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Physiological and biochemical features of local strains of lactic acid bacteria

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Abstract: *The main physiological and biochemical properties of two lactic acid bacteria (LAB) strains isolated from healthy calves faeces and identified as Enterococcus hirae and Lactobacillus reuteri were studied. Their ability to ferment different types of carbohydrates was determined. Both strains were identified as heterofermentative LAB as they produced CO_2 from glucose. At the same time protease, catalase and amylase activities of the strains were not detected. Both strains showed the ability of growth at the wide temperature rates (from 20°C up to 50°C), in the presence of NaCl (up to 6%) and bile (up to 40%). Isolated LAB strains have a potential as starter cultures in creation of new bio preparations with probiotic characteristics in Uzbekistan.*

Keywords: *Enterococcus hirae, fermentation, heterofermentative lactic acid bacteria, Lactobacillus reuteri.*

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I. Introduction

Among the many microorganisms that have a practical value, lactic acid bacteria are of a huge interest. Being important inhabitants of the intestinal tract of human and other vertebrate animals, they prevent the development of intestinal infections by inhibiting the growth of pathogenic microflora, stimulate the immune system and the synthesis of vitamins.

Their antimicrobial potential comes out not only in production of several antimicrobials during carbon source metabolism, but also in competing with other species by acidifying their environment and by rapidly depleting the nutrients. Besides these relatively simple antagonistic mechanisms, some lactic acid bacteria also produce potent antibiotic compounds via complex secondary metabolism pathways. Among these are bacteriocins (e.g. nisin), antibiotics (e.g. reutericyclin and reuterin) [1].

Lactic acid bacteria are common in the areas where they can provide their high nutrient requirements including the large amounts of carbohydrates. The literature data report on isolation of *Lactobacillus* strains from young calves faeces and oral cavity, goatling stomach, tea leaves, papaya and other sources [2-5].

There have been isolated local strains of probiotic microorganisms belonging to the genera *Lactobacillus* from one and three month aged healthy calves faeces [6]. Their main physiological and biochemical properties must have been defined for their identification and subsequent application. The common indicators for describing the characteristics of lactic acid bacteria are the followings: fermentation of wide range of carbohydrates, growth at temperature of 15°C and 45°C, growth after heating at temperature of 60°C and 65°C during 30 minutes, growth in the presence of 4, 6, 8% NaCl and 20, 40% bile, carbon dioxide production from glucose and ammonia production from arginine [7].

The study of the formation of CO_2 gas is necessary to determine to which group of bacteria (either homofermentative or heterofermentative) this strain belongs. In general, homofermentative LAB convert carbohydrates into lactate using the Embden-Meyerhof Pathway (EMP), whereas heterofermentative LAB produce lactate, ethanol, and carbon dioxide using the Phosphoketolase Pathway (PKP) [8].

The aim of this work was to study physiological and biochemical properties of isolated probiotic bacteria in order to assess their resistance to low and high temperatures, to various concentrations of salt and bile, as well as the presence of proteolytic, amylolytic and catalase activities.

II. Materials And Methods

Strains *Enterococcus hirae* and *Lactobacillus reuteri* were stored in lyophilized form at temperature of 7°C. 24-hour samples, cultivated in MRS nutrient media, were used as inoculates.

The biochemical characterization was defined by fermentation of different carbohydrates in MRS-3 nutrient medium containing following ingredients (gms/litre):

Peptone – 10.00; yeast extract – 5.00; ammonium citrate – 2.00; sodium acetate – 5.00; magnesium sulphate – 0.10; manganese sulphate – 0.05; dipotassium phosphate – 2.00; cysteine – 0.2; polysorbate 80 – 1.00; appropriate substrate – 5.00; indicator chlorophenol red – 0.04; agar – 1.50.

The ability of CO_2 production from glucose was defined in MRS-7 nutrient medium containing ingredients (gms/litre):

Peptone – 10.00; beef extract – 10.00; yeast extract – 5.00; glucose – 50.00; ammonium citrate – 2.00; sodium acetate – 5.00; magnesium sulphate – 0.10; manganese sulphate – 0.05; dipotassium phosphate – 2.00; cysteine – 0.2; polysorbate 80 – 1.00; agar – 5.00.

The sterilized MRS-7 was spilled into the tubes and after the inoculation, nutrient agar medium was layered onto the MRS-7. In case of gas production, the tap of nutrient agar lifted up in 3-5 days of cultivation.

Qualitative reactions to catalase, protease and amylase were carried out according to the standard methods [9 - 11].

