Identification and studying of the biochemical properties of Lactobacillus strains

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Abstract. Phenotypic and biochemical properties of the strain isolated from fresh pepper were studied. Antimicrobial activity of isolated strain against *E. coli* B-6954 was examined. It has been found that the microorganisms are Gram-positive non-spore forming rods and they belong to *Lactobacillus pentosus* strain. Resistance of *Lactobacillus pentosus* strain to chloramphenicol antibiotics, tetracycline, kanamycin was shown, but it is not resistant to streptomycin, penicillin, lysozyme. The results obtained by the analysis of antimicrobial activity showed high antagonist activity of *Lactobacillus pentosus*.
[Zimina M.I., Prosekov A.J., Babich O.O., Sukhih S.A. **Identification and studying of the biochemical properties of Lactobacillus strains.** *Life Sci J* 2014;11(11):338-341] (ISSN:1097-8135). http://www.lifesciencesite.com. 54

Keywords: antimicrobial activity, lactic acid bacteria, vegetables, fruits, pathogenic microorganisms, biopreservative

Introduction

Spoilage is a complex process associated with change in physiological and biochemical parameters, leading to microbial degradation of the product, which in turn leads to deterioration of color, texture and taste[1]. Microbial spoilage is the most common cause of food spoilage [2]. Spoilage is a serious problem for the food industry because it causes significant economic losses and can have serious impact on health [3].

At present we know many ways to increase the shelf life of fruits and vegetables (ultrasound, protective coating, canning) [4]. But lately, consumers become more demanded to food and more concerned about their health. They want to consume fresh and healthy food products without chemical preservatives. But due to the lack of modern and efficient technologies for the storage of food, the most popular methods are canning and freezing. These methods allow extending the shelf life of products significantly, but there is a significant loss of nutrients. For example, in canning retained only 60% of nutrients, in freezing it is 70% [5].

Biopreservation is an alternative to existing methods. Biopreservation based on the using of more natural or controlled flora and / or antibiotics.

Currently there are a lot of papers about using biopreservatives for extending the shelf life of fruits and vegetables [6, 7]. The most relevant for using as biopreservative are lactic acid bacteria isolated from dairy products, meat, fish and vegetables, they have antibacterial properties because of their metabolic end-products such as lactic acid, acetic acid, hydrogen peroxide, acetaldehyde, reuterin and bacteriocins [8].

Moreover, the lactic acid bacteria can not only eliminate the pathogenic and spoilage organisms, but also to give the product more useful properties for the human health, because these bacteria are probiotics.

This paper is aimed to identifying species of lactobacillus isolated from fresh pepper and analysis of its biochemical properties.

Methods

Object of study is a strain of microorganisms isolated from fresh pepper.

We determined morphological characteristics for identification the species, their study was performed by microscopy. Colony diameter measured in millimeters, color, shape, texture, structure, surface, edge character contour were examined.

Milk fat content of 0.5 % was taken as a nutrient medium for the isolation of pure cultures of lactic acid bacteria from plant samples. Milk was dispensed into 10 ml vials, which were closed with cotton plugs and sterilized in an autoclave.

For isolation of the bacteria cultures from samples plant tissue was ground and triturated in sterile porcelain mortar, after which the tube was inoculated by a sterile skim milk with plant prepared by the described method. Seeding was produced from each sample in the 3 tube, which were incubated in an incubator at 30°C, 37°C and 45°C.

Daily seeding were performed until getting in tubes with sterile skim milk smooth, dense bunch without breaks or gas bubbles in order to obtain pure cultures of bacteria.

The final selection of pure cultures of bacterial strains to acidify lactose, performed by seeding clot milk of hydrolysed milk in Petri dishes in order to obtain isolated colonies, which are screened on agar slant [9].

The study of cultural and morphological properties were carried out on a dense medium - MRS agar, g / l: bactopeptone - 10.0; meat extract - 10.0; Yeast extract - 5.0; Glucose - 20.0; twin - 1.0; ammonium citrate - 2.0; Sodium acetate - 5.0; sodium phosphate - 2.0; magnesium sulphate 7-water - 0.1; manganese sulphate aqueous 5 - 0.05; agar - 20.0.

We used standardized test system API 50 CHL with identification software Apiweb VioMerieux (France) for studying of biochemical properties of the strain. This test system includes 50 biochemical tests for studying of carbohydrate metabolism of microorganisms and it is used for identifying bacilli, enterobacteria and vibrios.

Resistance of strains to the following antibiotics: chloramphenicol, streptomycin, tetracycline, kanamycin, penicillin, lysozyme was determined. Analysis was performed by diffusion method based on the usage of discs with antibiotics. Bacterial suspension was applied on a nutrient agar in petri dishes; discs with a concentration of 0.4% antibiotic were placed in a petri dishes too. Diffusion of the antibiotic in the agar led to the formation of the inhibition zone of the microorganisms around the disk. The a petri dishes were incubated at 37°C for 24 h, then formed zone around the antibiotic disks was measured.

