



The Hypoglycemic Activity of Lactic Acid Bacteria Isolated From Medicinal Plants of Uzbekistan and Their Probiotic Potential

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed to evaluate the hypoglycemic activity of lactic acid bacteria isolated from the medicinal plants possessing antioxidant and hypoglycemic properties growing in Uzbekistan and analyze their probiotic properties.

Four plant isolates of lactic acid bacteria (LAB) and three commercial strains were used in the study. Alimentary hyperglycemia was induced in white outbred rats, and glucose level was measured before and after treatment with lactic acid bacteria according to the experiment scheme using an intraperitoneal glucose tolerance test.

The investigation was shown that strains of *Lactobacillus kunkeei* 1, *L. plantarum* TK1, *L. plantarum* KA3, *Enterococcus faecium* effectively reduce postprandial hyperglycemia in rats. Moreover, evaluation of their probiotic properties: sensitivity to antibiotics, simulated gastric juice, simulated juice of small intestine, bile, and elevated concentration of sodium chloride, demonstrated that the strains *Enterococcus faecium* 1, *Lactobacillus kunkeei* 1, *L. plantarum* TK1, *L. plantarum* KA3 meet the criteria for probiotics.

These strains could be considered promising candidates for the preparation of probiotic preparations intended not only to correct gut microbiota but also to maintain normal blood glucose levels.

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1. INTRODUCTION

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

Diabetes can damage blood vessels, eyes, kidneys, and nerves, significantly increase the risk of heart disease and stroke [4], are a significant 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]. Although there are many antidiabetic drugs, the therapies for this pathology are not perfect. Most people with diabetes follow a lifestyle and diet plan to improve the effectiveness of their treatment, and for the most part, they prefer using natural medicines and traditional therapies. There is a large amount of evidence that the intestinal microflora composition is associated with the development of T2DM [6]. A close interaction was shown between T2DM and compositional changes in the gastrointestinal tract (GIT) microbiota, 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].

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 diabetes mellitus [12]. Probiotic preparations from local microorganisms on the Uzbek market have not been previously studied, 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 [13], 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 the ability to maintain normal blood glucose levels.

The aim of this research is to study the hypoglycemic activity and probiotic properties of lactic acid bacteria isolated from local flora and commercial preparations.

2. MATERIALS AND METHODS

2.1 Microorganisms

The *Lactobacillus plantarum* TK1 strain isolated from *Jerusalem artichoke* root; the 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 based on known antioxidant and hypoglycemic properties. De Mann, Rogosa, Sharp (MRS) medium (HiMedia) was used for isolation, purification, and growth of lactobacilli. All strains were stored in the freeze stock of the Laboratory. The dried culture was restored by double culture in MRS broth to study the microbial properties, and a suspension containing 109 CFU / ml was used [13].

2.2 Determination of Hypoglycemic Activity

Healthy 115 white outbred rats (both sexes) weighing 150-180 g were used for the experiments. They were quarantined for at least 10-14 days [14, 15] and divided into 23 groups, each containing five animals (Table 1). The study of the hypoglycemic activity of the samples (preparations) was carried out using an intraperitoneal glucose tolerance test [16]. Briefly, 30 minutes after the administration of the tested bacterial suspension to the groups No 1-23, 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). Fifteen minutes after the glucose injection in all

animals in 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.

The blood was placed in a serological tube without anticoagulant and centrifuged at 3000 rpm for 10 minutes for the glucose concentration measure. 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]. Briefly, the dots of test strains were grown on the surface of the MRS agar and covered with the second layer of the soft agar containing 10^7 CFU/ml of the indicator strain. The plates were incubated for 24 hours at 37°C. The

presence of the clear zones around the test strain dot was indicative of antagonistic activity.