III. Results And Discussion

21 types of carbohydrates were tested for studying the biochemical characterization of isolated LAB strains. The results shown in Table 1 demonstrate that *Enterococcus hirae* intensively fermented L-arabinose, cellobiose, dextrose, dulcitol, galactose, fructose, maltose, mannose, sucrose in 24-48 hours of cultivation. Also this strain fully fermented lactose, mannitol, trehalose in 120 hours and partially inulin, melibiose, salicin and L-histidine. It did not ferment adonitol, inositol, raffinose, rhamnose, sorbitol and xylose. There was a moderate production of gas (CO_2) at the end of glucose metabolism (fig.1).

At the same conditions *Lactobacillus reuteri* intensively fermented L-arabinose, cellobiose, dextrose, dulcitol, galactose, fructose, maltose, mannitol, mannose, sorbitol, sucrose, trehalose. The strain fully fermented lactose, melibiose, raffinose in 120 hours and partially inulin, rhamnose, salicin, L-histidine and L-serine. Other carbohydrates were not fermented. The strain also produced CO_2 as a result of glucose fermentation (fig.1).

Major lactic acid bacteria do not dilute gelatin, i.e. do not display protease activity [7]. In this case, the protease, amylase and catalase activities of studied strains were not observed as well.

Table 1. Fermentation of carbohydrates by isolated localstrains ofLAB.

Substrate		*E. hirae*	*L. reuteri*
Carbohydrates	adonitol	–	–
	L-arabinose	+	+
	cellobiose	+	+
	dextrose	+	+
	dulcitol	+	+
	galactose	+	+
	fructose	+	+
	inositol	–	–
	inulin	±	±
	lactose	(+)	(+)
	maltose	+	+
	mannitol	(+)	+
	mannose	+	+
	melibiose	±	(+)
	raffinose	–	(+)
	rhamnose	–	±
	salicin	±	±
	sorbitol	–	+
	sucrose	+	+
	trehalose	(+)	+
	xylose	–	–
CO_2 production from glucose		+	+
Gelatin dilution		–	–
Starch hydrolysis		–	–
Catalase test		–	–

"+" - ferments intensively, fully; "–" - does not ferment; "(+)" - ferments fully but slowly; "±" - ferments partially, poorly.

fig.1. CO_2 production from glucose: 1 - control (without inoculation); 2 –*Enterococcus hirae*; 3 –*Lactobacillus reuteri*; 4 - homofermentative bacterium *Lactobacillus plantarum.*

In order to function normally and adhesion in the gastrointestinal tract, LAB should express high tolerance to bile and bile salt. There are several researches data in literature describing bile and bile salt tolerant strains of *L. reuteri* isolated from different sources [12 -14].

Isolated strains were tested on ability of growth in different concentrations of natural bile and NaCl salt. Both LAB strains grew in the presence of NaCl at the concentration of 4 and 6%. Besides *L. reuteri* demonstrated higher resistance to salt as grew in medium containing 8% NaCl. Also both strains were tolerant to bile of high concentrations up to 40% (Table 2).

Table 2. The growth of isolated local LAB strains in presence of salt and bile.

substrate	*E. hirae*	*L. reuteri*
4% NaCl	+	+
6% NaCl	+	+
8% NaCl	–	±
10% NaCl	–	–
20% bile	+	+
30% bile	+	+
40% bile	+	+

"+" - intensive growth; "–" - growth absence; "±" - passive growth.

As a rule, the maximal temperature of lactobacilli cultivation in researches is 45°C. But isolated cultures were tested at the higher temperatures. They grew actively at 50°C. *E. hirae* was even more thermotolerant and grew at 55°C as well as after storage at 60°C during 30 minutes (Table 3).

Table 3. The effect of temperature on the growth of isolated localstrains of LAB.

	E. hirae	*L. reuteri*
growth at:		
20°C	+	+
50°C	+	+
55°C	±	–
growth after heating at (during 30 minutes):		
60°C	+	–
65°C	–	–

"+" - intensive growth; "–" - growth absence; "±" - passive growth.

IV. Conclusion

Therefore, both strains being adapted to a sharply continental climate, demonstrated the ability to grow at high temperature rates. Herewith *Enterococcus hirae* was more resistant to high temperature rates, while *Lactobacillus reuteri* was more resistant to higher salt concentration. The strains did not possess neither protease, amylase nor catalase activities. Summarizing, new isolated lactic acid bacteria have a prospect in creation of new biopreparations for cattle breeding in Central Asian region.

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