

УДК 579.672

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### CHARACTERISTICS OF BACTERIA OF THE GENUS *PSEUDOMONAS*, ISOLATED FROM FOODS

Twenty two strains of bacteria of the genus *Pseudomonas* were isolated from contaminated food (milk, meat, poultry and fish). Their morphological and physiology-biochemical characteristics which allowed to identify nine strains are studied. Six strains are members of *P. fluorescens*, and three strains – *P. aeruginosa*.

The isolated bacteria identification tests were based on such properties as their dependence on molecular oxygen, ability to form specific pigments, catalase activity, ability to grow at different temperatures, nitrates and nitrites reduction, hydrolysis of starch, as well as morphology of cells.

The result of this investigation was the creation of collection of bacteria *P. fluorescens* and *P. aeruginosa* which would be the base for study and design of food-protection agents from spoilage.

**Key words:** foods, microbiological spoilage, bacterial strains, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, pyoverdin, pyocyanin.

**Introduction.** Microbiological spoilage is the main type of food losses in the world. In this regard, the actual tasks of modern microbiology are to identify microorganisms and to find ways to prevent damage [1].

Bacteria of the genera *Escherichia*, *Proteus*, *Clostridium*, *Achromobacter*, *Pseudomonas*, *Bacillus*, *Lactobacterium*, *Mycobacterium*, yeast of the genera, *Candida*, *Debaryomyces*, *Mycoderma*, *Rhodotorula*, filamentous fungi of the genera *Penicillium*, *Aspergillus*, *Rhizopus*, *Thamnidium*, *Cladosporium* can cause spoilage of protein-containing food [2].

Bacteria *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* play a key role in the deterioration of the meat of slaughtered animals and poultry, eggs, milk and fish. [3]

When typing bacteria of the genus *Pseudomonas* the tests like oxidation-fermentation, oxidase activity, mobility, and other tests are used.

The purpose of this work is to extract bacteria of *Pseudomonas* genus from the spoiled protein-containing products, their identification and the establishment of the collection that will provide the basis for the study and development of means of protection food from spoiling.

**Main part.** The objects of the study were spoiled milk, meat, poultry and fish.

A quantity of 20 g of the ground investigated material was placed in a conical flask of 100 ml, poured in 5 ml of sterile physiological solution (PS). It was incubated at 30°C for 3 days. Plating on the dense elective synthetic nutrient medium MM9 was performed from the resulting enriched culture to isolate bacteria of the genus *Pseudomonas* [4]. As elective factors we used the ability of microorganisms to grow in the presence tryptophane, it is sole carbon and energy source and in the presence of oxygen.

The resulting colonies were replated into differential diagnostic medium 'Gloss' to eliminate pathogenic *Pseudomonas*, which formed colonies with a metallic gloss. Non-pathogenic bacteria of the genus *Pseudomonas* were identified by morphological, physiological and biochemical characteristics.

To determine the relationship of bacteria to molecular oxygen, the medium of Hugh-Leyfson was used [5].

Differentiation of bacteria *P. fluorescens* and *P. aeruginosa* was carried out in the King B medium to form pyoverdin and pyocyanin pigments, respectively [6].

Bacterial catalase activity was observed when the gas bubbles were formed in the presence of hydrogen peroxide [7].

Morphological characteristics of bacterial cells were determined by conventional methods [8].

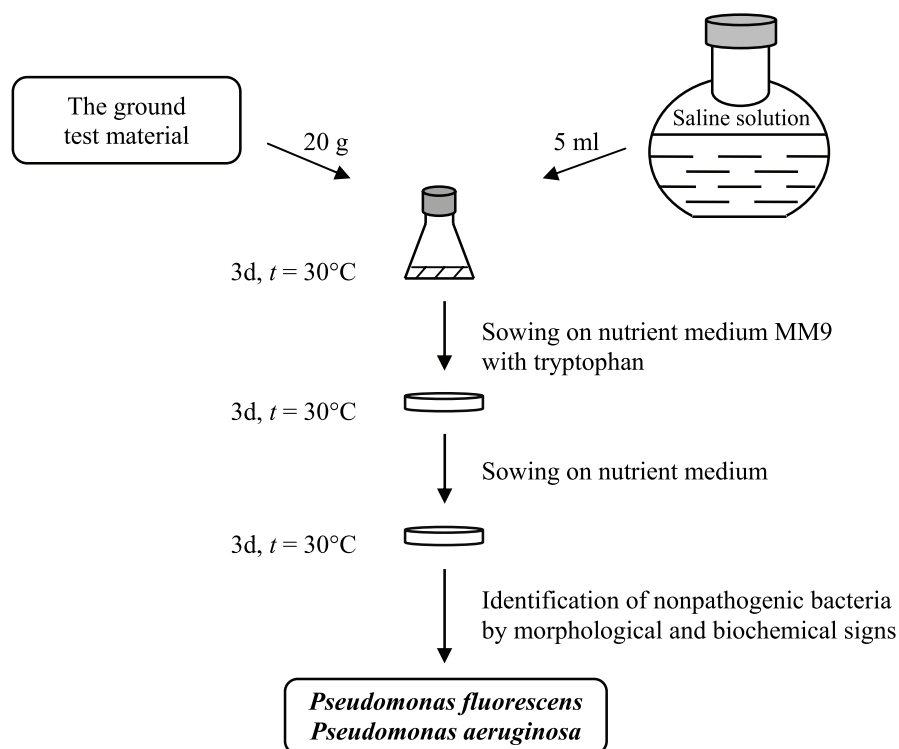
The assignment to the genus *Pseudomonas* bacteria was due to the ability to reduce nitrates and nitrites, and hydrolyze starch [7].

