



Original Research Article

Study on the ability of some *Lactobacillus* strains to assimilate oligosaccharides

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A B S T R A C T

Six *Lactobacillus* strains were isolated from two types of Balkan homemade yogurt and cheese. They were tested for their ability to utilize and grow two different oligosaccharides – fructooligosaccharides – FOS and glucooligosaccharides – GOS. The results clearly demonstrated that the growth rates on different oligosaccharides- OS showed very different preferences. The results from the tested antimicrobial activity demonstrated that these sugars induce it and it is strain specific. The aims of this work were to determine ability to utilization and growth on two different OS – FOS and GOS for strains *Lactobacillus* sp. isolated from traditional Bulgarian milk products. A total six *Lactobacillus* sp. was isolated from two types sheep and cow Balkan homemade yogurts and cheeses. The strains were originated from the northeast part of Bulgaria, in a village near to Shumen. The strains were cultured overnight (16–18h) on Mann Rogosa Sharpe (MRS) broth at 37°C and in limitation of oxygen. Initial identification of all the strains was performed by API 59 CHL system (BioMerieux, Craaponne, France), according to the manufacturer's instruction. The fermentation profiles were read after incubation at 37°C in anaerobic condition, for 3 days. Antimicrobial test: *Escherichia coli* HB 101, *Bacillus cereus* ATCC 6633 and *Staphylococcus aureus* ATCC 39592 were treated for 48 hours with neutralized supernatants obtained after 24h preculture in mMRS-FOS and mMRS-GOS. The antibacterial activity was assayed by the well diffusion method. Microbial growth: Bacterial growth was measured by a turbidimetric method at 600 nm and calibrated against cell dry weight using a spectrophotometer. This study has demonstrated that 6 lactic acid strains identified *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus helveticus* and *Lactobacillus casei* isolated from artisanal dairy products can utilize two different oligosaccharides –FOS and GOS to support their growth *in vitro*. Now it is well known that while most bifidobacteria strains can readily use oligosaccharides, only a few strains from other genera, including lactobacilli, possess this ability. The antimicrobial activity determined after cultivation on oligosaccharides also indicate that the system of uptake of unusual sugars influence in a specific way the production of antimicrobial substances. One of the interesting observation from this study is that when cultivated on different oligosaccharides as FOS and GOS strains showed very different preferences. Their specific growth rates differs significantly. LAB accumulate sugars by secondary active transport (mainly by PMF), the PTS, or an ATP-mediated systems. A binding-protein dependent multiple sugar metabolism (MSM) transport system was described that transported the trisaccharides, raffinose and isomaltotriose and disaccharide melibiose. The MSM genes coding for this system are organized as a cluster typical of ABC operons in that it contains genes coding for ATP- and substrate-binding proteins, and two membrane-spanning domains. The genetic bases for how LAB metabolizes oligosaccharides has not yet been established, biochemical and physiological data support the presence of and inducible and specific ATP-dependent transport systems. However, more studies should be conducted to elucidate the pathways of utilization of oligosaccharides in these lactobacillus strains

Keywords

Antimicrobial activity,
Growth rate,
Glucooligosaccharides,
Fructooligosaccharides,
Lactobacillus sp.

Introduction

The ability of specific dietary substances to influence the gastrointestinal microflora by increasing the population of beneficial bacteria has attracted considerable research attention. These so-called prebiotics substances are defined: “A prebiotics is a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health” (Gibson *et al.*, 2004; Roberfroid, 2007).

Many nondigestible oligosaccharides (OS), such as FOS, GOS and galactooligosaccharides, have been reported to beneficially affect human health. Importantly, among the bacteria that metabolize prebiotic OS are strains of *Lactobacillus* spp. (Ignatova-Ivanova *et al.*, 2009) and *Bifidobacteria* spp., organisms that are generally considered to be desirable members of the colonic microbiota (Kaplan and Hutkins, 2000).

