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INFLUENCE OF LACTOSE CONCENTRATION ON THE α-GALACTOSIDASE AND β-GALACTOSIDASE ACTIVITY OF *LACTOBACILLUS PLANTARUM*

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Abstract

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Twenty seven lactic acid bacteria (LAB) strains identified as *Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus sakei,* isolated from sausages were tested for their ability to utilize and grow on lactose at concentration by 10%, 12.5%, 15% and 5% lactulose. Results clearly demonstrated that the growth-rates on high percent of lactose showed very different preferences. It could be propose that the studied β-galactosidase catalyzes trans-glycosylation reaction at concentration of lactose 12.5% and 15%. The activity of α-galactosidase is 10 times lower than β-galactosidase on modified MRS (mMRS) with 12.5% lactose concentration. On media with 5% lactulose, α-galactosidase activity of the studied strain S26 is 20% lower than measured β-galactosidase activity. It could be concluded that lactulose is better inductor for α-galactosidase than lactose.

Key words: lactose, lactulose, prebiotic potential, *Lactobacillus*, prebiotics, β-galactosidase, α-galactosidase

Introduction

Several strains from genus *Lactobacillus* have been considered as probiotics due to their beneficial effect on the host by improving the intestinal microflora, helping in the immune system maturation, and presenting inhibitory activity toward the growth and adhesion to epithelial cells or intestinal mucus of pathogenic microorganisms (Tannok et al., 2004; Mnnoz et al., 2012).

It has been shown also that different non-digestible di- and oligosaccharides as fructo-oligosaccharides (FOS), galacto–oligosaccharides (GalOS), xylo-oligosaccharides (XOS), lactulose, etc. act as prebiotics. GalOS are mixtures consisting of numerous different oligosaccharides varying in their degree of polymerization (DP), structure and glycosidic linkage (Iqbal et al., 2010a).

β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) is an enzyme that catalyzes two basic reactions, hydrolysis of lactose and structurally related galactosides and transglycosylation reactions, resulting for example, in a mixture of galacto-oligosaccharides when lactose is the starting material for the latter reaction (Iqbal et al., 2010b). α-Galactosidase enzymes (α-D-galactosil galactohydrolases, E.C. 3.2.1.22) hydrolise the α-1,6 linkages that join the residue of galactose to the glucose present in raffinose, producing free galactose and sucrose. Possible sources of these two enzymes are plant, bacteria, fungi, animal organisms and moulds. Lactic acid bacteria have been studied intensively with respect to their enzymes for various different reasons including their GRAS status. *Lactobacillus plantarum* is a versatile lactic acid bacterium, which is encountered in a range of environmental niches including dairy, meat, and vegetable fermented foods (Saarela et al., 2003).

The aim of the present work was to study the activity α-galactosidase and β-galactosidase from different *Lactobacillus species,* cultivated in medium with more than 10% lactose and 5% lactulose as sole carbon substrate.

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Materials and Methods

Bacterial strains and culture conditions

A total of 27 *Lactobacillus* strains (22 of *L. plantarum*; 3 of *L. brevis*. and 2 of *L. sakei*) from the collection of Department of Microbiology, Sofia University, Bulgaria were used (Stoyanovski et al., 2009). The strains were cultured overnight (16-18 h) on MRS (de Mann Rogosa Sharpe broth, Merck, Darmstadt, Germany) at 37ºC and in limitation of oxygen (BBL® Gas Pak anaerobic system Envelopes, Becton Dickinson).

Carbohydrates used in this study

Lactose (Merck, Germany) and lactulose (Lactulose-Crystals EP, Viccio, Italy) contained lactulose 97.5%, galactose 0.5%, lactose 0.5%, epilactose 0.5%, tagatose 0.5%, fructose 0.5%. Glucose (purity 99%, Merck, Germany) was used as a control as well as lactose (Merck, Germany). Each carbohydrate was sterilized on 0.2 μm sterile filter (Sartorius), and pH was not adjusted. All examinations were performed at least twice.

