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Evaluation of probiotic potential of *Bifidobacterium animalis* subsp. lactis strains: an *in vitro* study

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Abstract

Probiotic potential of two bifidobacterial strains isolated from feces of healthy adults and identified as *Bifidobacterium animalis* subsp. *lactis* was evaluated using *in vitro* testing. The analyzed strains were able to ferment a broad spectrum of carbohydrates, produced bioactive exopolysaccharides, demonstrated high survival rate in model GIT conditions, under heat and oxidative stresses, inhibited growth of a wide range of pathogenic and opportunistic bacteria, and proved to be safe for biotechnological application. Based on the complex phenotypic characteristics tested, *Bifidobacterium animalis* subsp. *lactis* may be regarded as prospective probiotic cultures.

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Introduction

Probiotic potential of two bifidobacterial strains isolated from feces of h**.** The human gastrointestinal tract (GIT) contains as many as ten times more microbial cells than human cells in the entire body, and the gut microbiota have significant implications in the health status of the host. During the whole life of each individual, the gut microbiota composition could be altered by lifestyle, diet, antibiotic therapies, or stress conditions, which may lead to acute and chronic disorders.

Probiotic ingestion is recommended as a preventive approach to maintain the balance of the intestinal microbiota and to enhance the human well-being. At present, one of best known probiotic organisms are bacteria of the genus *Bifidobacterium*, naturally present in human GIT, which provide many beneficial effects on the host health (1-3).

Aim of the study – characterization of probiotic properties of two bifidobacterial strains, isolated from feces of healthy Belarusian adults.

Materials and Methods

Identification of bifidobacteria was performed using PCR with genus-specific primers, sequencing of 16S rDNA and transaldolase gene, MALDI-TOF MS protein profiling and biochemical testing with ANAEROtest 23 kit. Antibiotic susceptibility of bifidobacteria was determined by disc-diffusion and broth micro-dilution methods, genes coding for antibiotic resistance were screened using PCR analysis. Probiotic characteristics of bifidobacteria were studied using standard methodologies.

Results and Discussion

Bifidobacterium animalis subsp. *lactis* strains isolated from feces of healthy Belarusian adults were phenotypically and genotypically characterized according to international guidelines for probiotics. Taxonomic affiliation of strains was confirmed based on the re-

sults of biochemical testing, 16S rRNA and transaldolase genes sequencing, MALDI TOF-MS protein profiling.

Analysis of carbohydrate fermentation patterns revealed that both strains utilize glucose, sucrose, maltose, raffinose, lactose, arabinose, and salicin, but do not ferment mannose, trehalose, cellobiose, galactose, mannitol, xylose, rhamnose, melezitose or sorbitol.

Both strains demonstrated high survival rate (up to 36% and 79% respectively) in model GIT conditions, were able to grow at pH 5.0 and in presence of 4.5 % oxgall, tolerated heat and oxidative stresses (survival rates 73% and 61%, aerobic/anaerobic growth coefficients 0.35 and 0.25).

Analysis of antagonistic activity of bifidobacteria revealed their ability to inhibit growth of a wide range of pathogenic and opportunistic bacteria, including *Escherichia coli* (inhibition zone 4-10 mm), *Pseudomonas aeruginosa* (3-10 mm), *Pseudomonas fluorescens* (1-2 mm), *Bacillus cereus* (1-3 mm), *Proteus vulgaris* (2-3 mm), and *Staphylococcus aureus* (4-12 mm), without inhibition of "useful" bacteria – lactobacilli and lactococci.

Both *B. animalis* subsp. *lactis* strains produced exopolysaccharides (EPS) – bioactive molecules involved in interaction with the host intestinal epithelia and modulation of the immune system. The level of EPS production by tested bifidobacterial strains depended on the carbon source and cultivation conditions. Genome analysis of the investigated strains revealed the presence of a gene cluster including genes coding for glycosyl transferases, probably involved in biosynthesis of membrane-associated exopolysaccharides.

Evaluation of lipid profiles of bifidobacteria demonstrated the presence of phosphatidylglycerol (Rf=0.45) as the predominat cellular phospholipid and unidentified lipids, including two major phosphoglycolipids (Rf=0.25; Rf=0.35), one nitrogen-containing phospholipid (Rf=0.40) and three glycolipids (Rf=0.14, Rf=0.22, Rf=0.8), which according to literature data may possess biological activity.

Biosafety of *B. animalis* subsp. *lactic* strains was studied using *in vitro* and *in vivo* techniques. Assessing the antibiotic resistance profiles of tested strains revealed their susceptibility to chloramphenicol (MIC 1-4 μg/ml), ampicillin (0.5-1 μg/ml), streptomycin (80-100 μg/ml), gentamycin (20-60 μg/ml), erythromycin (1-4 μg/ml), and resistance to tetracycline (8-10 μg/ml). Molecular analysis of antibiotic resistance by PCR and whole genome sequencing confirmed the presence of single copies of chromosomal *miaA* and *tetW* genes, coding for tetracycline resistance, in both strains. As detected genes have identical sequence and the same location in all currently sequenced *B. animalis* subsp. *lactis* genomes, the possibility of their horizontal transfer and impact in spread of antibiotic resistance would be minor.

To support the *in vivo* strains safety, a 14-day repeated oral dose study in normal laboratory rats was conducted. Oral administration of bifidobacteria resulted in no changes in general condition and no clinically significant changes of biochemical and haematological markers of safety, relative to control-treated animals, suggesting biosafety of the tested strains.

Conclusion

Complex *in vitro* testing of two *Bifidobacterium animalis* subsp. *lactis* strains isolated from feces of healthy adults revealed that the analyzed strains possess a number of useful characteristics and may be regarded as prospective probiotic cultures.

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Conflict of Interest Statement

There are no conflicts of interest with respect to any of the authors.

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