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EXPERIMENTAL ARTICLES

Patterns of Growth and β-Galactosidase Production by Bifidobacteria

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Abstract—We investigated the patterns of growth and β-galactosidase production in the strains *Bifidobacterium adolescentis* GO-13, MS-42, 91-BIM, and 94-BIM and *B. bifidum* No.1, LVA-3, 791 on media with various carbon sources. The synthesis of β-galactosidase was shown to be associated with exponential growth of the cultures involved. The maximum specific rate of β-galactosidase synthesis of 0.20 U mg^{-1} h^{-1} was observed in *B. bifidum* LVA-3 after 3–6 h of cultivation. This value for *B. adolescentis* 91-BIM and 94-BIM was lower and amounted to 0.03–0.08 U $mg^{-1}h^{-1}$. On the medium with lactose, the highest specific growth rates for *B. bifidum* LVA-3 and *B. bifidum* No.1 were 0.38 and 0.60 h^{-1}, respectively, after 3–6 h of cultivation. For *B. adolescentis* 91-BIM and 94-BIM, this parameter peaked at 12–15 h of cultivation at 0.13 and 0.22 h^{-1}, respectively. The hydrolytic activity of β-galactosidase in the growth medium decreased during the stationary growth phase of the tested cultures.

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Key words: bifidobacteria, β-galactosidase activity, growth rate.

The main catalytic function of β-galactosidase (β-D-D-galactoside-galactohydrolase, lactase, EC 3.2.1.23) is to hydrolyze β-D-galactosides, detaching the non-reducing β-D-galactose residue. The enzyme cleaves terminal β-galactoside bonds, liberating galactoside residues from the non-reducing side of galactosides, including oligosaccharides, polysaccharides, glycolipids, glycopeptides, glycoproteins, and mucopolysaccharides [1]. The enzyme β-galactosidase is present in many bacteria, particularly in milk-utilizing microflora, which consumes the natural β-galactosidase substrate (lactose and its metabolites). These bacteria include various species of *Propionibacterium*, *Lactobacillus*, and *Streptococcus* [2–4]. The β-galactosidase of *Escherichia coli* has been especially well-characterized; this enzyme is used in studies on the mechanism of protein synthesis regulation in prokaryotes and in research related to molecular biology and genetics [5, 6]. However, researchers still devote much attention to this enzyme. The transferase reaction catalyzed by β-galactosidases is known to result in the formation of β-galactooligosaccharides with various degrees of polymerization and bond types [7]. These enzymes are also involved in the metabolism of complex galactosides (glycoprotreins and glycolipids). Endogalactosidases can affect blood-group-specific substances; i. e., they are involved in immune reactions. Apart from this, β-galactosidase is of importance for metabolic processes in nerve tissues [8].

The considerable attention given to the research on the structural and catalytic properties of bacterial β-galactosidases used as a component of probiotic preparations is due to their importance for the curative properties of such preparations [9].

Further research is necessary for understanding the physiological functions of these enzymes, as well as the occurrence of multiple forms of β-galactosidases in various bacteria, their location, substrate specificity, and genetic expression.

The goal of this work was to investigate the characteristics of growth and β-galactosidase formation by various strains of bifidobacteria.

MATERIALS AND METHODS

Bacterial strains. Strains *Bifidobacterium adolescentis* MC-42, *B. adolescentis* GO-13, *B. bifidum* No. 1, *B. bifidum* 791, *B. bifidum* LVA-3, and *B. longum* B379M, were kindly provided by the workers of the All-Russian Research Control Institute of Microbiology and Immunology and of the G.N. Gabrichevsky Research Institute of Epidemiology and Microbiology. Strains *B. adolescentis* 91-BIM and 94-BIM, which were obtained at the Laboratory for Biochemistry of

† Deceased.

