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# Valuable Biotechnological Properties of *Lactobacillus acidophilus* Strains

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**Abstract.** One of the most common probiotic cultures is *Lactobacillus acidophilus*. This bacterium is characterized by a high antimicrobial activity against pathogenic and opportunistic microorganisms. The search of effective *L. acidophilus* strains with biotechnologically valuable properties is becoming especially important, because competition in the promising market of probiotics is high. This research aimed to study the acid forming ability (active acidity (AA), titratable acidity (TA) and final acidity (FA)) and organoleptic characteristics of three lactobacilli strains from Russian National Collection of Industrial Microorganisms (VKPM). The bacterial strains *L. acidophilus* B-4107, *L. acidophilus* B-2585 and *L. acidophilus* B-8153 were used in this study. Milk fermented by *L. acidophilus* B-2585 strain was possessed by the most pronounced technologically valuable properties (AA –  $3.47 \pm 0.30$ , TA –  $236.53 \pm 13.15$  °T, FA –  $308.33 \pm 11.06$  °T, pleasant sour milk taste and odor, dense clot).

## INTRODUCTION

*Lactobacillus acidophilus* is one of the most studied species of the genus *Lactobacillus* and is generally recognized as safe (GRAS) [1]. This bacterium belongs to probiotic microorganisms and has a high antimicrobial activity against *Candida albicans* [2], *Penicillium expansum*, *Aspergillus flavus* [3], *Listeria monocytogenes*, *Clostridium sporogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and other pathogens. The antagonistic effect of *L. acidophilus* is due to the synthesis of lactic acid, diacetyl, ammonia and bacteriocins (lactocin B, lactacin F, acidocin B and others) [4]. In addition, lactobacilli are cholesterol-lowering, resistant to bile, stimulate the immune system and actively metabolize lactose. Therefore, functional foods and nutritional supplements for humans and animals based on *L. acidophilus* are actively commercialized [5].

The global market of probiotics for 2011 was estimated at \$15 billion, of which 10% was made up by the market of drinks fermented by lactic acid bacteria [6]. In 2019, the world probiotic products market was already \$49 billion and would reach \$74.69 billion by 2025 according to Fortune Business Insights forecasts [7].

Currently, many of milk processing enterprises are trying to expand their product line by probiotics. The search for effective *L. acidophilus* strains with biotechnologically valuable properties is becoming important, because competition in the promising market of probiotics is high [8].

Starter cultures of acidophilic lactobacilli for probiotics should inhibit the growth of technically harmful microorganisms, give a product a pleasant taste and have a pronounced acid forming activity. It also should have an optimal rate of clot formation and the high number of CFU (colony forming units) per unit volume of product [9].

This research aimed to investigate the valuable biotechnological properties (active acidity (AA), titratable acidity (TA) and final acidity (FA), and organoleptic characteristics) of *L. acidophilus* strains from Russian National Collection of Industrial Microorganisms (VKPM).

## MATERIALS AND METHODS

### The Studied Strains

The bacterial strains *L. acidophilus* B-4107, *L. acidophilus* B-2585 and *L. acidophilus* B-8153 from VKPM were used in this study.

### Media, Reagents and Culture Conditions

Skimmed milk (115 g of powdered milk per 1 liter of distilled water) was used for the cultivation of lactobacilli. Cultures of *L. acidophilus* were grown in a thermostat Binder BD 115 at 40 °C for 18 hours. Titratable acidity of the starter culture was determined with 0.1 N NaOH solution. 1% phenolphthalein solution was used as an indicator. Microscopic preparations were stained with Gram reagents.

### Morphological and Biotechnological Properties

Lactobacilli were grown in 100 ml of sterile skimmed milk to assess their properties. Cultures concentration was 3 %. The morphological characterization of *L. acidophilus* strains was carried out microscopically. The active acidity of fermented milk was determined by potentiometric method using a pH meter (Mettler-Toledo FiveGo FG2) [10]. Titratable acidity and final acidity of the starter culture were determined by the indicator method using a digital titrator (Biotrate DE-M 18). Acidity was determined by the formula (1) and expressed as Turner degrees (°T).

$$TA = V \times 10 \quad (1)$$

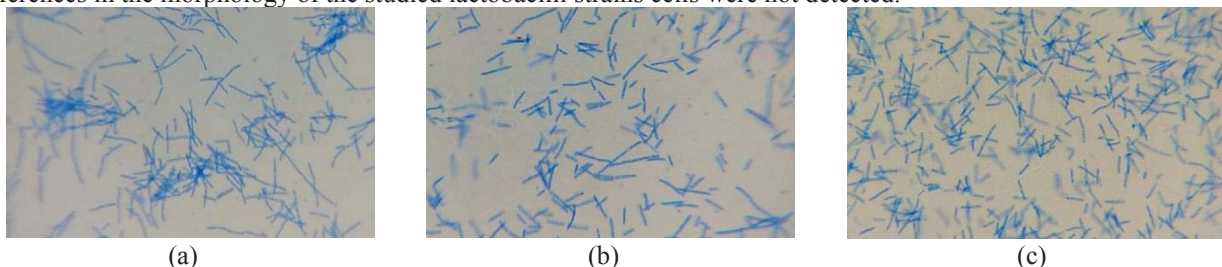
where V is volume of 0.1 N NaOH which was consumed to the neutralization of 10 ml acidophilus milk; 10 is coefficient for conversion to 100 ml of milk [11].