The aims of this work were to determine ability to utilization and growth on two different OS – FOS and GOS for strains *Lactobacillus* sp. Isolated from traditional bulgarian milk products.

Materials and Methods

Strain: A total six *Lactobacillus* sp. was isolated from two types sheep and cow Balcan homemade yogurts and cheeses. The strains with prefics “K” and “O” correspond to strain isolated from cow and sheep yogurts and cheeses respectively. The strains were originated from the northeast part of Bulgaria, in a village near to Shumen. The strains were cultured overnight (16–18h) on Mann Rogosa Sharpe (MRS) broth at 37°C and in limitation of oxygen.

Media: The strain cultivated in media of MRS (de Mann Rogosa Sharpe) in composition, per liter: glucosa – 20; Tween 80 – 1; pepton from casein – 10,0; meat extract – 8,0; yeast extract – 4,0; K₂HPO₄ – 2,0; sodium acetat – 5,0; amonium citrate – 2,0; MgSO₄.7H₂O – 0,2 and MnSO₄ – 0,05 g/l. The pH of media was adjusted to 6,5 with 1N NaOH. The basic media was sterilized by autoclaving at 121°C for 20 min, and carbohydrates supplemented were sterilized using 0.22 µm filters (Manisart®). An mMRS agar medium with different OS was prepared by adding 2% (w/v) FOS and GOS to MRS agar.

API 50CHL system assay: Initial identification of all the strains was performed by API 59 CHL system (BioMerieux, Craaponne, France), according to the manufacturer`s instruction. The fermentation profiles were read after incubation at 37°C in anaerobic condition, for 3 days.

Antimicrobial activity: Antimicrobial assay was performed as previously described (Bertrand-herb *et. al.*, 2003) by the well diffusion method by using soft 0.8% agar. After adjusting the pH at 6.5 by NaOH, the activity of the collected samples (60µL) was checked against *Escherichia coli* HB 101 on Luria-Bertain agar media, *Bacillus cereus* ATCC 6633 on meat extract agar media and *Staphylococcus aureus* ATCC 39592 on meat extract agar media. The plates were incubated overnight at 37°C. Antimicrobial activity of 24h hydrolyzed samples was checked on the strains cultivated on media containing 2% glucose. The neutralized supernatants (pH 6.5) obtained after 24h preculture in mMRS-FOS and mMRS-GOS and 24h culture were also checked for their activity against *E. coli*, *B. cereus* and *S. aureus*. All experiments were performed in triplicate.

Microbial growth: Bacterial growth was measured by a turbidimetric method at 600 nm and calibrated against cell dry weight using a spectrophotometer (UV/vis Shimadzu, Kyoto, Japan). For each experiment, data were analyzed using the Excel statistical package. The OD reading and standard deviations were calculated from duplicate samples from three separate experiments. Growth of each strain was monitored by measuring the OD of the cultures at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30 and 48h at 600nm.

Result and Discussion

API Tests

API tests showed that strains isolated from cow cheeses were identified as *Lactobacillus helveticus-K* and strains isolated from sheep cheeses were *Lactobacillus casei-O*. From cow yogurt were identified as *Lactobacillus delbrueckii-K5* and *Lactobacillus plantarum-K1*. From sheep yogurt were identified *Lactobacillus delbrueckii-O42* are *Lactobacillus plantarum-O1*.

Microbial growth

The specific growth rate of 6 bacterial *Lactobacillus* strains in modified mMRS broth supplemented with different carbohydrate sources is showed in Table 1.

Growth was evaluated in terms of maximum optical density of 600 nm and specific growth rate archived during a fermentation period of 24 hours. For control was used the growth kinetics on glucose. All studied *Lactobacillus* strains fermented FOS and GOS in different manner. Only 2 strains *L. delbrueckii-O43* and *L. casei-O* were able to grow on a medium containing GOS. FOS was fermented only 3 strains *L. delbrueckii-K2*, *L. plantarum-K1*, and *L. casei-O*.

