Review Article

The Hypoglycemic Activity Of Lactic Acid Bacteria Isolated From Medicinal Plants Of Uzbekistan And Their Probiotic Potential

ABSTRACT

The study of the hypoglycemic activity strains of the lactic acid bacteria isolated from plants with antioxidant and hypoglycemic properties are given in this article. Investigation was shown that strains of Lactobacillus kunkeei 1, L. plantarum TK1, L. plantarum KA3, Enterococcus faecium effectively reduce postprandial hyperglycemia in rats, for example their probiotic properties: sensitivity to antibiotics, simulated gastric juice, simulated juice of small intestine, bile and elevated concentration of sodium chloride. It was demonstrated that the strains Enterococcus faecium 1, Lactobacillus kunkeei1, L. plantarum TK1, L. plantarum KA3 meet the criteria for probiotics and can be utilized for the planning of probiotic arrangements.

Keywords: [Diabetes, bacteria, glucose, strain, probiotic potential}

1. INTRODUCTION

It is known that diabetes is a common metabolic disease, occurs when the pancreas does not produce enough insulin (hormone that regulates blood glucose), or when the body does not respond to the insulin produced [1]. In last decades, the incidence of diabetes has been steadily increasing and it was 451 million patients in 2017 [2], 5 million people died by diabetes in this year [3].

Diabetes can damage blood vessels, eyes, kidneys and nerves greatly increase the risk of heart disease and stroke [4], is a major economic and social problem. Type 2 Diabetes Mellitus (T2DM is characterized by an increase in fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c), which indicates impaired glucose metabolism [5]. Despite the fact that, there are large number of antidiabetic drugs, the therapiesy for this pathology areis not perfect. Most people with diabetes follow a lifestyle and diet plan to improve the effectiveness of their treatment, and for the most part prefer using natural medicines in addition to traditional therapies. In the published data, there areis a large amount of evidence that the composition of the intestinal microflora is associated with the development of T2DM [6]. A close interaction was shown between T2DM and compositional changes in the microbiota of the gastrointestinal tract (GIT), with a relative decrease in the number of *Firmicutes* and an increased concentration of *Bacteroidetes* and *Proteobacteria* in patients with T2DM [7, 8]. Recent studies demonstrate that some strains of lactic acid bacteria of the species *Lactobacillus rhamnosus, L. plantarum, L. gasseri,* along with probiotic properties, have both the ability to lower blood glucose levels and antioxidant properties and have great potential for the treatment of type 2 diabetes [9, 10, 11].

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According to WHO, in Uzbekistan 8.7% of the population suffers from diabetes mellitus and 2% of the total number of deaths is caused by the result of~~from~~ diabetes mellitus (World Health Organization - Diabetes profiles in countries, 2016). Probiotic preparations from local microorganisms on the Uzbek market have not been previously studied as hypoglycemic agents. Taking into consideration that microorganisms isolated from the geographical area in which they will be used have a competitive advantage over other "foreign" representatives [12], the study of the ability of local strains of bacteria to reduce blood glucose levels will allow the selection of effective strains which, along with the probiotic properties will possess ability to maintain normal blood glucose levels.

~~**Purpose of the research**~~

The aim of this research is to study the hypoglycemic activity and probiotic properties of bacteria *Lactobacillus kunkeei 1, Enterococcus faecium 1, Lactobacillus plantarum TK1, Lactobacillus plantarum MAL*, isolated from medicinal plants~~local sources~~.

2. MATERIAL AND METHODS ~~/ EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)~~

2.1. Microorganisms. The *Lactobacillus plantarum* TK1 strain isolated from *Jerusalem artichoke* root; strain of *Lactobacillus plantarum MAL*, isolated from flowers of low mallow (Málva neglecta); *Lactobacillus kunkeei 1*, isolated from dandelion flowers (Taraxacum officinale); *Lactobacillus plantarum KA3*, isolated from the leaves of the Ayuga Turkestanica; *Lactobacillus rhamnosus D*, *Enterococcus durans 1* and *Enterococcus faecium 1*, containing in the commercial preparations Lactobacterin and Bifidumbacterin PL have been used in the study.