Antimicrobial activity of strain against *E. coli* B-6954 was studied. Test cultures were plated on LB medium by dense lawn. Lactic acid bacteria were grown in MRS-broth for 24 hours at 37°C. The culture broth was centrifuged at 7000 rev/min for 10 min and the supernatant was separated. The supernatant was neutralized with 1M NaOH to reach values pH 7.0. The supernatant was filtered through filter Millex-GV (0.22 micron, Nihon «Millipore», USA) for cell separation. Paper discs were dipped in liquid culture, squeezing excess. Antimicrobial discs impregnated by metabolites of lactobacilli were applied to the agar with test - culture aseptically. Discs were placed so that the distance between centers of discs was not less than 24 mm. After placing the discs on agar plate discs were pressed by sterile forceps or needle until complete contact with the surface of the medium.

Cups were inverted and incubated in an aerobic atmosphere at temperature from 35 to 37°C for 24 hours after 15 minutes of the discs placement. Zones of full diameter were measured by incubation (data observation with the naked eye), including the diameter of the disc, to the nearest whole millimeter with calipers, rulers or template designed for this purpose.

The main part

The culture was obtained from a natural source. It was necessary to verify the identity of the resulting strain on the morphological, cultural and physiological and biochemical properties, in order to study the morphological features of this strain and to determine to which genus includes this culture of microorganisms. Such indicators as size, shape and arrangement of cells, spores, related to on Gram stain were studied in determining of the morphological properties of the isolated strains. Cultural characteristics were determined by the nature of growth on nutrient media. They are important diagnostic attributes because constant for each species of bacteria and growth characteristics of microorganisms on various culture media may be pre indirectly judge of the species of microorganisms, the data presented in Table 1.

Table 1. Characteristics of the phenotypic properties of the selected strain

Size	5-10 microns
Form	Rod, no spore
Character of an edge contour	Edge is smooth, profile of colonies is convex
Relief	Relief
Surface	scabrous
Color	The surface is white, not transparent
Structure	Patterned, granular
Consistency	Consistency of colonies is dense with a white grainy coating, it can be easily removed from the agar

Studying of the physiological properties of microorganisms is necessary not only to increase of biomass. By culture conditions and medium composition we can determine features of the microorganism used in taxonomy, ecological niche, to investigate the possibility of using it to solve practical problems. Finally growth management of cultures underlies biotechnological processes. All this emphasizes the importance of studying the nature of growth and nutritional needs of bacterial cultures. In order to determine the species of bacteria of *Lactobacillus* genus, series of biochemical studies using the test system API 50 were carried out. Results of physiological and biochemical properties of cultures of studied bacterial strains are listed in Table. 2.

Table 2. Results of biochemical testing of *Lactobacillus* genus microorganisms

#	Name	Test results strain	#	Name	Test results strain
1	Glycerol	+	26	Salicin	+
2	Erythritol	–	27	Cellobiose	+
3	D-arabinose	–	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	–
6	D-xylose	+	31	Saccharose	+
7	L-xylose	–	32	Trehalose	+
8	Ribitol	–	33	Inulin	–
9	B-methyl-ksilozid	–	34	Melezitose	–
10	Galactose	+	35	D-raffinose	–
11	D-glucose	+	36	Starch	–
12	D-fructose	+	37	Glycogen	–
13	D-munoz	+	38	Xylitol	–
14	L-sorbose	–	39	B-gentsibioza	+
15	Rhamnose	+	40	D-turanoza	–
16	Galactitol	–	41	D-lyxose	–
17	Inositol	–	42	D-tagatose	–
18	Mannitol	+	43	D-fucose	-
19	Sorbitol	–	44	L-fucose	–
20	A-methyl-D-manozid	–	45	D-arabitol	–
21	A-methyl-D-glucoside	–	46	L-arabitol	–
22	N-acetyl-glyukozoamin	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	–
24	Arbutin	+	49	5-keto-gluconate	–
25	Esculin	+	50	Control	–

The sensitivity of *Lactobacillus pentosus* strain to antibiotics was studied. Microbial resistance to antibiotics due to the presence of the corresponding enzymes, modifying or cleaving antibiotics (which leads to inactivation of the latter), or the development of the properties of the surface membrane and cell wall to reduce the penetrating ability of antibiotics or their rapid withdrawal from the cell to the outside.

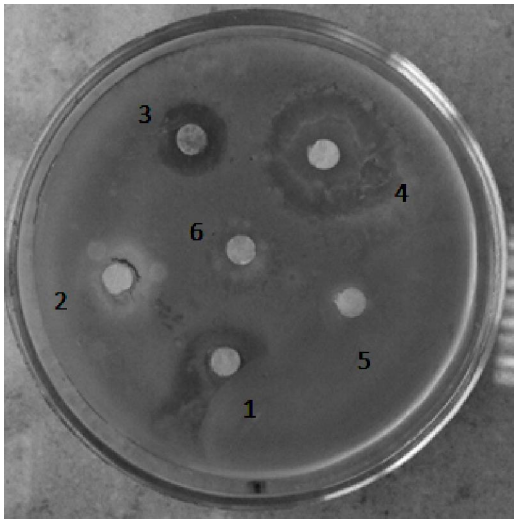

Figure 1. Antibiotic sensitivity of the strain *Lactobacillus pentosus* 1 - chloramphenicol, 2 - streptomycin, 3 - tetracycline, 4 - kanamycin, 5 – penicillin, 6 – lysozyme