Opportunistic and pathogenic strains *C. freundii*, 002801/27, *Pseudomonas aeruginosa* 003841/114, *Serratia marcescens* 367, *Listeria monocytogenes* ATCC 1911, *Escherichia coli* 002673/477, *Candida albicans*, *Enterococcus* - 46, *B. subtilis*, *S. aureus* D8 were used as an indicator. All indicator strains were stored at + 4 °C onto 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 bile and different concentrations of NaCl tolerance in the environment

The bile tolerance of bacteria, their ability to grow at increased concentrations of sodium chloride were determined according to the Guidelines 4.2.2602-10. [18]. Briefly, 1 ml of 109 CFU/ml suspension of the tested culture was added to the growth medium containing 0,2; 0,3; 0,4 and 0,6% of ox bile (HiMedia) and 2; 4; 6,5% of NaCl. After incubation at 37°C for 24 hours, the number of viable cells was measured by serial dilution assay.

Table 1. Hypoglycemic activity tests experimental groups

No	Group No	Microbe tested	Dose (mg/kg)	Volume taken (ml/200g)
1	Intact group	animals without test modeling		
2	Control group	animals with test modeling, but without exposure to the drug		
3	No. 1	<i>Lactobacillus kunkeei</i> 1	500	1
4	No. 2	<i>Lactobacillus kunkeei</i> 1	1000	2
5	No. 3	<i>Lactobacillus kunkeei</i> 1	1500	3
6	No. 4	<i>Lactobacillus plantarum</i> TK1	500	1
7	No. 5	<i>Lactobacillus plantarum</i> TK1	1000	2
8	No. 6	<i>Lactobacillus plantarum</i> TK1	1500	3
9	No. 7	<i>Lactobacillus plantarum</i> KA3	500	1
10	No. 8	<i>Lactobacillus plantarum</i> KA3	1000	2
11	No. 9	<i>Lactobacillus plantarum</i> KA3	1500	3
12	No. 10	<i>Enterococcus faecium</i> 1	500	1
13	No. 11	<i>Enterococcus faecium</i> 1	1000	2
14	No. 12	<i>Enterococcus faecium</i> 1	1500	3
15	No. 13	<i>Lactobacillus plantarum</i> MAL	500	1
16	No. 14	<i>Lactobacillus plantarum</i> MAL	1000	2
17	No. 15	<i>Lactobacillus plantarum</i> MAL	1500	3
18	No. 16	<i>Enterococcus durans</i> 1	500	1
19	No. 17	<i>Enterococcus durans</i> 1	1000	2
20	No. 18	<i>Enterococcus durans</i> 1	1500	3
21	No. 19	<i>Lactobacillus rhamnosus</i> D	500	1
22	No. 20	<i>Lactobacillus rhamnosus</i> D	1000	2
23	No. 21	<i>Lactobacillus rhamnosus</i> D	1500	3

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]. The simulated gastric juice with pH=2 and simulated intestinal juice with pH=8 were prepared according to Cunha et al. [20]. 1 ml of test culture suspension containing 109 CFU/ml was added to the 9 ml of simulated juice, and viable cells number was determined in 30; 60; 90 min for gastric juice and in 1; 2 and 3 hours for intestinal juice by serial dilution assay.

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 for preclinical study for selection, verification, and storage of industrial strains used in the production of probiotics [17].

3. RESULTS

3.1 Evaluation of the 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 glucose level in the blood serum was observed, which was a sign of hyperglycemia. However, the preventive intake of different strains of lactobacilli leads to a decrease in glucose levels (Table 2).

3.2 Probiotic Properties

Probiotic properties ensure the survival of microorganisms in the gastrointestinal tract and their beneficial effect on the host organism. For the probiotic properties characterization, 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.

