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FERMENTATIVE CAPACITY OF *LACTOBACILLUS* STRAINS CULTIVATED ON LACTOSE AND ITS DERIVATIVES

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Abstract

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The aim of this study was to determine the capacity of two strains *Lactobacillus* to utilize lactose and its unabsorbable derivatives with prebiotic potential – lactulose and lactitol. The strains, named *L. fermentumO* and *L. sakeiTA61*, were cultivated in different modified media, containing these saccharides. Effect on the growth of lactobacilli was studied, as well as the production of α- and β-galactosidases. Antibacterial activity was tested by the agar diffusion method. It was determined that the studied strains saved and showed very similar antibacterial activity, as grown on glucose against the test food patogens. The origin of carbon sources influenced activity of the studied enzymes, its maximum was found in the early log growth phase.

Key words: lactose derivatives, prebiotic potential, *Lactobacillus,* fermentation

Introduction

Lactose derivatives are obtained from lactose by chemical, enzymatic or microbial modifications. Lactobionic acid, lactitol, lactulose, and lactosucrose are commercially produced for a variety of food and pharmaceutical applications (Kontula, 1999). Also, a large variety of oligosaccharides consisting β-linked moieties with carbohydrates other than glucose or lactose at the reducing end are produced by β-galactosidases from lactose when the appropriate acceptor carbohydrate is present.

Utilization of these saccharides is connected with their hydrolyses. The main enzymes involved in this process are α-galactosidases and β-galactosidases. Lactulose, lactitol and other derivatives are compounds that can be produced from lactose (or whey) and which, unlike lactose, are not absorbed in the small intestine of lactose-absorbing subjects. They reach the proximal colon unaltered, where they are selectively metabolized by bifidobacteria and lactobacilli, giving rise to the formation of carbon dioxide, hydrogen gas, and short-chain fatty acids (SCFA), causing an increase in fecal biomass and a decrease in pH (Olano and Corzo, 2009). Thus, all these compounds have potential to function as prebiotics, substrates that promote the growth of beneficial microbes in the large intestine (Saarela, 2002).

We aimed to obtain information about the structure–function relationship of lactulose and lactitol, and the influence of linkages that may have on the fermentability of these substrates.

Materials and Methods

Microorganisms, cultures and conditions

The growth characteristics of two Lactobacillus strains isolated from different fermented products, allifiated to *L. fermentum* and *L. sakei*, were studied for their potential to use lactose and its derivatives as a carbon sources. The strains were called *L. fermentumO* and *L. sakeiTA61.*

To study the ability of these strains to utilise lactose derivatives carbohydrate-free MRS broth was used as a basal growth medium. Stock solutions (**20**%) of glucose, lactulose, lactitol and lactose (all from Merck, Germany) were

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prepared in ion-exchanged water and filter-sterilised. Sterile substrate solutions were added into basal MRS-medium to obtain final carbohydrate concentrations of **2**%. The pH of the supplemented MRS was checked and adjusted if necessary. Their growth kinetics were evaluated at 600 nm during 48 hours.

Antimicrobial assay

The effect of lactose and its derivatives on the antimicrobial activity was tested using the following indicator strains: *Salmonella cholerae* 3591/31.10, *Enterobacter aerogenes* 3690/31.10, *Staphylococcus aureus* 746/31.10, *E. coli* 3398/31.10, *Listeria monocytogenes* 863/31. 10 (all from the indicator strain collection of "LB Bulgaricum" PLC). Wells (8 mm) were made in the agar plates and CFS (100 µl) was placed in them and allowed to diffuse through the agar for 20-40 min at room temperature prior to incubation overnight (Schillinger and Lucke, 1989 with slight modification). After which inhibitory zones were measured, at least 2 mm in diameter were recorded as positive. All experiments were prepared in triple.

Genetic analysis

Genetic analysis were provided, using Sigma's Gen Elute™ Bacterial Genomic DNA NA 2110 kit; PCR protocol and electrophoretic analysis, to show the presence of α-galactosidase (primers: LCB22ACI_2F and LCB22ACI_2R, fragment size 339bp) and β-galactosidase (primers: LCB23DEL_1F and LCB23DEL_1R, fragment size 559bp) gene' fragments. For the PCR-reaction volume was 50 µl (Taq master mix – 25 µl, Primer F-2µl, Primer R – 2 µl, PCR water-19 µl, DNA-2 µl) for the programme with 35 cycles.

Enzymatic assay

β-galactosidase activity was determined during 48 hours, using o-nitrophenyl-β-d-galactopyranoside (oNPG) as the substrate and α-galactosidase activity, using p-nitrophenyl-α-d-galactopyranoside (pNPG) (Sigma's protocols). Activity of β-gal was determined by the amount of o-nitrophenol (oNP) released, as measured by absorbance at 405 nm (method: Spectrophotometric Stop Rate Determination). One unit of oNPG activity was defined as the amount of enzyme releasing one micromole of oNP per minute under the described conditions. The same is for α-galactosidase determination with pNPG.

Results and Discussion

The growth kinetics evaluated at 600 nm during 48 hours show typical growth like when the strains are cultivated on glucose. Maximum growth rates (µmax) and lag phase were calculated (Figure 1).

Both two strains cultivated on MRS-glucose medium have antimicrobial activity against the food patogenes that were used. Grown on lactose, lactulose and lactitol, they save their antimicrobial activity with slight modifications – most significant results are with indicator strain *Enterobacter aerogenes 3690/31.10* (Figure 2).