Active acidity and titratable acidity of acidophilus milk were established after 18 hours of incubation as well as after 7 days of storage at 4–6 °C (final acidity). For all numerical values, the arithmetic mean (M) and standard deviation (m) were determined. A sensory evaluation of acidophilus milk was carried out.

## RESULTS AND DISCUSSION

### Morphological Characteristics of *L. acidophilus* Strains

As a result of microscopic analysis of the studied strains (Fig. 1), it was found that they are characterized by non-spore-forming, gram-positive cells in the form of long rounded rods (about 5–6 µm), occurring singly, in pairs or in chains. This corresponds to the description of *L. acidophilus* species in Bergey's Manual [12]. Significant differences in the morphology of the studied lactobacilli strains cells were not detected.



**FIGURE 1.** Micropreparations ( $\times 1000$ ) of *L. acidophilus* strains: (a) B-4107, (b) B-2585, (c) B-8153.

## Valuable Biotechnological Characteristics of *L. acidophilus* Strains

After 18 hours of incubation, all studied strains gave milk clots of high density. Active acidity significantly reduced, while titratable acidity significantly increased compared to the sterile milk (AA and TA of skimmed milk were  $6.56 \pm 0.05$  and  $37.30 \pm 4.53$  °T, respectively). The results of the study are shown in table 1.

**TABLE 1.** Characterization of technologically valuable properties of the studied *L. acidophilus* strains.

Characteristics		Strains		
		B-4107	B-2585	B-8153
Odor		Sour milk	Pronounced sour milk	Not sour milk enough
Taste		Fermented milk with a metallic taste	Fermented milk with a metallic taste	Not fermented milk enough, slightly sharp
Structure and consistency		Homogeneous, with dense clot and a small amount of serum, not viscous	Homogeneous, with dense clot and a small amount of serum, not viscous	Viscous, with not enough dense clot
Color		Light cream, uniform throughout the mass	Light cream, uniform throughout the mass	Light cream, uniform throughout the mass
AA	18 hours	$3.77 \pm 0.34$	$3.47 \pm 0.30$	$3.76 \pm 0.28$
(M $\pm$ m, pH)	7 days (FA)	$3.29 \pm 0.14$	$3.16 \pm 0.13$	$3.26 \pm 0.07$
TA	18 hours	$184.60 \pm 34.22$	$236.53 \pm 13.15$	$224.47 \pm 18.34$
(M $\pm$ m, °T)	7 days (FA)	$271.33 \pm 28.68$	$308.33 \pm 11.06$	$286.33 \pm 12.66$

When evaluating organoleptic indicators, it was found that strains *L. acidophilus* B-4107 and *L. acidophilus* B-2585 possess the most valuable characteristics in this regard. These strains have a pleasant sour milk flavor with a metallic taste, due to a significant accumulation of lactic acid, a non-viscous consistency and form dense homogeneous clots. However, the strain *L. acidophilus* B-2585 has a brighter sour-milk smell than the strain *L. acidophilus* B-4107. The strain *L. acidophilus* B-8153 in terms of organoleptic characteristics loses to the other studied strains, as it has a not enough sour milk taste and aroma as well as a viscous texture and fragile clot. Slightly sharp taste of *L. acidophilus* B-8153 strain is presumably due to its biochemical activity, as the inoculation was carried out under aseptic conditions and no extraneous microflora was detected during microscopy.

Separately, it is worth noting that after storage for 7 days, the organoleptic characteristics of acidophilus milk did not actually change. Only the sour milk flavor became more pronounced.

According to the obtained results, all the studied strains had good acid forming ability and acid resistance. When storing samples for seven days, an increase in titratable acidity and a decrease in the pH value were noted. This is due to the production of lactic acid by bacteria during fermentation. The highest final acidity was noted for *L. acidophilus* B-2585 strain.

Despite a similar morphology and belonging to the same species, the studied lactobacilli showed different cultural and biochemical properties. Based on such studies, strains with the most promising characteristics for the production of probiotics can be selected.

## CONCLUSION

Milk fermented by *L. acidophilus* B-2585 strain possesses the most pronounced technologically valuable properties (optimal organoleptic characteristics, active acidity  $3.47 \pm 0.30$ , titratable acidity  $236.53 \pm 13.15$  °T, final acidity  $308.33 \pm 11.06$  °T). This strain of lactobacilli can be recommended for inclusion in the composition of probiotic fermented milk products and also be a reference strain for researchers and developers of probiotic dairy products.

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