Table 2. Hypoglycemic effect of lactobacilli

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

Hypoglycemic effect of bacteria *in vivo*

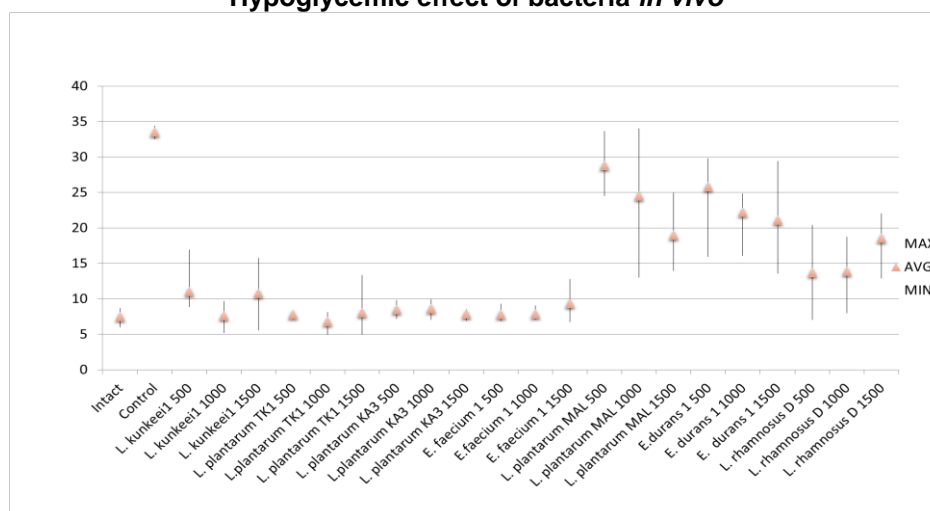


Fig. 1. The blood glucose level (mMol/ml) in rats with alimentary hyperglycemia after preventive treatment with different concentrations of lactobacilli

Table 3. Antagonistic activity

Investigated isolate	Diameter of the zone of inhibition of the growth of the indicator strain, mm									
	<i>C. freundii</i> , 002801/27	<i>P. aeruginosa</i> 003841/114	<i>S. marcescens</i> 367	<i>L. monocytogenes</i> ATCC 1911	<i>E. coli</i> 002673/477	<i>C. albicans</i>	<i>E. faecalis</i>	<i>S. aureus</i> 003594/wood 46	<i>B. subtilis</i>	<i>S. aureus D₈</i>
<i>Lactobacillus plantarum</i> 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
<i>Lactobacillus plantarum</i> KA3	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
<i>Enterococcus faecium</i> 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
<i>Lactobacillus kunkeei</i> 1	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

3.2.1 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 ten 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).

3.2.2 Resistant to various concentrations of bile. All studied strains showed resistance to the presence of 0.6% bile. The number of living cells was at least 10⁹ for all studied cultures.

Table 4. Resistance to various concentrations of bile

Culture	Control	Concentration of bile			
		0,2%	0,3%	0,4%	0,6%
<i>Enterococcus faecium</i> 1	10 ⁹	1,0 x10 ¹⁰	4 x10 ⁹	2 x10 ⁹	2 x10 ⁹
<i>Lactobacillus kunkeei</i> 1	10 ⁹	1,0 x10 ¹⁰	1,3 x10 ⁹	1,0 x10 ⁹	1,0 x10 ⁹
<i>Lactobacillus plantarum</i> TK1	10 ⁹	2x10 ⁹	2x10 ⁹	2x10 ⁹	1x10 ⁹
<i>Lactobacillus plantarum</i> KA3	10 ⁹	5x10 ⁹	3x10 ⁹	3x10 ⁹	1x10 ⁹

