shift in the reaction of the medium to the alkaline side. So, at a pH of 9.0, microbial growth was not observed. The growth of the culture also stopped with a decrease and an increase in the concentration of common salt in the medium. The most intensive growth of microbes was noted at a temperature of 37 ^{0} C. The optimal condition for these microorganisms is cultivation in a broth medium with a slightly acidic or neutral reaction, at a temperature of 37 ^{0} C.

St. aureus grew well on BCH with pH 5.0 and 7.2. At pH 9.0 no microbial growth was observed. A

decrease in the salt concentration in the medium also had a detrimental effect on microorganisms. A slight growth was observed when the medium was saturated with salt. The most intensive growth was noted at the usual content of NaCl.

An increase in temperature to 45 ^{0} C inhibited the growth of microbes, a decrease in temperature to 18 ^{0} C significantly delayed the growth of the culture. The optimal conditions for the growth of these microorganisms are a temperature of 37 ^{0} C, MPB with a pH of 7.2, with a 0.85% sodium chloride content. This culture can grow at a temperature of 18 ^{0} C.

Cultivation of E.Coli on BCH at various pH values of the medium showed that the shift of the reaction to the acidic or alkaline side did not have an inhibitory and detrimental effect on the growth of the culture. Intensive growth was also observed at pH 7.2. But these microorganisms were more sensitive to changes in the incubation temperature. So, at a temperature of 45 ^{0} C, growth of the culture was not observed, growth was significantly delayed at a temperature of 18 ^{0} C.

Also, an increase in the concentration of common salt in the medium had a detrimental effect on the growth of culture, although these microorganisms were not sensitive to a decrease in salt content. Optimal conditions are a temperature of 37 ^{0} C with a low salt content in the broth medium. These microorganisms can grow in a wide range of medium pH gradients.

The quality of the composition of fish microbiocenosis is of great importance, since the dominance of opportunistic and pathogenic microflora against the background of a weakening of the protective forces of the macroorganism can lead to the onset of an epizootic [8,9].

The structure of the microbial community is very dynamic and subject to rapid change; therefore, changes in its composition and the prevalence of pathogens may indicate changes in environmental conditions even before they occur. Microbiome balance is known to be key to maintaining the overall health of fish, as changes in the microbiome in response to stress can be precursors to disease. The composition of the microbial community influences digestion, synthesis and absorption of nutrients, resistance to pathogens, morphogenesis, survival, and more. There is a significant variation in the abundance of bacteria in the intestines of different fish of the same species and fish of the same species from different habitats [1, 2].

The aim of the work is to isolate microorganisms from the gastrointestinal tract (GIT) of various types of healthy fish and to characterize pure cultures.

Objects and methods of research

The objects of research were samples of different parts of the intestines of fish, a farm in the Tashkent region.

After sampling, samples of various sections of the fish intestine were placed in test tubes with saline, followed by inoculation on elective nutrient media to isolate bifidus, lacto, and spore bacteria. Lactobacilli were grown on MRS medium (HiMedia). Bifidobacteria were grown on Blaurock's medium and Bifidobacterium broth (HiMedia). Spore bacteria were isolated on meat-peptone broth (MPB) and meat-peptone agar (MPA).

Agar medium was used to obtain pure cultures. On dense nutrient media, bifidobacteria and lactobacilli were incubated in an anaerostat at 37 $^\circ$ C. Spore bacteria in a thermostat at 37 $^\circ$ C .

Isolation of pure culture was carried out in 3 stages:

1. Obtaining an enrichment culture;
2. Isolation of pure culture;
3. Determination of the purity of the isolated culture.

The nature of growth in liquid and agar nutrient media was determined using methods generally accepted in microbiology.

Cell morphology was studied by immersion microscopy of preparations of isolated cultures at a magnification of 10 × 100.

The titer of cells of one or another physiological group of microorganisms was determined by the method of successive tenfold dilutions (Netrusov 2005).

Research results

Actively feeding bony fish have a bacterial flora in the digestive tract similar to that of the skin and gills, often 10^{3} -10^{8} cells. per gram of wet body weight [3] . Lactic acid bacteria of the genus Lactobacillus - in the intestines of fish are part of the normal microflora, while not being dominant species [4].

From the fisheries of the Tashkent region, 15 enrichment cultures were selected from the gastrointestinal tract (GIT) of various fish species of different ages; 2-year-old scaly carp, 2-year-old white carp , 2-year-old white carp, 2-year-old bighead carp, 2-year-old silver carp.