Antimicrobial activity

The supernatant received after fermentation on MRS-GOS and MRS-FOS were tested for antimicrobial activity after pH adjustment to 6,5. The results are presented on Fig. 1 and Fig. 2. It could be concluded that the zone of inhibition of *E. coli* were considerably bigger for strains *L. plantarum-K1*, *L. plantarum-O* and *L. casei-O* cultivated in the presence on FOS. The other strains *L. delbrueckii-O42*, *L. plantarum-K1* and *L. casei-O* cultivated in the presence on GOS. The two strains were active against *B. cereus* – *L. delbrueckii-K5* and *L. casei-O*, cultivated in the presence on FOS. Two strains cultivated in the present GOS have very bigger zone of inhibition - *L. delbrueckii-O43* and *L. casei-O*. Four strains could be were considerably bigger zone of inhibition of *St. aureus* – strains *L. delbrueckii-K2*, *L. plantarum-O* and *L. casei-O* cultivated in the presence on FOS, and strain *L. delbrueckii O43* cultivated in the presence on GOS.

From these results it is clear that the different energy source induced the production of antimicrobials substances. This study has demonstrated that 6 lactic acid strains identified *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus helveticus* and *Lactobacillus casei* isolated from artisanal dairy products can utilized 2 different oligosaccharides – FOS and GOS to support their growth *in vitro*. Now it is well known that while most bifidobacteria strains can readily use oligosaccharides, only a few strains from other genera, including lactobacilli, possess this ability. One of the interesting observation from this study is that when cultivated on different oligosaccharides as FOS and GOS strains showed very different preferences. Their specific growth rates differs significantly.

Table.1 Oligosaccharide utilization by *Lactobacillus* strains

Growth rate (μ^{-1})/strains	<i>L. delbrueckii</i> O43	<i>L. delbrueckii</i> K2	<i>L. plantarum</i> K1	<i>L. plantarum</i> O1	<i>L. helveticus</i> K	<i>L. casei</i> O
μ on MRS-glucose	0,26±0,012	0,30±0,005	0,32±0,008	0,37±0,014	0,25±0,012	0,28±0,017
μ on MRS-FOS	0,10±0,012	0,20±0,009	0,10±0,017	0,29±0,008	0,09±0,005	0,29±0,005
μ on MRS-GOS	0,27±0,005	0,11±0,012	0,10±0,014	0,10±0,005	0,13±0,008	0,25±0,017

Results were obtained \pm SEM ofrom three sepearte trails
Determined within the time interval from 0 to 24h

Figure.1 Inhibitory activity of strains *Lactobacillus* cultivated in mMRS – FOS:
1 - strain *L. delbrueckii*-O, 2 - strain *L. delbrueckii*-K2; 3 - strain *L. plantarum*-K1; 4 - strain *L. plantarum*-O; 5 - strain *L. helveticus*-K; 6 - strain *L. casei*-O.