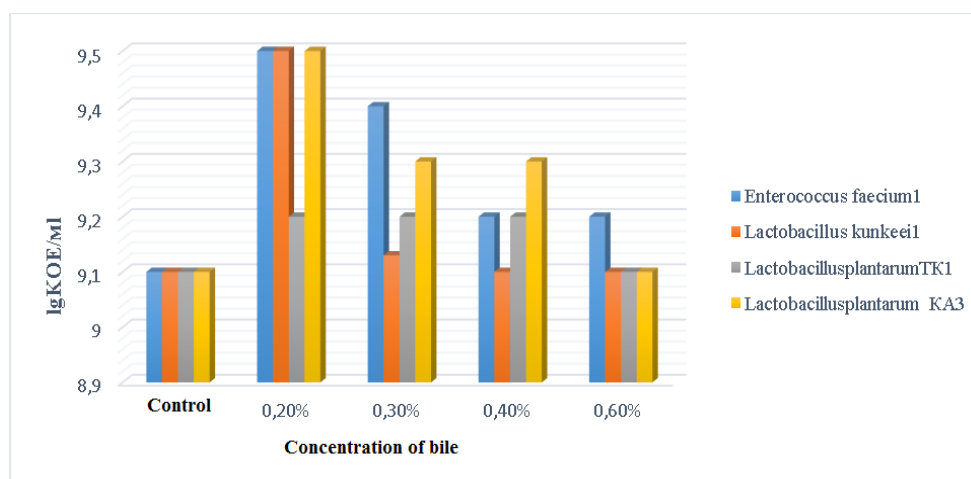


Fig. 2. Resistance to various concentrations of bile

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
<i>Enterococcus faecium</i> 1	1 x10 ⁹	1 x10 ⁹	3x10 ⁵	2x10 ⁵	1x10 ⁴	1 x10 ⁹	2 x10 ⁹	1 x10 ⁹	1x10 ⁹
<i>Lactobacillus kunkeei</i> 1	7 x10 ⁹	4 x10 ⁹	3x10 ⁷	2x10 ⁶	2x10 ²	6 x10 ⁸	5x10 ⁸	5 x10 ⁸	1 x10 ⁸
<i>Lactobacillus plantarum</i> 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
<i>Lactobacillus plantarum</i> KA3	1,1 x10 ¹⁰	4x10 ⁷	2x10 ⁴	2x10 ²	1x10 ¹	2x10 ⁹	2x10 ⁹	2x10 ⁹	1x10 ⁹

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 lactic acid bacteria 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 [21]. Considering these physiological characteristics of the organism, we established the duration of the treatment of cultures with simulated gastric juice.

The investigation of the resistance of *Lactobacillus plantarum* KA3, *Lactobacillus plantarum* TK1, *Enterococcus faecium* 1, and *Lactobacillus kunkeei* 1 isolate to simulated

gastric juice with a pH 2.0 showed that the studied bacteria exhibit different sensitivity.

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 h hours, the number of living cells was 1×10^9 , 1×10^8 , and 1×10^9 , respectively.