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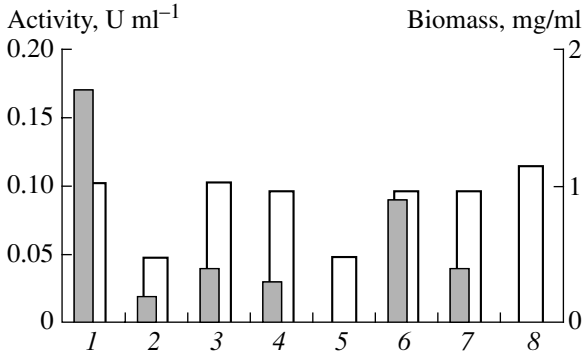

Fig. 1. β-Galactosidase activity of collection cultures of bifidobacteria. Cultivation on a lactose-containing medium. Dark columns, β-galactosidase, U ml^{-1}; light columns, biomass, mg ml^{-1}. *1*, *B. bifidum* No. 1; *2*, *B. bifidum* 791; *3*, *B. bifidum* LVA-3; *4*, *B. adolescentis* GO-13; *5*, *B. adolescentis* MC-42; *6*, *B. adolescentis* 91-BIM; *7*, *B. adolescentis* 94-BIM; *8*, *B. longum* B379 M.

Microorganisms by autoselection; are presently stored as *B. adolescentis* BIM B-91 and *B. adolescentis* BIM B-87 at the Scientific Collection of Type Strains and Industrial Nonpathogenic Microorganisms of the Institute of Microbiology of the National Academy of Sciences of Belarus (Belorussian Collection of Non-pathogenic Microorganisms).

Cultivation of the bacteria was carried out under microaerophilic conditions at 37°C on a medium with peptone and yeast extract [10]. Depending on the goal of the experiments, the medium was supplemented with different carbon sources. Physiologically active 18-h-old cultures were used as inocula. 5% (v/v) of the inoculum was added to the medium and samples were taken at intervals of 1.5, 3, 6, and 24 h for 2–4 days.

Activity of β-galactosidase was determined colorimetrically from the amount of *o*-nitrophenol liberated from *o-nitrophenyl*-β-D-galactopyranoside (*o*-NPG) at 37°C after 15–60 min of incubation. The reaction mixture contained 1.0 ml 0.14 M citrate–phosphate buffer (pH 5.0), 0.5 ml of 0.1 % *o*-NPG solution, and 0.5 ml of the enzyme solution [11]. One activity unit (U) corresponded to the amount of the enzyme that catalyzed the degradation of 1 μmol of substrate per minute at 37°C.

Biomass of bacteria was determined as weight by drying the washed cells at 105°C to constant weight, and nephelometrically, from the optical density of a bacterial cell suspension at 590 nm.

Number of viable bacterial cells per 1 ml of suspension (the number of colony-forming units, CFU) was estimated by inoculating diluted cell suspensions on nutrient media with 0.2% of agar.

Active acidity was measured potentiometrically.