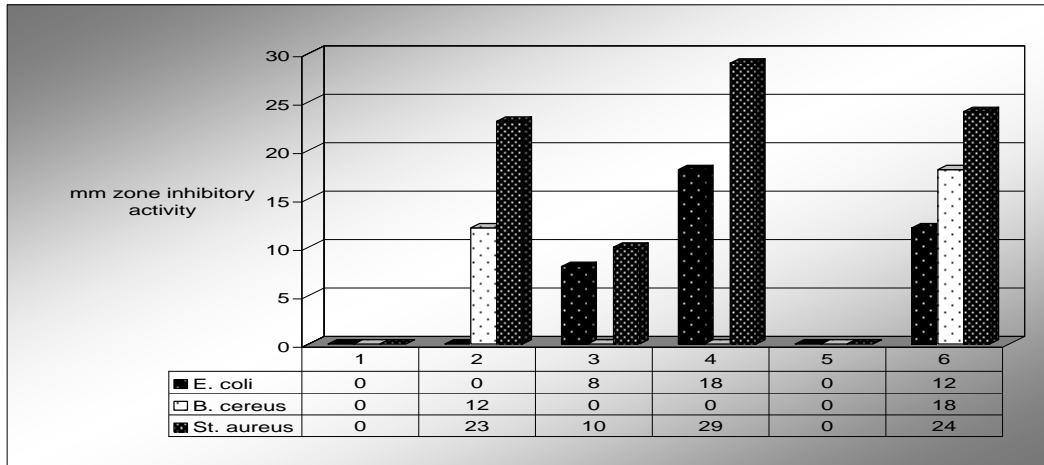
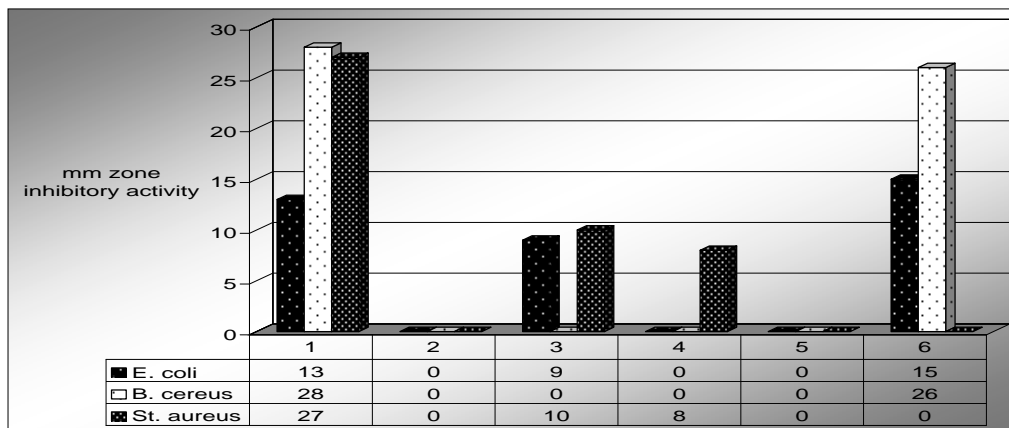


Figura.2 Inhibitory activity of strains *Lactobacillus* cultivated in mMRS – GOS:
1- strain *L. delbrueckii*-O43; 2 - strain *L. delbrueckii*-K2; 3 - strain *L. plantarum*-K1; 4 - strain *L. plantarum*-O; 5 - strain *L. helveticus*-K; 6 - strain *L. casei*-O



The most interesting capacity showed strains *Lactobacillus plantarum-O* and *Lactobacillus casei-O* isolated from sheep yogurt and chesse. The antimicrobial activity determined after cultivation on oligosaccharides also indicate that the system of uptake of unusual sugars influence in a specific way the production of antimicrobial substances. Some similar data were received in the study of (Leroy *et al.*, 2006).

LAB accumulate sugars by secondary active transport (mainly by PMF), the PTS, or an ATP-mediated systems. A binding-protein dependent multiple sugar metabolism (MSM) transport system was described that transported the trisaccharides, raffinose and isomaltotriose and disaccharide melibiose. The MSM genes coding for this system are organized as a cluster typical of ABC operons in that it contains genes coding for ATP- and substrate-binding proteins, and two membrane-spanning domains. The genetic bases for how LAB metabolizes oligosaccharides has not yet been established, biochemical and physiological data support the presence of and inducible and specific ATP-dependent transport systems (Kaplan and Hutkins, 2003).

It's well known that peptide bacteriocins are exported across the cytoplasmic membrane by a dedicated ABC-binding cassette/ABC transporter. Some speculation on the dual action of ABC system could be made in the case of FOS transport and bacteriocin production in the studied strains. However, more studies should be conducted to elucidate the pathways of utilization of oligosaccharides in these *Lactobacillus* strains.

Conflict of Interest

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