Plants used for the isolation of lactic acid bacteria were selected on the basis of known antioxidant and hypoglycemic properties. MPC medium (HiMedia) was used for isolation, purification and growth of lactobacilli. All strains were~~are~~ stored in the freeze stock of the ~~laboratory "~~Microbiology and Bb~~b~~iotechnology of Pp~~p~~robiotics "Laboratory of the Institute of Microbiology at~~of~~ the Academy of Sciences of the Republic of Uzbekistan. To study the properties, the dried culture was restored by double culture in MRS broth and~~; for experiments,~~ a suspension containing 10^9 CFU / ml was used [13].

2.2. Determination of hypoglycemic activity.

Healthy animals quarantined for at least 10-14 days was used for the experiments [14, 15]. The study of the hypoglycemic activity of the samples (preparations) was carried out using an intraperitoneal glucose tolerance test [16]. The experiments were carried out on 115 white outbred rats (both sexes) weighing 150-180 g, followed by division into groups of 5 animals each (Table 1).

Table 1. Hypoglycemic activity tests experimental groups

No	Group No	Microbe tested	Dose	Volume taken
1	Intact group	animals without test modeling		
2	Control group	animals with test modeling, but without exposure to the drug		
3	Experimental group No. 1	*Lactobacillus kunkeei1*	500 mg / kg	1 ml / 200 g

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4	Experimental group No. 2	*Lactobacillus kunkeei1*	1000 mg / kg	2 ml / 200 g
5	Experimental group No. 3	*Lactobacillus kunkeei1*	1500 mg / kg	3 ml / 200 g
6	Experimental group No. 4	*Lactobacillus plantarum* TK1	500 mg / kg	1 ml / 200 g
7	Experimental group No. 5	*Lactobacillus plantarum* TK1	1000 mg / kg	2 ml / 200 g
8	Experimental group No. 6	*Lactobacillus plantarum* TK1	1500 mg / kg	3 ml / 200 g
9	Experimental group No. 7	*Lactobacillus plantarum* KA3	500 mg / kg	1 ml / 200 g
10	Experimental group No. 8	*Lactobacillus plantarum* KA3	1000 mg / kg	2 ml / 200 g
11	Experimental group No. 9	*Lactobacillus plantarum* KA3	1500 mg / kg	3 ml / 200 g
12	Experimental group No. 10	*Enterococcus faecium* 1	500 mg / kg	1 ml / 200 g
13	Experimental group No. 11	*Enterococcus faecium* 1	1000 mg / kg	2 ml / 200 g
14	Experimental group No. 12	*Enterococcus faecium* 1	1500 mg / kg	3 ml / 200 g
15	Experimental group No. 13	*Lactobacillus plantarum MAL*	500 mg / kg	1 ml / 200 g
16	Experimental group No. 14	*Lactobacillus plantarum MAL*	1000 mg / kg	2 ml / 200 g
17	Experimental group No. 15	*Lactobacillus plantarum MAL*	1500 mg / kg	3 ml / 200 g
18	Experimental group No. 16	*Enterococcus durans* 1	500 mg / kg	1 ml / 200 g
19	Experimental group No. 17	*Enterococcus durans* 1	1000 mg / kg	2 ml / 200 g
20	Experimental group No. 18	*Enterococcus durans* 1	1500 mg / kg	3 ml / 200 g
21	Experimental group No. 19	*Lactobacillus rhamnosus* D	500 mg / kg	1 ml / 200 g
22	Experimental group No. 20	*Lactobacillus rhamnosus* D	1000 mg / kg	2 ml / 200 g
23	Experimental group No. 21	*Lactobacillus rhamnosus* D	1500 mg / kg	3 ml / 200 g

30 minutes after the administration of the drugs, the animals of all groups (except for the intact one) were injected with glucose in the form of an 8% solution, at a dose of 2 g / kg (5 ml / 200 g). 15 minutes after the injection of glucose in all animals in a state of ether anesthesia (ether was administered by inhalation), blood was taken from the cardiac region. The criterion for assessing the pharmacological activity was the normalization of blood glucose levels.