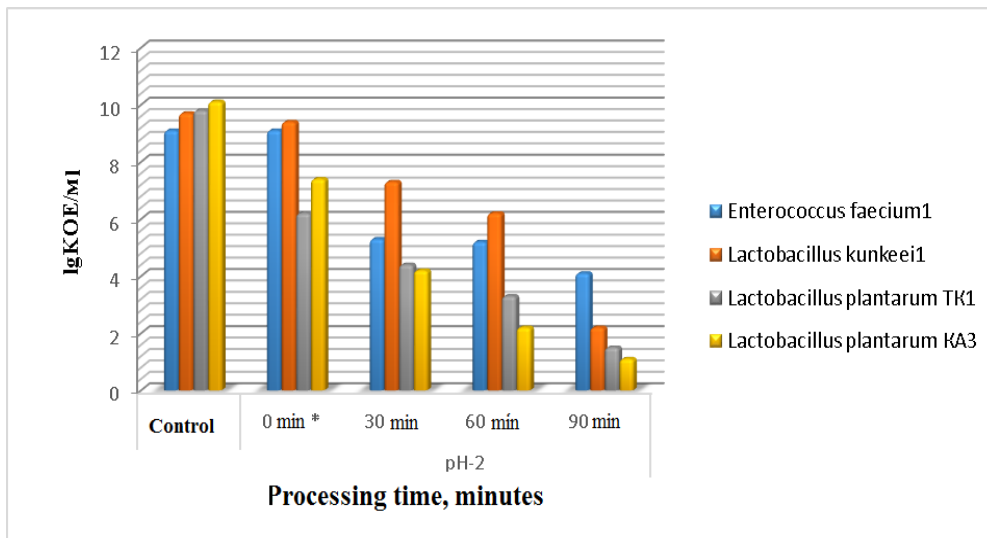


Fig. 3. Resistance to the simulated gastric juice

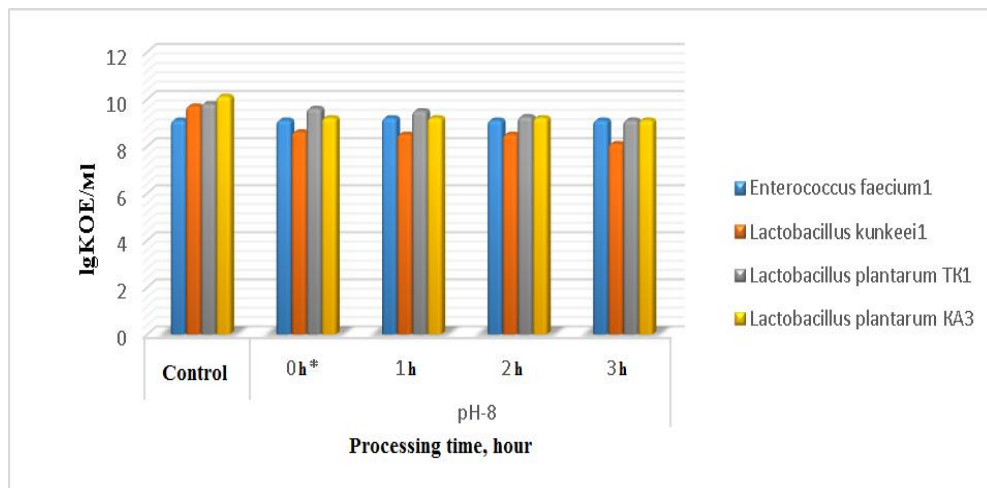
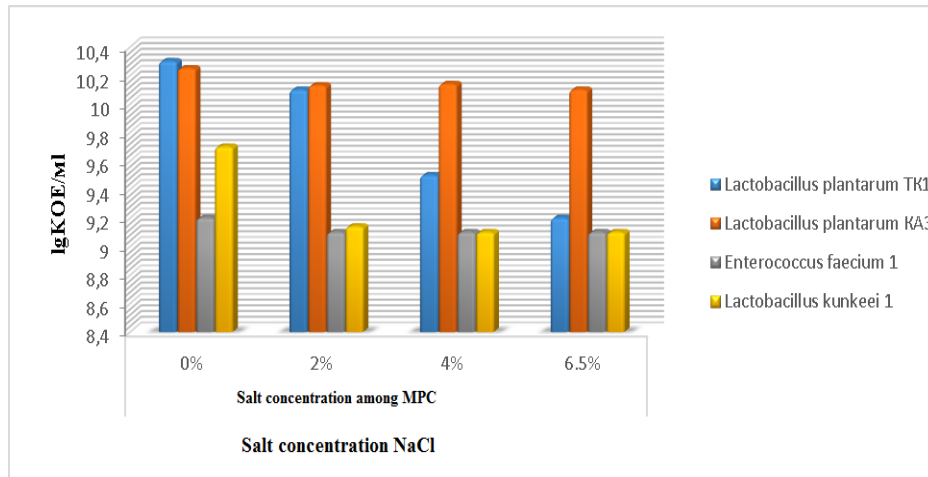


Fig. 4. Resistance to the simulated juice of the small intestine

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

Isolate	NaCl concentration in MRS medium			
	0%	2%	4%	6.5%
<i>Lactobacillus plantarum</i> TK1	3×10^{10}	1×10^{10}	5×10^9	2×10^9
<i>Lactobacillus plantarum</i> KA3	$2,5 \times 10^{10}$	$1,3 \times 10^{10}$	$1,4 \times 10^{10}$	1×10^{10}
<i>Enterococcus faecium</i> 1	2×10^9	1×10^9	1×10^9	1×10^9
<i>Lactobacillus kunkeei</i> 1	7×10^9	$1,4 \times 10^9$	1×10^9	1×10^9

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

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

3.2.3 Resistance of strains to various concentrations of NaCl

The results showed that all cultures are resistant to 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.

