RESEARCH ARTICLE

Utilization of xylooligosaccharides from different *Lactobacillus* strains

Maria Ananieva [1]
Tanya Mandadzhieva [1]
Irina Koliandova [1]
Sasho Stoyanovski [2]
Ilia Iliev [3]
Iskra Ivanova [1]

Authors' addresses:
[1] Department of General and Applied Microbiology, Faculty of Biology, Sofia University, Sofia, Bulgaria.
[2] Bitola Veterinary School, Bitola, Macedonia.
[3] Department of Biochemistry and Microbiology, Plovdiv University, Plovdiv, Bulgaria.

Correspondence:
Iskra Ivanova
Department of General and Applied Microbiology, Faculty of Biology, Sofia University,
8 "Dragan Tzankov" Blvd.,
1164 Sofia, Bulgaria,
Tel.: +359 2 816 7341
e-mail: vitanova@biofac.uni-sofia.bg

ABSTRACT

Xylooligosaccharides (XOS) have prebiotic potential when consumed as a part of diet and can be incorporated into many food products. They affect the host by selectively stimulating the growth of limited number of bacteria such as *Bifidobacteria and Lactobacillus* and hence improve the health. Thirty strains of lactic acid bacteria (LAB) were isolated from Bulgarian dry sausages – Lukanka. They were identified through standard techniques and sequenced for 16S ribosomal genes and were referred to the following types *Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus sakei.* To investigate the utilization of xylo-oligosaccharides, modified media containing 2% xylo-oligosaccharides was used. The microbial growth on xylooligosaccharides was compared with such on glucose .The obtained results revealed that some of the identified strains can utilize XOS and glucose with similar efficiency.

Key words: Lactic acid bacteria (LAB), xylooligosaccharides, utilization

Introduction

Xylo-oligosaccharides (XOS) have great prebiotic potential and can be incorporated into many food products. XOS seem to exert their nutritional benefits in various animal species, which by definition have an intestinal tract populated by a complex, bacterial intestinal ecosystem. Xylo-oligosaccharides (XOSs) have emerged in recent years as valuable prebiotics (health-promoting non-digestible food ingredients) because of their beneficial effects on *Bifidobacterium* and *Lactobacillus* (Reddy & Krishnan, 2010).

Main source of energy for Lactobacilli are different carbohydrates and especially glucose. During fermentation of glucose they produce lactic acid and some antibacterial substances. Several studies have shown the ability of Lactobacilli to ferment prebiotic carbohydrates, especially xylooligosaccharides. (Aachary & Prapulla, 2011) Utilization of XOS by Lactobacilli is not studied in details. Nevertheless xylo-oligosaccharides (XOS) have also been referred as emerging prebiotics that may present the same or more desirable properties than the established prebiotics.

Materials and Methods

Bacterial strains and culture conditions

In this study we use thirty strains from Lactic acid bacteria isolated from Bulgarian dry sausages – Lukanka. These strains were identified using standard techniques. All strains were cultivated on MRS medium overnight (16-18 h) on 37°C.

Detection techniques

All isolated strains were identified to belong to genera *Lactobacillus*. Initial identification of all the strains was performed by API 50CHL system (BioMerieux, France), according to the manufacturer's instructions

To identify all strains we use standard PCR techniques, using strain specific primers. After identification via PCR, all strains were sequenced for 16S ribosomal genes.

RESEARCH ARTICLE

The strains were referred to the following species: *Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus sakei.*

Carbohydrate using in this study

To compare utilization of XOS and glucose by Lactobacilli we used mix of XOS and MRS (Merck Germany).

Fermentation and dynamic of bacterial growth

Overnight growth cells were washed twice with 0.85% NaCl solution. 200µl from bacterial suspension was used to inoculate modified MRS broth medium (pH 6.8), containing 2% XOS.

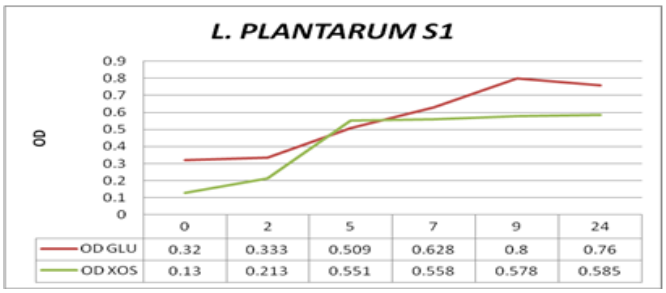
As control was the same amount of 200 µl from bacterial suspension was used to inoculate MRS broth medium. All stains were grown on media containing XOS at 37°C for 48 h. The same conditions were used for strains cultured on glucose.