To determine the glucose concentration, the blood was placed in a serological tube without anticoagulant and centrifuged at 3000 rpm for 10 minutes. Next, the concentration of glucose in the obtained serum was determined on a biochemical analyzer "HUMALYZERPrimus" (semi-automatic), manufactured by "Human GmbH" (Germany), with metrological characteristics: 340, 405, 500, 546, 620 nm, reagent consumption 400 µl.

2.3. Study of the probiotic properties of strains.

2.3.1 Determination of antagonistic activity.

The antagonistic properties of the studied cultures were determined by the method of spots on agar [17].

Opportunistic and pathogenic strains *C. freundii*, 002801/27, *Pseudomonas aeruginosa* 003841/114, *Serratia marcescens* 367, *Listeria monocytogenes* ATCC 1911, *Escherichia coli* 002673/477, *Candida Sture albicans*, *Enterococcus* -46, *B. subtilis*, *S. aureus* D8 were used for test. All indicator strains were stored at + 4 ° C on nutrient agar slant. Before the experiment, the test cultures were renewed two times in meat-peptone broth (BCH) (HiMedia, India) and incubated at 37° C for 24 hours.

2.3.2 Determination of resistance to bile and to different concentrations of NaCl in the environment.

The resistance of the tested strains to the bile, their ability to grow at increased concentrations of sodium chloride was determined according to the Guidelines 4.2.2602-10. [18].

2.3.3 Study of resistance to gastric juice and small intestine juice.

Survival in the presence of simulated gastric juice and simulated small intestine juice was studied according to the method described by B.M. Corcoran et al. [19].

2.4. Study of physiological and biochemical properties of strains. The test for catalase activity, for the production of lecithinase, hemolysin, gelatinase, and amylase was carried out according to Guidelines 4.2.2602-10 [18].

3. RESULTS ~~AND DISCUSSION~~

3.1 Evaluation of hypoglycemic activity of microorganisms in the model of alimentary hyperglycemia in rats.

As a result of intraperitoneal administration of glucose in animals, a significant increase in the level of glucose in the blood serum was observed, which was a sign of hyperglycemia. However, the intake of different strains of lactobacilli lead to decrease in glucose level (Table 2).

Table 2. Hypoglycemic effect of lactobacilli

Tested strain	Decrease in glucose level		
	500 mg / kg	1000 mg / kg	1500 mg / kg
Lactobacillus kunkeei	67.14%	77.36%	67.76%
Lactobacillus plantarum TK1	76.81%	79.63%	75.96%
Lactobacillus plantarum KA3	74.71%	74.36%	76.55%
Enterococcus faecium 1	76.75%	76.51%	72.09%
Lactobacillus plantarum MAL	14.08%	26.79%	43.31%
Enterococcus durans 1	28.87%	33.70%	37.13%
Lactobacillus rhamnosus D	54.17%	58.57%	44.60%

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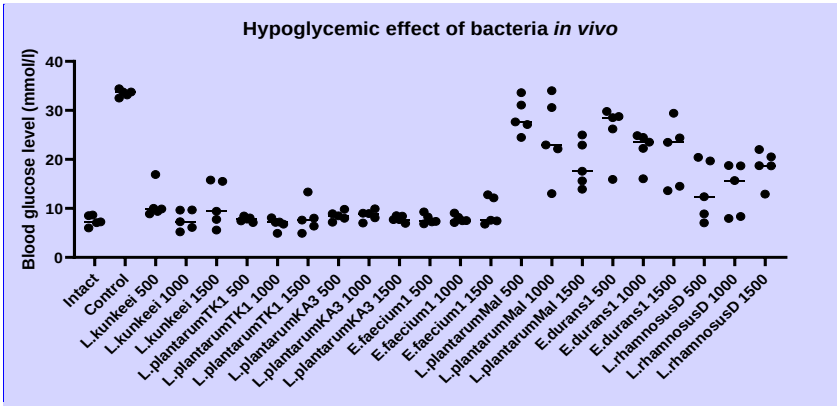