Specific growth rate (μ) was calculated using the formula

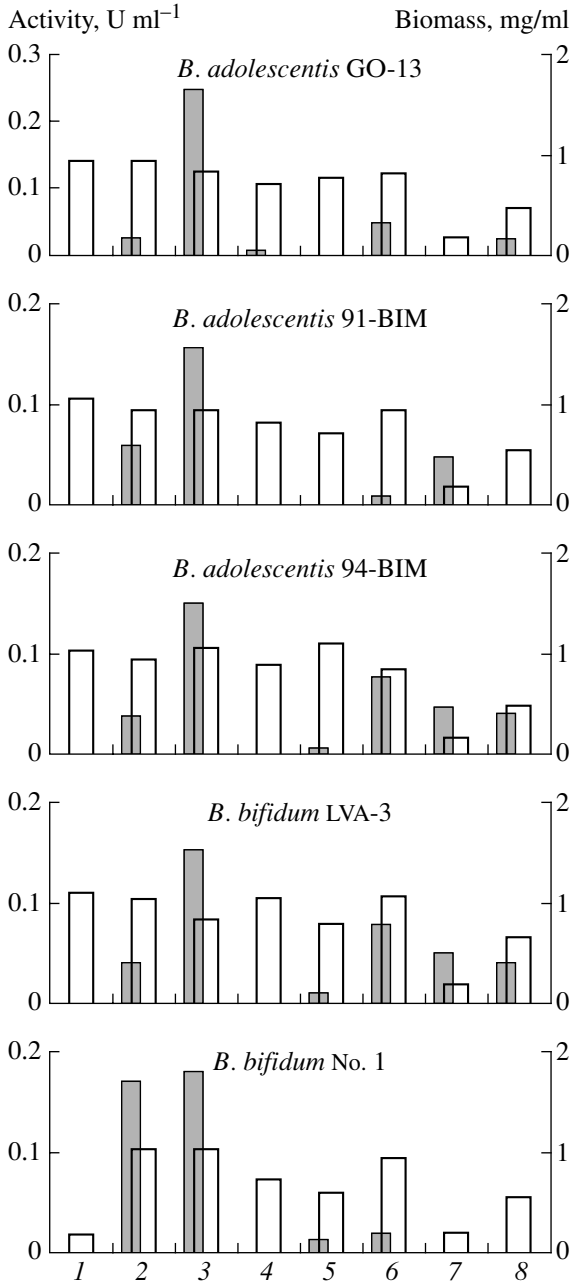

Fig. 2. β-Galactosidase activity (dark columns, U ml^{-1}) and growth (light columns, mg ml^{-1}) of bifidobacteria on media with various carbon sources. *1*, sucrose; *2*, lactose; *3*, galactose; *4*, glucose; *5*, fructose; *6*, maltose; *7*, arabinose; *8*, cellobiose.

$$\mu = dx x^{-1} dt^{-1},$$

where dx is the change in the biomass (x) during the time interval dt.

Specific rates of enzyme synthesis (ε) were calculated using the formula

$$\varepsilon = dE dt^{-1} x^{-1},$$

where x is the biomass (mg ml^{-1}) and dE the activity change during the time interval dt [12].