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

3.3 Physiological and Biochemical Properties of Strains

In the cultivation process, 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 (hyaluronidase, fibrinolysin, plasma coagulase, hemolysin, lecithinase C, lysozyme) neuraminidase) [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 forming 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 as well as the socio-economic development of the country. 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 finding 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) [21].

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 [22], *Lactobacillus rhamnosus* CCFV0528 [21], *L. rhamnosus* NCDC17 [23], *L.casei* CCFM419 [24].

It 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 determining 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 possessing 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.

Our experiment 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 robust 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 rhamnosus* 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 due to 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. That indicates that not all lactic acid bacteria exhibit the antidiabetic effect, which is strain-dependent. 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].

Our study 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.

The aim of screening the hypoglycemic properties of lactic acid bacteria was to elaborate the probiotic preparations to help keep the average glucose level in the blood. Although lactic acid bacteria have a GRAS (generally recognized as safe) status, not all could be considered probiotics. In order to claim that a bacterial strain is a potential probiotic, the FAO/WHO [30] has established guidelines with safety and functional criteria. They include antagonistic activity and strain behavior under conditions that mimic the GIT. That allows a selection of strains likely to survive such conditions, further investigating their potential as probiotic cultures.

We analyzed these probiotic properties of the isolates, which demonstrated hypoglycemic activity. Strains showed good adaptation to bile, intestinal juice at pH=8 and NaCl, and moderate to low gastric juice tolerance. *E. faecium* and *L. kunkeei* were able better survive simulated stomach juice (2×10^5 and 2×10^6 in 1 hour) rather than *L. plantarum* TK1 and *L. plantarum* KA3 (4×10^4 and 2×10^4 in 30 min). A strain-dependent tolerance to conditions similar to those found in the GIT was also observed in *L. plantarum* strains isolated from Bulgarian cheeses [31] and Fiore Sardo cheese [32].

Safety assessment must also include the lack of harmful activities, such as catalase, gelatinase, and hemolysin activity. Hemolysis is a common virulence factor among pathogens, facilitating iron availability to the microorganism and causing anemia and edema in the host [33]. Iron is a micronutrient that acts as a cofactor for several enzymes and is thus required for the growth of these microorganisms [34]. Lactobacilli can grow without iron, giving them an advantage in the natural environment, in competition with pathogenic bacteria [35]. However, some studies [36, 37] have shown lactobacilli strains with hemolytic activity. The hemolytic activity was not detected in the four tested strains in our study.

These results agree with those found by Cunha et al. [20], who also detected no hemolytic activity in 30 Lactobacilli strains isolated from the stools of a Brazilian newborn infant. Similarly, Kröll et al. [46], did not detect hemolytic activity in 93 lactobacilli strains isolated from Estonian and Swedish children (1–2 y old).

Lactobacilli produce metabolites such as organic acids, fatty acids, hydrogen peroxide, and bacteriocins, which can inhibit the growth of

pathogenic bacteria. In our study, four lactobacilli were assayed for antimicrobial activity against ten pathogenic bacteria. All of them exhibited strong antagonistic activity. The nature of this activity will be investigated in our further researches. These results are in agreement with Cunha et al. [20], Martin et al. [38-42], Maragkoudakis et al. [36] that also detected inhibitory activity of lactobacilli isolates.

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 will be further investigated in the author's laboratories to evaluate their other probiotic safety characteristics to be considered as a new probiotic strain with a set of valuable properties

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 kunkeei*1, *L. Plantarum* TK1, *L. Plantarum* KA3, meet the criteria for probiotics and can be used to prepare probiotic preparations.

The hypoglycemic properties of *Lactobacillus kunkeei*1, *Enterococcus faecium*1, *L. plantarum* TK1, *L. plantarum* KA3, together with their probiotic properties and the absence of virulent enzymes, makes it possible to consider them as promising candidates for the preparation of probiotic preparations intended not only to correct gut microbiota but also to maintain normal blood glucose levels.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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