Fig. 1. The hypoglycemic effect of the studied isolates in the model of alimentary hypoglycemia in rats

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3.2 Probiotic properties

Probiotic properties ensure the survival of microorganisms in the gastrointestinal tract and their beneficial effect on the host organism. To characterize the probiotic properties, isolates with the most pronounced hypoglycemic properties were selected and their antagonistic activity, resistance to bile, gastric juice, small intestinal juice and NaCl were studied.

3.2.1 Antagonistic activity of strains against conditionally pathogenic microorganisms.

The antagonistic activity of *Lactobacillus kunkeei 1, Enterococcus faecium 1, Lactobacillus plantarum TK1, Lactobacillus plantarum KA3* against 10 cultures of pathogenic and opportunistic microorganisms was studied.

The studied isolates showed high antagonistic activity against *Serratia marcescens* 367 (the diameter of growth inhibition for the antagonistic strains- ranges from 44 to 28 mm), *E. coli* 002673/477 (from 38 to 25 mm), *Pseudomonas aeruginosa* 003841/114 (from 34 to 30 mm), *B. subtilis* (from 38 up to 20mm), *E. faecalis* (from 30 to 35mm), *Listeria monocytogenes* ATCC 1911 (from 34 to 16mm). The strains also effectively suppressed the growth of the clinical isolate *S. aureus* D8 (from 35 to 14 mm), *S. aureus* 003594 / wood 46 (from 36 to 14 mm). All tested strains exhibited antimicrobial activity against all indicator cultures (Table 3).

Table 3. Antagonistic activity

Investigated isolate	Diameter of the zone of inhibition of the growth of the indicator strain, mm									
	C. freundii, 002801/27	*P. aeruginosa 003841/114*	*S. marcescens 367*	*L. monocytogenes ATCC 1911*	*E. coli002673/477*	*C. albicans*	*E. faecalis*	*S. aureus 003594/wood 46*	*B. subtilis*	*S. aureus D8*
Lactobacillus plantarum TK1	30±0.3	34±0.24	35±0.20	26±0.22	36±0.23	34±0.24	30±0.3	30±0.2	20±0.3	34±0.2

Lactobacillus plantarumKA3	28±0.2	30±0.6	44±0.4	34±0.2	38±0.22	34±0.6	35±0.5	36±0.4	25±0.3	35±0.2
Enterococcus faecium 1	12±0,25	34±0,1	40±0,35	18±0,4	30±0,3	19±0,5	31±0,1	18±0,1	34±0,2	18±0,3
Lactobacillus kunkeei1	14±0,3	30±0,22	28±0,1	16±0,5	25±0,2	17±0,4	30±0,3	14±0,3	38±0,2	14±0,5

3.2.2 Resistant to various concentrations of bile. All studied strains showed resistance to presence of 0.6% bile. The number of living cells was at least 10^9 for all studied cultures.

Table 4. Resistance to various concentrations of bile

Culture	control	0,2%	0,3%	0,4%	0,6%
Enterococcus faecium 1	10^9	$1,0 \times 10^{10}$	4×10^9	2×10^9	2×10^9
Lactobacillus kunkeei 1	10^9	$1,0 \times 10^{10}$	$1,3 \times 10^9$	$1,0 \times 10^9$	$1,0 \times 10^9$
Lactobacillus plantarum TK1	10^9	2×10^9	2×10^9	2×10^9	1×10^9
Lactobacillus plantarum KA3	10^9	5×10^9	3×10^9	3×10^9	1×10^9