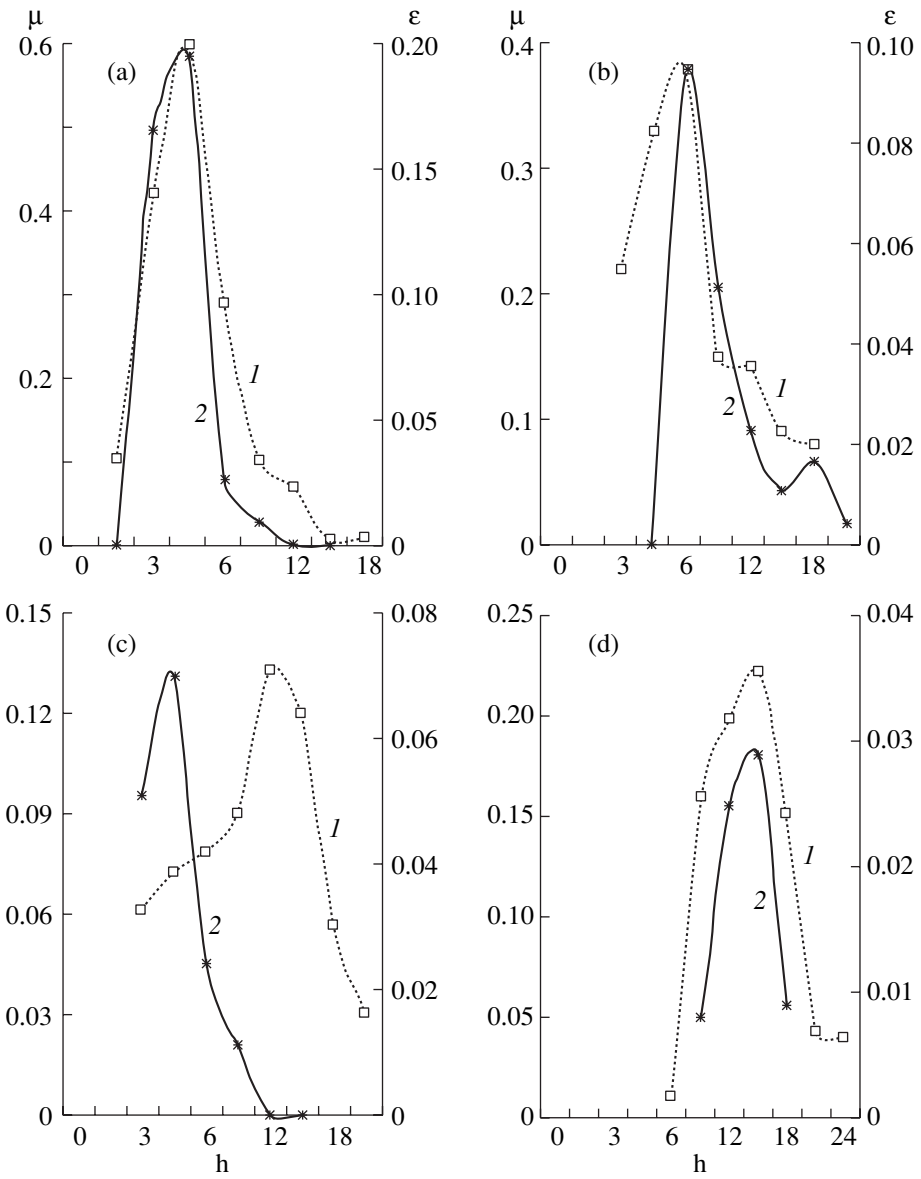

Fig. 3. Kinetics of growth and β-galactosidase synthesis in *B. bifidum* LVA-3 (a), *B. bifidum* No. 1 (b), *B. adolescentis* 91-BIM (c), and *B. adolescentis* 94-BIM (d). *1*, μ, specific growth rate of bifidobacteria, h^{-1}; *2*, ε, specific β-galactosidase synthesis rate, $U/(mg^{-1} h^{-1})$.

RESULTS AND DISCUSSION

The data presented in the literature indicate that β-galactosidase is an inducible enzyme in most of the tested microbial strains [8]. Our analysis of β-galactosidase activity in the culture fluid of various strains of bifidobacteria maintained on a medium with glucose revealed that all of the tested strains except *B. longum* B379M displayed β-galactosidase activity after substituting lactose (as a carbon source) for glucose (Fig. 1). The highest β-galactosidase activity level occurred in *B. bifidum* No. 1 and *B. adolescentis* 91-BIM. We selected the following cultures for subsequent study: *B. bifidum* No. 1, LVA-3, and No. 1; and *B. adolescentis* GO-13, 91-BIM, and 94-BIM.

It is well-known that microorganisms form numerous polyfunctional metabolites while utilizing various substrates. Importantly, the influence of medium components on the cellular regulatory system is indirect. All these substances undergo metabolic changes, and the real regulatory metabolites, e.g., inducers, still remain unidentified in most cases [8].

We investigated β-galactosidase production during the cultivation of bifidobacteria on media with various carbon sources. We found that the cultures grew well with glucose, sucrose, and fructose, but no β-galactosi-

dase activity was detected in the medium. Galactose, a product of lactose hydrolysis, caused an increase in β-galactosidase yield in all tested *B. adolescentis* strains (Fig. 2). This activity increased from 0.03 to 0.25 U ml^{-1} in *B. adolescentis* GO-13. As for arabinose, a significant β-galactosidase yield was revealed in *B. adolescentis* 91-BIM and 94-BIM and also in *B. bifidum* LVA-3, which does not use arabinose as a carbon source [13] (Fig. 2). The data on the increase in β-galactosidase activity in various strains of bifidobacteria in response to the addition of lactose, galactose, or arabinose suggest that the mechanisms of regulating β-galactosidase synthesis or galactose transport into the cell are different.

On the medium with lactose, the highest specific growth rate (μ) occurred in *B. bifidum* LVA-3 and *B. bifidum* No. 1 (Figs. 3a, 3b). The maximum μ values were achieved after 3–6 h of cultivation; they were 0.38 and 0.60 h^{-1}, respectively. *B. adolescentis* 91-BIM and 94-BIM had lower growth rates on this medium; the maximum values (0.13 and 0.22 h^{-1}, respectively) occurred at 12–15 h of cultivation (Figs. 3c, 3d).