Bacterial growth was measured by turbidimetric method at 600 nm and calibrated against MRS broth using spectrophotometer (Uv/Vis Shimadzu, Japan). The OD readings and standard deviations were calculated from duplicate samples from two separate experiments. (Ignatova et al., 2009)

Results Discussion

The growth kinetics of *Lactobacillus plantarum* (2 strains), *Lactobacillus brevis* (3 strains) and *Lactobacillus sakei* (4 strains) was evaluated in the terms of OD 600 nm during 24 h of fermentation. (Mandadzhieva et al., 2009). On Figure 1, Figure 2 and Figure 3 is shown utilization of XOS and compared with utilization of glucose. The presented results reviled that the utilization of XOS is strain specific and the examined strains utilize XOS and glucose in the same manner. The examined 4 strains *Lactobacillus sakei* cannot utilize XOS. The inability of utilization of XOS by *Lactobacillus sakei* could be due to its homofermentative pattern of fermentation.

From the presented results on Table 1 is clear that the 2 strains *L. plantarum* had similar specific growth rates on glucose and XOS. In the case of *L. brevis* strain S8 and S39 had relatively low growth rate on XOS.



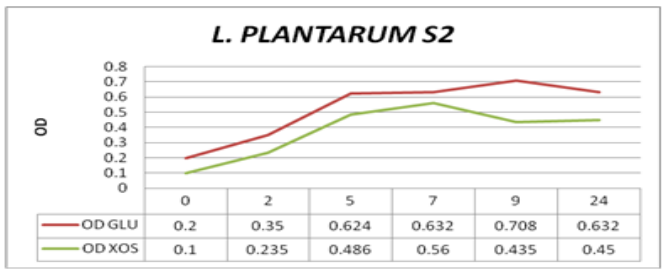
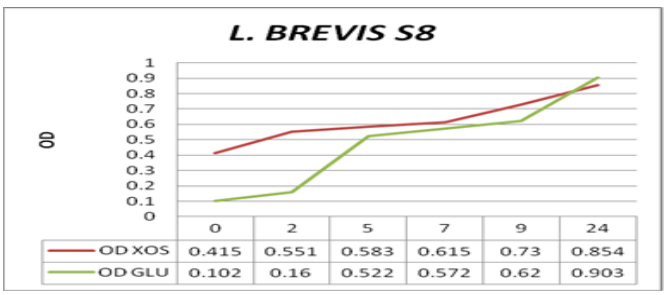
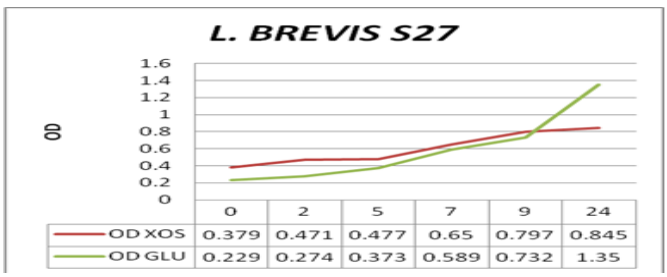


Figure 1. *Growth on XOS and glucose by Lactobacillus plantarum S1 and Lactobacillus plantarum S2*





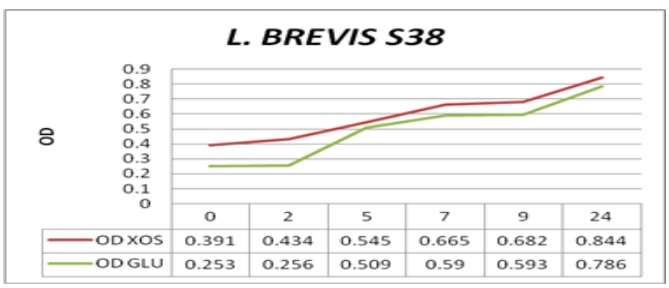


Figure 2. *Growth on XOS and glucose by Lactobacillus brevis S8, Lactobacillus brevis S27, Lactobacillus brevis S38*