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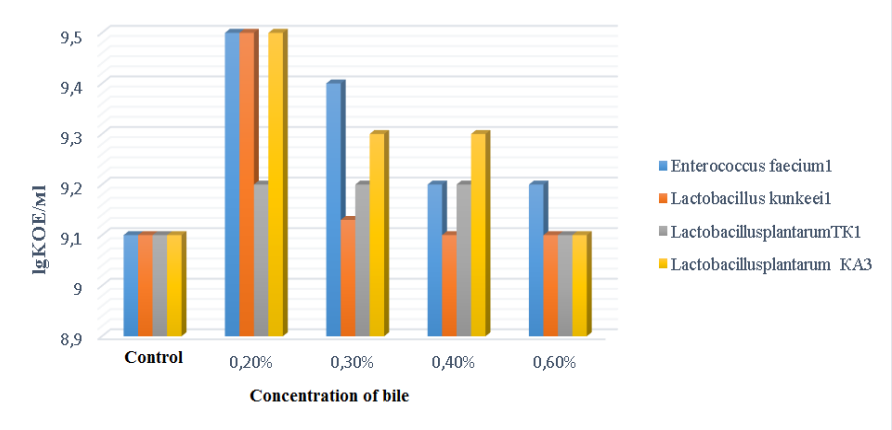

Fig. 2. Resistance to various concentrations of bile

3.2.3 Resistance of the studied isolates to simulated gastric juice (pH 2.0) and simulated small intestine juice (pH 8.0).

The survival rate of LAB at pH 3.0 for 2 hours and with a bile content of 1 g / l (0.1%) are optimal for probiotic cultures. Moreover, The transit time of food through the stomach is 90 minutes [20]. Taking into account these physiological characteristics of the organism, we established the duration of the treatment of cultures with simulated gastric juice.

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The investigation of resistance of *Lactobacillus plantarum KA3*, *Lactobacillus plantarum TK1, Enterococcus faecium 1* and *Lactobacillus kunkeei 1* isolates to simulated gastric juice with a pH 2.0, ~~it was~~ showed~~n~~ that the studied bacteria exhibit different sensitivity.

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Table 5. Resistance to simulated gastric juice and the juice of the small intestine

Culture	control	pH-2	pH-8

		0 min*	30 min	60 min	90 min	0 h*	1 h	2 h	3 h
Enterococcus faecium1	1 x10^{9}	1 x10^{9}	3x10^{5}	2x10^{5}	1x10^{4}	1 x10^{9}	2 x10^{9}	1 x10^{9}	1x10^{9}
Lactobacillus kunkeei1	7 x10^{9}	4 x10^{9}	3x10^{7}	2x10^{6}	2x10^{2}	6 x10^{8}	5x10^{8}	5 x10^{8}	1 x10^{8}
Lactobacillusplantarum TK1	8 x10^{9}	2 x10^{6}	4 x10^{4}	3 x10^{3}	5 x10^{1}	6x10^{9}	5x10^{9}	2,5x10^{9}	1 x10^{9}
Lactobacillusplantarum KA3	1,1 x10^{10}	4x10^{7}	2x10^{4}	2x10^{2}	1x10^{1}	2x10^{9}	2x10^{9}	2x10^{9}	1x10^{9}