The assignment to the genus *fluorescens* and *aeruginosa* of *Pseudomonas* bacteria was determined by the growth capacity at optimum temperatures [9].

Scheme of isolation and identification of bacteria of the genus *Pseudomonas*, causing the spoilage of protein-containing products is shown in the figure.

As a result of the screening of the milk samples of ground beef, fish (carp, silver carp and frozen mackerel), chicken breasts and wings, which undergone spoilage, 22 strains of *Pseudomonas* bacteria were selected; their identification was carried out according to the source [10].

Characteristics of some isolated bacterial strains are presented in the Table.



Scheme of isolation and identification of bacteria of the genus *Pseudomonas*, causing protein-containing food spoilage

#### Characteristics of some strains of bacteria isolated by morphological, physiological and biochemical characteristics

Strain	Relation to oxygen		Pigments	Coloration by Gram	Types of cells	Catalasa	Growth		Reduction of nitrates and nitrites	Hydrolysis of starch
	Aer.	Anaer.					4°C	41°C		
Gi-1	A	-	+	-	R	+	+	-	+	-
Mi-1	A	-	+	-	R	+	+	-	+	-
Fi-1	A	A	-	+	Co	+	+	+	-	+
Fi-2	A	A G	-	+	Co	+	+	+	-	+
Me-1	A	A	+	-	R	+	+	-	-	+
Mi-2	A	-	+	-	R	+	+	-	+	-
Gi-2	A	-	+	-	R	+	+	-	+	-
Fi-3	A	-	+	+	R	+	+	-	-	-
Fi-4	-	A G	-	+	Co	+	-	-	-	+
Me-2	A	-	+	-	R	+	-	+	+	-
Fi-5	A	A	-	+	Co	-	+	-	-	+
Fi-6	-	-	-	-	Co	+	-	-	-	-
Fi-7	A	-	+	-	R	+	-	+	+	-
Gi-4	-	-	-	+	Co	+	+	-	-	+
Gi-5	A	-	+	-	R	+	-	+	+	-
Me-3	A	-	+	-	R	+	+	-	+	-
Fi-8	-	-	-	+	Co	+	-	-	-	+
Gi-6	-	-	-	-	Co	+	+	-	-	-
Me-4	A	-	+	-	R	+	+	-	+	-
Gi-7	-	-	-	+	Co	+	-	-	-	+
Gi-8	-	-	-	+	Co	+	-	-	-	+
Gi-9	A	-	-	-	R	+	+	+	-	-

Notes: Gi – gills; Mi – milk; Fi – fish; Me – meat; Aer. – aerobic bacterium; Anaer. – anaerobic bacterium; A – gluconic acid formation; G – gas formation; R – rod bacterium; Co – cocci.

As it is seen from the table, nine strains belonging to *Pseudomonas* genus are aerobic (glucose is oxidized to gluconic acid); they form specific pigments, they are gram-negative bacilli, they possess catalase activity, reduce nitrates and nitrites, do not hydrolyze starch.

Most isolates of *P. fluorescens* bacteria form colorless or yellowish, convex, smooth, shiny colonies. A characteristic feature of the colony is the outer microstructure, which has a net or cellular texture. Cells were stained the medium into a greenish-yellow color due to the production of pigments, i.e. pioverdin and fluorescein.

Bacteria *P. aeruginosa* form colorless or yellowish flat colonies. Cells stained the medium into the blue-green color due to production of pyocyanin pigment.

Bacteria *P. fluorestsens* grew at 4°C, and the bacteria *P. aeruginosa* – at 41°C.

The studies have shown that the strains Gi-1, Mi-1, Mi- 2, Gi-2, Me-3, Me-4 belong to *fluorescens* genus, strains Me-2, Fi-7, Gi-5 belong to *aeruginosa* genus.

**Conclusion.** 22 strains of *Pseudomonas* bacteria of the genus isolated during the experimental work caused the spoilage of protein-containing foods. Their morphological, physiological and biochemical characteristics were set, 9 strains were identified on their basis. Formation of specific pigments and ability to growth at different temperatures allowed us to identify the bacteria of the genus *Pseudomonas* as belonging to species *fluorestsens* and *aeruginosa*.

The collection of strains of bacteria *P. fluorescens* and *P. aeruginosa* will be the basis for the study and development of safety measures from food spoilage in the course of further investigation.

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Received 20.02.2015