RESEARCH ARTICLE

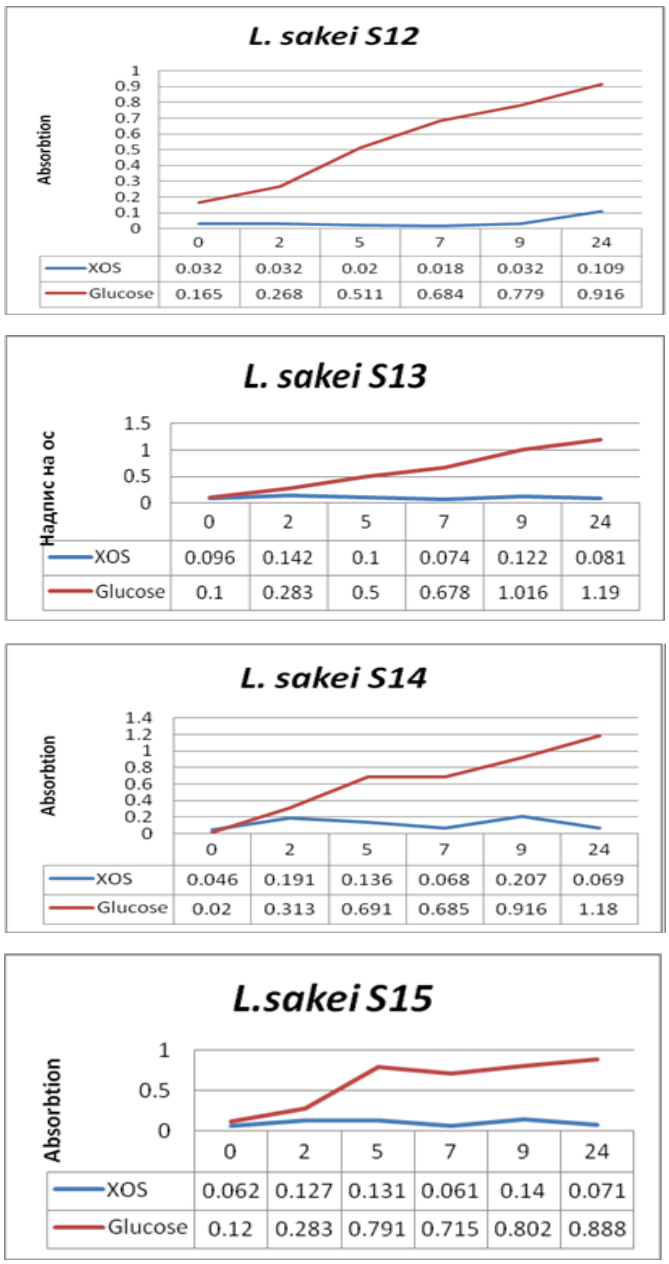


Figure 3. *Growth on XOS and Glucose by Lactobacillus sakei S12, Lactobacillus sakei S13, Lactobacillus sakei S14 and Lactobacillus sakei S15*

As it is seen from the results on Table 2, *L. plantarum* S1 and S2 and *L. brevis* S8, S27 and S38 did not show any activity against *E. coli* when the studied strains were grown on glucose. It is very interesting to note that all the studied strains when cultivated on XOS had antimicrobal activity. In the case of the other test culture most of the strains showed low antimicrobal activity, grown on glucose. It could be noted that the induction of activity in the presence of XOS was higher especially for *L. plantarum* S2 and *L. brevis* S8 and S38.

Study of the antimicrobial activities of these LAB indicated that the system of uptake of unusual sugars influenced in a specific way the production of antimicrobial substances specific against *E.coli*.

The utilization of XOS is strain specific and the obtained results may help to explain the ability of Lactobacillus strains to compete with other bacteria in the ecosystem of the human gastro-intestinal tract.

Table 1. *Specific growth rate of Lactobacilli*

Strain	Specific growth rate h^{-1}	
	Glucose	+ 2% XOS
L. plantarum S1	0.125	0.316
L. plantarum S2	0.193	0.242
L. brevis S8	0.394	0.022
L. brevis S27	0.168	0.128
L. brevis S38	0.229	0.085

Table 2. *Antimicrobal activity of studied Lactobacilli*

Strain	Antimicrobal activity on 24 h			
	E.coli - glucose	*E.coli - XOS*	*B. subtilis - glucose*	*B. subtilis - XOS*
L. plantarum S1	-	++	-	+
L. plantarum S2	-	++	+	+++
L. brevis S8	-	+	+	+++
L. brevis S27	-	++	+	++
L. brevis S38	-	+	+	+++

Legend: "-" - Not detected; "+" – diameter of halo – 12 mm "++" - diameter of halo – 14 mm; "+++" – diameter oh halo – 15 mm

Acknowledgement

This work was supported by research grant of NSF Bulgaria BG RNF02/2-2009.

References

Aachary AA, Prapulla SG. 2011. Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. Comprehensive Reviews in Food Science and Food Safety, 10: 2011.

RESEARCH ARTICLE

Ignatova T, Iliev I, Kirilov N, Vassileva T, Dalgalarrondo M, Haertlé T, Chobert J-M, Iskra I. 2009. Effect of Oligosaccharides on the Growth of Lactobacillus delbrueckii Subsp. bulgaricus Strains Isolated from Dairy Products. J. Agric. Food Chem., 57(20): 9496-9502.

Mandadzhieva T, Ignatova-Ivanova T, Kambarev S, Iliev I, Ivanova I. 2009. Utilization of different prebiotics by *Lactobacillus spp*. and *Lactococcus spp*. Biotechnol . & Biotechnol. Eq., 25/4, Suppl., 117-120.

Reddy S, Krishnan C. 2010. Production of prebiotics and antioxidants health food supplements from lingo cellulosic materials using multienzymatic hydrolysis. Int. J. Chem. Sci., 8(3): S535-S549.