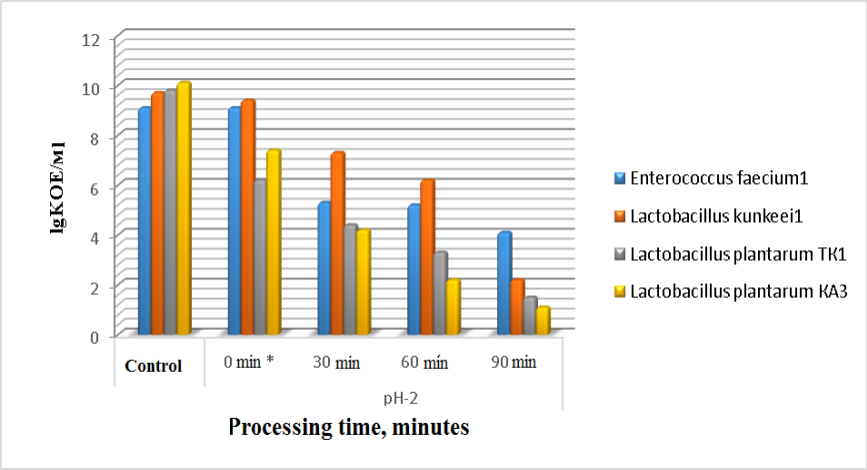

Fig. 3. Resistance to the simulated gastric juice

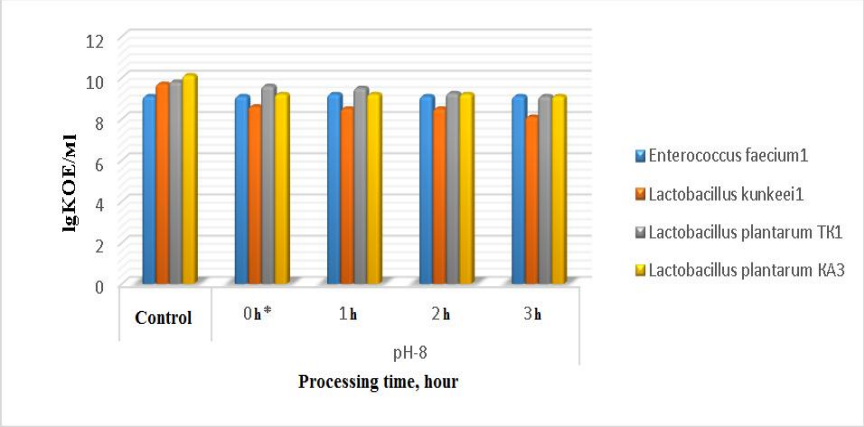

Fig. 4. Resistance to the simulated juice of the small intestine

Among the cultures studied, resistance to simulated gastric juice was observed in all isolates, where the cells remained viable after 90 minutes of co-cultivation.

When studying the survival of the studied isolates under the influence of simulated small intestine juice with a pH value of 8, all strains of *Enterococcus faecium 1, Lactobacillus kunkeei 1, Lactobacillus plantarum TK1, Lactobacillus plantarum KA3* showed high resistance - after 3 hours the number of living cells was $1x10^9$, $1x10^8$ and $1x10^9$, respectively.

All the studied strains proved to be resistant to both simulated gastric juice and small intestine juice, which ensures their survival under stressful conditions during gastric passage.

3.2.3 Resistance of strains to various concentrations of NaCl.

The results showed that all cultures are resistant to ~~the presence of~~ 2%, 4% and 6.5% NaCl in the medium, with an increase in salt concentration to 6.5%, the cells number did not fall below 10^9. In the case of *Enterococcus faecium 1, Lactobacillus kunkeei 1 and Lactobacillus plantarum KA3* the number of viable cells at a salt concentration of 6.5% did not differ from the initial one; in *Lactobacillus plantarum TK1*, it decreased by 1 log.

Table 6. Survival of isolates in the presence of different concentrations of NaCl

Isolate	NaCl concentration in MRS medium			
	0%	2%	4%	6.5%
Lactobacillus plantarum TK1	$3x10^{10}$	$1x10^{10}$	$5x10^9$	$2x10^9$
Lactobacillus plantarum KA3	$2,5x10^{10}$	$1,3x10^{10}$	$1,4x10^{10}$	$1x10^{10}$
Enterococcus faecium 1	$2x10^9$	$1x10^9$	$1x10^9$	$1x10^9$
Lactobacillus kunkeei 1	$7x10^9$	$1,4x10^9$	$1x10^9$	$1x10^9$