The specific β-galactosidase synthesis rate (ε) was comparatively high during the first 3–6 h of cultivation. The maximum ε value (0.20 U mg^{-1} h^{-1}) was characteristic of *B. bifidum* LVA-3 (Fig. 3a). The specific β-galactosidase synthesis rates of *B. adolescentis* 91-BIM and 94-BIM were 0.08 and 0.029 U mg^{-1}h^{-1}, respectively (Fig. 3c and 3d). The data on the relationship between the growth rate and the β-galactosidase synthesis rate significantly contribute to our knowledge concerning bifidobacteria and are in agreement with the information on the development of bacterial populations presented in the literature [13].

Our research on the growth dynamics of the tested cultures on the medium with lactose revealed a relationship between the growth of bifidobacteria and the level of β-galactosidase activity in the medium (Fig. 4). The biomass of bifidobacteria accumulates within 24 h of cultivation. By the end of this period, the log [CFU/ml] reaches maximum values (from 8.08 for *B. adolescentis* 94-BIM to 9.04 for *B. bifidum* No. 1). After 48 h of cultivation, a decrease in viable cell numbers was detected; after 96 h, they drop by 40–60%. In all the tested strains of *B. adolescentis* and *B. bifidum*, active β-galactosidase synthesis during the exponential growth phase was followed (after 18–24 h of cultivation) by a decrease in β-galactosidase yield and activity level in the medium (Fig. 4). At the onset of the stationary phase, the decrement in β-galactosidase activity was still insignificant, but it progressively decreased at later cultivation stages. In *B. bifidum* LVA-3, β-galactosidase activity was 0.12 U ml^{-1} (90% of the maximum value) after 9 h. It dropped to 0.02 U ml^{-1} (15% of the maximum value) after 21 h of growth (Fig. 4).

The decrease in the viable cell number and in the β-galactosidase activity of bifidobacteria is likely to be due to the decline in proliferation intensity, a change in cell sensitivity to deleterious factors, and the effect of extremely high concentrations of hydrogen ions that are, in part, supplied by acidic metabolic products functioning as autoinhibitors [15].

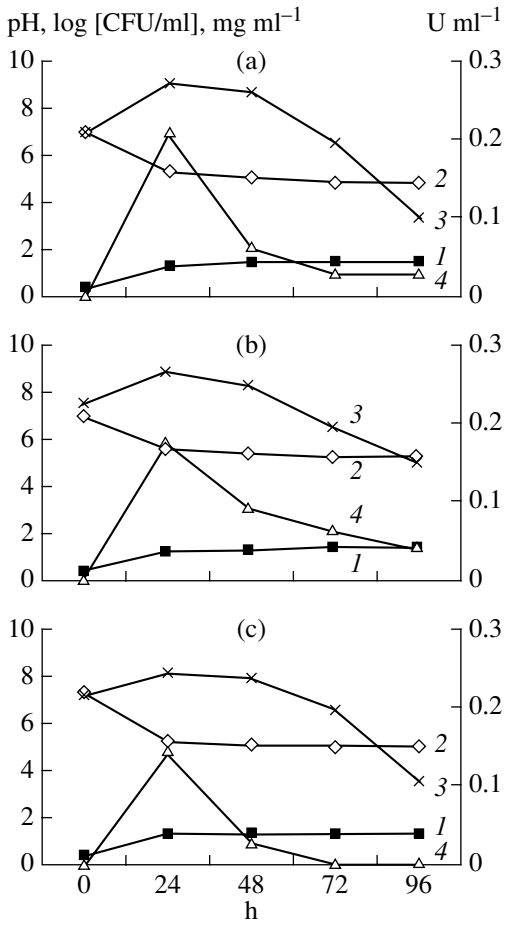

Fig. 4. Growth (*1*, mg ml^{-1}), acid formation (*2*, pH), viability (*3*, log CFU/ml), and β-galactosidase activity (*4*, U ml^{-1}) during the development of populations of *B. bifidum* No. 1 (a), *B. adolescentis* 91-BIM (b), and *B. adolescentis* 94-BIM (c).

The data obtained agree with the conclusion drawn in an earlier work that populations of bifidobacteria at late developmental stages are characterized by hypometabolism, i.e., a deceleration of metabolic processes, a decrease in substrate utilization rate, and a decline in the production of extracellular and cell-wall-associated hydrolytic enzymes [13, 16].

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