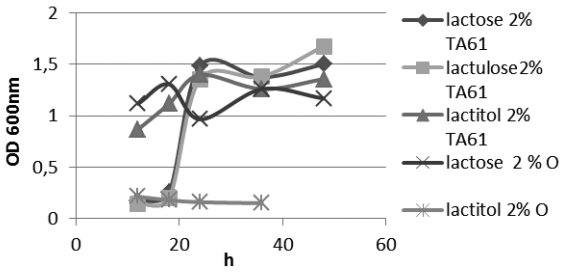

Fig. 1. Growth curves

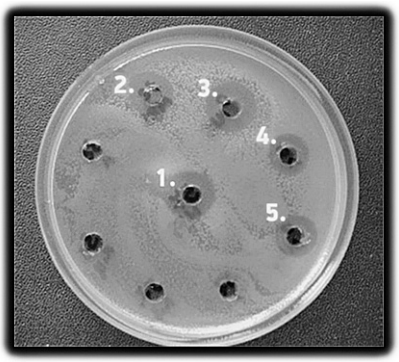

Fig. 2 Antimicrobial activity, overnight cultures – in the center *L. sakeiTA61* grown in lactose(1), in lactulose (3) and lactitol (4) as carbon sources; *L. fermentumO* grown in lactulose (2) and lactitol (5). Indicator strain *Enterobacter aerogenes 3690/31.10* ("LB Bulgaricum" PLC)

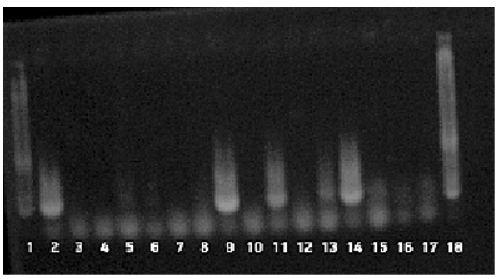

Fig. 3. Electrophoretic analysis of β-galactosidase (fragment size 559bp, from 1-18) On the position 2 and 10, is *L. fermentumO*, cultivated in lactulose and lactitol; on the position 9 and 14 is *L. sakeiTA61* again grown in lactulose and lactitol

Electrophoretic analysis of β-galactosidase and α-galactosidase show the presence of the needed fragments, respectively genes for α- and β-galactosidase, that we are measured (Figures 3 and 4).

The activity of the studied enzymes was evaluated during the growth of the strains. Maximum of activity of both enzymes was found in the early log growth phase. It should be noted that the activity of β-galactosidase was highest when the strains are cultivated on lactose (Figures 5 and 6).

Lactose derivatives can be utilised in varying extent by different Lactobacillus and Bifidobacterium species/ strains (Sahota et al., 1982; Smart et al., 1993; Kneifel et al., 2000). The obtained results have shown the variations in fermentation properties of lactulose and lactitol providing an initial assessment of their prebiotic potential and should be completed by evaluating *in vivo* their fermentation properties before being finally used as a functional ingredient for improving the composition of gut microflora.

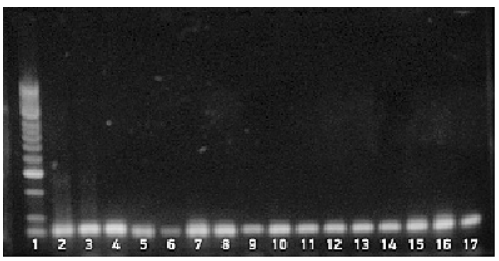

Fig. 4. α-galactosidase (fragment size 339bp, from 1-17). On the position 2 and 3 is *L. fermentumO*, cultivated in lactulose and lactitol; on the position 7 and 10 is *L. sakeiTA61* grown in lactulose and lactitol

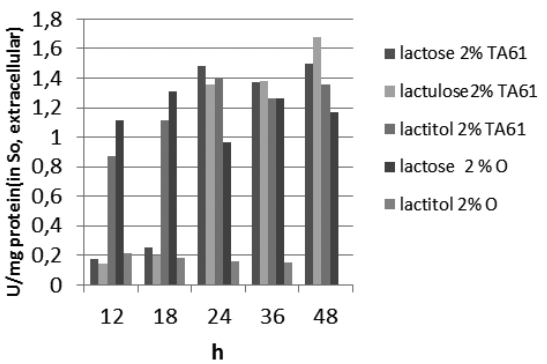

Fig. 5. β-galactosidase activity

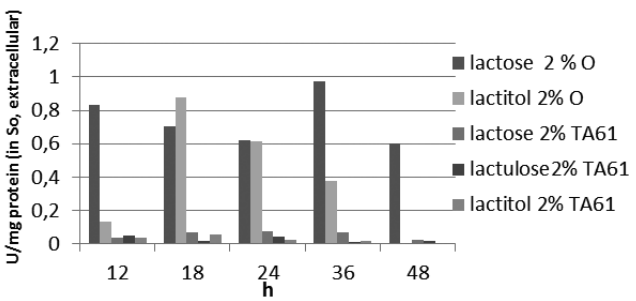

Fig. 6. α-galactosidase activity

Conclusion

Present results furthermore indicate that finding synbiotic (combination of prebiotic and probiotic) pairs where the prebiotic would benefit the specific probiotic strain, e.g. during production and formulation into foods, is not a simple task. However, although no *in vitro* benefit is seen, *in vivo* the synbiotic pair may act differently – for example, we have found in another study (Kontula et al., 2002), that *in vivo* lactulose prolonged the persistence of a strain *L. rhamnosus* in the human GI-tract after discontinuation of the probiotic feeding.

Acknowledgements

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