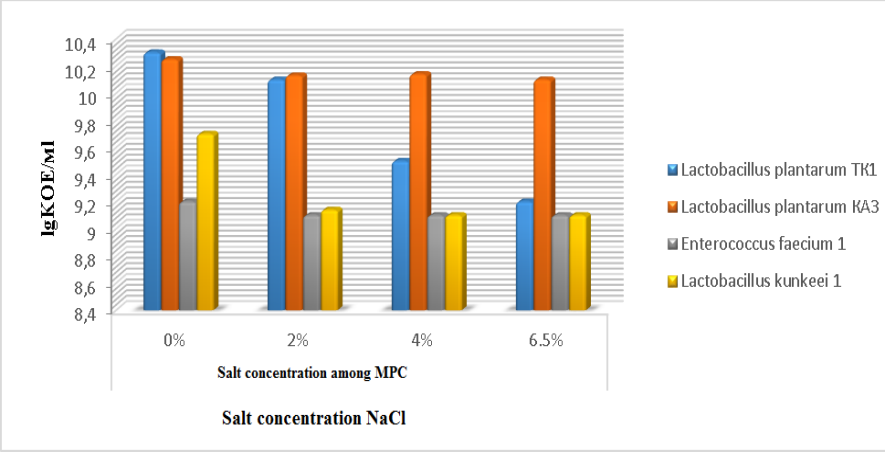

Fig. 5. Survival of isolates in the presence of different concentrations of NaCl

All studied cultures showed resistance to the presence of 6.5% salt in the medium, which is an indicator of their stability during the production process.

3.3 Physiological and biochemical properties of strains. In the process of cultivation, microorganisms secrete various proteolytic enzymes into the external environment, which can be divided conditionally into two groups: the first group should include enzymes that take part in the metabolism of microorganisms (respiration, nutrition). They break down carbohydrates, proteins, peptides, amino acids, resulting in the formation of food and metabolic products easily digestible by microorganisms - acids, peroxides, indole, hydrogen sulfide, etc. The second group should include enzymes related to pathogenic factors (for example, hyaluronidase, fibrinolysin, plasma coagulase, hemolysin, lecithinase C, lysozyme, neuramidase) [18]. To determine the absence of synthesis of enzymes that are virulence factors, we studied catalase, lecithinase, and hemolytic activity.

- Catalase activity. All studied isolates are catalase negative.
- Lecithinase production test. None of the studied strains showed the ability to produce lecithinase.
- Hemolysin test. The studied isolates: Lactobacillus plantarum TK1, Lactobacillus plantarum KA3, Enterococcus faecium 1, Lactobacillus kunkeei 1 do not produce hemolysins.

3.4 Enzymes involved in metabolism. *Gelatinase production test.* The investigated strains do not thin the gelatinous medium.

Amylase production test. Amylase production is judged by the formation of transparent hydrolysis zones around crops in potato agar. The results showed that the cultures of *Enterococcus faecium 1, Lactobacillus kunkeei 1* produce amylase. *Lactobacillus plantarum KA3* and *Lactobacillus plantarum TK1* did not give clear zones of starch hydrolysis around the inoculation on potato agar.

All studied cultures showed the absence of pathogenic factors.

4. DISCUSSION

Diabetes is a metabolic disease characterized by hyperglycemia, which strongly affects both the patient's health and the socioeconomic development of the country (Kulleretal., 2000). As of today, there are no cures for diabetes. The high cost and side effects of hypoglycemic drugs, and the increasing number of diabetics have led to the need to find alternative, natural treatments. The mechanisms of action of the drugs currently used are as follows: a decrease in the flow of glucose into the blood (α-glucosidase inhibitors and biguanide); an increase in the amount of insulin (insulin and sulfonylurea injections) and an increase in insulin sensitivity (glucagon-like peptide-1, GLP-1).

It is known that the consumption of probiotics can lead to a decrease in hyperglycemia. These results were obtained when studying the antidiabetic effect of lactic acid bacteria *Lactobacillus reuterii* GMNL-263 [21], *Lactobacillus rhamnosus* CCFV0528 [22], *L. Rhamnosus* NCDC17 [23], *L.casei* CCFM419 [24].