Enzymes metabolizing antibiotics are coded by genes comprising respective mobile elements (plasmids, transposons), which are transferred from one bacterium to another, and ultimately lead to the stability of the total population of bacteria to the antibiotic. Thus the sensitivity of microorganisms to antibiotics is a specific feature that characterizes each strain. Determination of the sensitivity of the selected strain to antibiotics is relevant to facilitate further work with the selected strain.

Results of determination of *Lactobacillus pentosus* resistance are shown in Figure 1 and Table 3.

Table 3. Antibiotic sensitivity of the strain *Lactobacillus pentosus*

#	Antibiotic	Antibiotic concentration, %	Zone of bacterial grow inhibition, R, см
1	Chloramphenicol	0,4	0,8
2	Streptomycin	0,4	0,1
3	Tetracycline	0,4	0,7
4	Kanamycin	0,4	1,7
5	Penicillin	0,4	-
6	Lysozyme	0,4	-

Antimicrobial properties are one of the main properties of lactobacilli due to the formation of organic acids, ethanol, diacetyl, H_2O, and protein compounds, known as bacteriocins [10]. The antagonistic activity of lactobacilli metabolites in relation to *E. coli* B-6954 was investigated on solid medium by diffusion method (Figure 2).

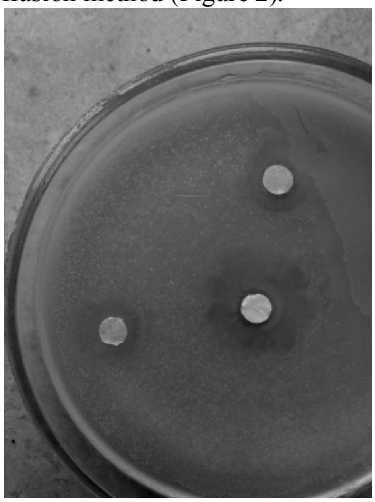

Figure 2. Antagonistic activity of *Lactobacillus pentosus* against *E. coli* B-6954

Conclusion

It has been found in result of analysis of phenotypic properties that the bacteria form a convex, circular, raised, rough colonies of irregular shape with white straight edges. Consistency of colonies is dense, dry white grainy coating, can be easily removed from the agar. Gram-positive bacteria with size 5-10 microns have the form of sticks, sometimes arranged in chains, when they grow in a nutrient medium. Sticks are not mobile. Culture is not a spore-forming.

The data presented in Table 1 indicate that microorganisms obtained from fresh vegetables are close to *Lactobacillus* bacteria genus by phenotypic indicators.

The data presented in Table 2 show that the Gram-positive bacillus ferment: glycerol, L-arabinose, ribose, D- xylose, galactose, D- glucose, D- fructose, D- mannose, rhamnose, mannitol, N-acetyl- glyukozoamin, amygdalin, arbutin (beta -D-glucopyranoside, esculin, salicin. Bacterial strain possesses the ability to produce cellobiose (4 - (β-glucoside)- glucose), maltose, sucrose, lactose, trehalose, β- gentsibiozu gluconate.

These biochemical characteristics are correspond to *Lactobacillus pentosus* by 98%, and the test for the type of *Lactobacillus plantarum* showed taxon belonging to the microorganism by 1.2%.

From the data presented in Table 3 and Figure 2 it follows that the selected culture is resistant to the three types of antibiotics (chloramphenicol, tetracycline, kanamycin), the radius of the inhibition zone is from 0.7 to 1.7 cm. Microorganisms possess low resistance to streptomycin, zone of inhibition was only 0.1 cm.

Figure 3 shows that the inhibition zone of *E. coli* B-6954 is 12-17 mm, this shows that the selected strain of microorganism *Lactobacillus pentosus* has high antimicrobial activity against *E. coli* B-6954.

The phenotypic and biochemical properties of the strain of microorganisms selected from fresh peppers were examined. In the result of the determination of morphological properties revealed that the isolated microorganisms are Gram-positive, their size is about 5-10 microns, and they are not spore-forming. Microorganisms form convex, round, raised, rough colonies of irregular shape with smooth edges white during cultivation. It has been found that biochemical properties of the identified strain correspond to the biochemical characteristics of *Lactobacillus pentosus* by 98 %. The sensitivity of the strain *Lactobacillus pento*sus to antibiotics was studied. An isolated strain of microorganism is resistant towards chloramphenicol, tetracycline, kanamycin. Antagonistic activity of isolated strain against *E. coli* B- 6954 was studied. It was established that the selected strain *Lactobacillus pentosus* has high antagonistic activity.

Acknowledgments

The work was funded by the Ministry of Education and Science of the Russian Federation within the framework of the project part

6/28/2014

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