Table 1. Cell titer of fish samples isolated from the intestine on elective media

fish specimens	No. of samples	Nutrient media		
		Wed.MRS _	Wed Blaurock	Wed BCH
Scaly carp - year old	1	$.5.10^{2}$	No growth	$2.5.10^{2}$
	2	$2.5.10^{2}$	6.10^{1}	$2.5.10^{5}$
	3	$2.5.10^{2}$	1.10^{1}	$2.5.10^{2}$
	4	No growth	No growth	No growth
	5	$2.5.10^{1}$	6.10^{1}	$2.5.10^{2}$
White carp - yearling	6	$2.5.10^{3}$	9.10^{2}	$2.5.10^{2}$
	7	$2.5.10^{2}$	No growth	$2.5.10^{2}$
Bighead carp - year old	8	$2.5.10^{1}$	1.10^{3}	$2.5.10^{3}$
	9	No growth	No growth	$2.5.10^{1}$
White silver carp yearling	10	$2.5.10^{4}$	9.10^{5}	$2.5.10^{5}$
Silver carp 2 years	eleven	No growth	No growth	No growth
	12	No growth	No growth	no growth
White carp -2 years, intestines	13	$2.5.10^{4}$	1.10^{1}	$2.5.10^{1}$
	14	No growth	No growth	No growth
White carp -2 years liver	15	No growth	No growth	No growth

From the data given in Table 1, it follows that the cell titer in all the studied samples was low, except for sample 10 (annual silver carp), the cell titer of which is 2.5x10 5 , and in the remaining samples it is even lower, $^{\text{mainly}}$ 10^{2} -10^{3} degree. The next stage of the work is devoted to obtaining pure cultures of the gastrointestinal tract of fish from enrichment cultures.

Pure cultures of spore bacteria were obtained by inoculating one loop of a suspension of enrichment cultures on beef-peptone broth (MPB) sequentially on 3 plates of beef-peptone agar (MPA), without burning the loop any more. Sowing was carried out from separately grown colonies into test tubes with slanted agar medium MPA. By successive inoculations from the agar medium to the broth medium and vice versa, microscopy of the grown cultures, the purity of spore cultures was determined (Netrusov, 2005).

Table 2 presents the description, size of the colonies, as well as the microscopic picture of the cells.

Table 2. Description of colonies and cell shape of cultures isolated from the intestines of fish of different species on MPA

No. of samples	shape, cell size, microns	Spore formation	Gram stain	Colonies on nutrient media
1	The cells are represented by rods. According to the location of the cells and their shape, the characteristic smell of jasmine, they can be	Dispute does not form	Gram (+)	White round shiny colonies, slightly convex with a clear edge. The diameter of the colonies is 2-2.5 mm.

	attributed to the genus Pseudomonas .			
2	Cells are represented by rods, 2 cells together, as well as chains.	Dispute does not form	Gram (-)	Colonies are white-cream in color, shiny, slightly convex with smooth edges with a diameter of 3-4 mm.
3	2 types of colonies were revealed. Colonies are round, white, slightly convex with smooth edges, rather small. Diameter 1.5-2 mm. and colonies are flat with astral outgrowths, similar to a hedgehog with open needles.	Dispute does not form Spore formation	Gram (+)	Cells of smooth round small colonies are represented by rods without spores and visible granules. Cells of astral colonies are represented by rods with a centrally located oval dispute.
4	Colonies are small white, convex matte. The diameter of the colonies is 1.2-1.5 mm.	Dispute does not form	Gram (-)	Cells are represented by cocci. Cocci - solitary, in pairs of small chains and clusters of various shapes.
5	The colonies are white, round in the center, raised and wrinkled. The edges are flat and slightly wavy. Colonies dry matte. Colony diameter 3-4 mm	Spore formation	Gram (+)	The cells are represented by short rods with an oval, subterminally located spore slightly exceeding the width of the cell.
6	Colonies are small, round, slightly convex, 1.5-2.0 mm in diameter.	Dispute does not form	Gram (-)	Cells are represented by small short rods without spores, but with granules. Often there are dividing and not yet dispersed lanceolate cells.
7	Colonies are large, white-cream in color, dry, not shiny. The edges are even. Diameter 3.5 mm.	Dispute does not form	Gram (-)	Cells are represented by rods. With granules, without spores, arranged singly or in pairs. Dividing cells are lanceolate.
8	Small, round, slightly convex colonies. Diameter 1.5-1.8 mm.	Dispute does not form	Gram (-)	Cells are represented by rods of different lengths up to filamentous, without spores, with granules.
9	Colonies are large, shiny, cream-colored, slightly convex. Diameter 4.5 mm.	Dispute does not form	Gram (-)	Cells are represented by rods of different lengths, with granules, without spores.

Thus, out of 15 fish samples, 2 spore cultures were isolated into a pure culture from sample No. 3 taken from scaly yearling carp - small round colonies and sample No. 6 grass carp - yearling - white colonies, dry, wrinkled in the center. Other cultures studied by us did not form spores.

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