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Its is also proved that potential beneficial microbes, when isolated from the local source, would be more resistant to particular spices and herbs, and have an advantage over other currently available probiotics in terms of stability, viability, and ultimately functionality after consumption [25] In this regard, we have devoted work to determine the hypoglycemic effect of 7 strains isolated from local sources, to study their probiotic potential. Some studied strains are already used in the composition of probiotic preparations produced in Uzbekistan (*Enterococcus durans, Enterococcus faecium*), others were isolated and studied by us in the framework of early experiments - *Lactobacillus plantarum mal* [26], and still others were isolated for the first time from medicinal plants with antioxidant and hypoglycemic properties (*Lactobacillus kunkeei, L. plantarum TK1, L. plantarum KA3, Lactobacillus rhamnosus D*). The ability of these microorganisms to lower blood glucose levels has been studied for the first time.

In our experiment, we studied the effect of a single dose of live bacteria cells on postprandial hyperglycemia in a model of alimentary hyperglycemia in rats. As a result, it was found that strains *Lactobacillus kunkeei 1, L. plantarum TK1, L. plantarum KA3, Enterococcus faecium 1* have a strong hypoglycemic effect; when administered to rats, a decrease in blood glucose levels to the level of intact rats (100%) was observed. A moderate effect was observed with the introduction of *Lactobacillus rhmanosus D* (58.57%) and relatively low - in *Lactobacillus plantarum MAL* and *Enterococcus durans* (43.31% and 37.13%, respectively). Most lactic acid bacteria use glucose as a food source. Therefore, we assume that the studied lactic acid bacteria reduce postprandial blood glucose by suppressing glucose adsorption as a result of its utilization. This assumption is also confirmed by the results obtained by Tabuchi et al. [27].

The degree of glucose reduction varied slightly depending on the dose, however, a significant difference was observed between the effects of different strains. This indicates that not all lactic acid bacteria exhibit the antidiabetic effect and it is strain dependanted. The same conclusion was made when the different effects on blood glucose were found in *L. Rhamnosus* and *L. Bulgaricus* strains in the work of Honda et al [28].

In our study, we used the *L. kunkeei* strain, which had not previously been isolated in Uzbekistan. The isolate is isolated from the medicinal plant *Taraxacum officinale*, which has medicinal properties such as antioxidant activity, lowering cholesterol and regulating blood sugar levels. *L. kunkeei* was firstly isolated from fermented wine and identified as a new species based on the 16SrRNA gene sequence in 1998 [29], and later the species was characterized as fructophilic lactic acid bacteria [29]. The properties of representatives of the species *L. kunkeei* are poorly studied, antidiabetic properties are shown in this study for the first time, and it is not previously used in commercial preparations. Considering the compliance of the isolate with the criteria of probiotic microorganisms (survival in SGS, the absence of pathogenic enzymes and high antimicrobial activity (Tables 1, 3), it can be considered as a new probiotic strain with a set of valuable properties.

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4. CONCLUSION

According to the result of the research work *Lactobacillus kunkeei, L. Plantarum TK1, L. Plantarum KA3, Enterococcus faecium* reduce postprandial hyperglycemia in rats and the antidiabetic effect depends on the bacterial strain.

The strains *Enterococcus faecium 1, Lactobacillus kunkeei1, L. Plantarum TK1, L. Plantarum KA3* meet the criteria for probiotics and can be used to prepare probiotic preparations.

The hypoglycemic properties of *Lactobacillus kunkeei1, Enterococcus faecium1, L. plantarum TK1, L. plantarum KA3*, together with their probiotic properties and the absence of virulent enzymes, makes it possible to consider themse strains as promising candidates for the preparation of probiotic preparations intended not only to correct gut microbiota, but also to maintain normal blood glucose levels.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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