Fermentation

Lactobacilli were routinely grown in MRS broth (Merck). Overnight grown cells were washed twice in saline (0.85% NaCl solution) and 10% of the bacterial suspension (107 cfu/mL^{-1}) was used to inoculate mMRS broth medium (pH 6.8) from 5% to 15% lactose and from 2% to 5% lactulose. The anaerobic fermentations were performed for lactobacilli in 50 mL PS bottles at 37ºC for 48 h (Mandadzhieva et al., 2011).

Analytical assays

Microbial growth

Bacterial growth was measured by a turbidimetric method at 650 nm and calibrated against cell dry-weight using a spectrophotometer (UV/Vis Beckman Coulter DU 800, USA). For each experiment, data was analyzed using Excel statistical package. The optical density (OD) readings and standard deviations were calculated from duplicate samples from two separate experiments.

The β-galactosidase activity assays were carried out using ONPG(ortho-Nitrophenyl-β-galactopyranoside)with substrate prepared in citrate-phosphate buffer solution. One β-galactosidase unit (U) was defined as the amount of enzyme which liberated 1 μmol of ONP(ortho-Nitrophenyl) per min per mg of protein at 37°C and pH 6.0 (Kneifel et al., 2000).

The α-galactosidase activity assays were carried out using PNPG(***p*-Nitrophenyl α-D-galactopyranoside**) with substrate prepared in citrate–phosphate buffer solution. One α-galactosidase unit (U) was defined as the amount of enzyme which liberated 1 μmol of ONP per min per mg of protein at 37°C and pH 6.5 (Kontula et al., 2002).

Proteins were assayed by the method of Bradford (1976) by using bovine serum albumin as standard (Olano et al., 2009).

Results

Several (27) *Lactobacillus* strains were screened for their capacity to use lactose in concentration 10% (data not shown). Only 8 strains were able to growth in a medium containing 10% lactose. The biomass formation, pH and enzyme activity were measured and compared during the cultivation. During the cultivation pH gradually decreased due to production of lactic and acetic acid, and finally the cultivation was stopped at pH 3.2 on medium with 10%, 12.5%, 15% lactose and 5% lactulose. The highest growth rate was reached to 15.4 g/l at the end of cultivation on medium with 12.5% lactose and 5% lactulose. Studying the fermentation profiles of the studied strains it could be concluded that activity of α-galactosidase and β-galactosidase was not strongly associated with cell growth (Table 1).

Table 1

Utilization of 10% lactose and 5% lactulose from Lactobacillus strains

Strain	mMRS 10% lactose			mMRS 5% lactulose			mMRS 10% lactose			mMRS 5% lactulose		
	Biomass,g/L			Biomass, g/L			pH			pH		
	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
Lactobacillus brevis – S8	1.1	1.0	1.6	0.8	0.8	1.4	5.67	5.80	4.98	5.89	5.89	5.89
Lactobacillus sake – S12	2.6	14.5	14.1	2.0	12.9	9.9	5.74	3.66	3.42	5.76	3.34	3.34
Lactobacillus plantarum – S25	1.0	1.1	2.0	0.3	12.4	12.3	5.75	5.39	5.05	6.43	3.41	3.23
Lactobacillus plantarum – S26	1.6	10.9	14.9	1.4	12.1	13.1	5.71	3.64	3.39	5.75	3.31	3.21
Lactobacillus brevis – S27	1.2	12.0	15.3	1.2	12.4	13.0	6.44	3.73	3.34	6.19	3.47	3.28
Lactobacillus plantarum – S29	0.5	5.9	13.9	0.2	6.6	11.9	6.31	4.83	3.48	6.49	4.19	3.24
Lactobacillus plantarum – S30	2.1	13.7	14.2	1.8	13.2	12.4	5.63	3.60	3.44	5.40	3.35	3.25
Lactobacillus plantarum – S34	1.1	1.6	11.1	1.8	12.3	12.4	6.39	6.04	3.69	5.85	3.40	3.17

Table 2

Growth of *Lactobacillus plantarum* S26 in mMRS medium with 10%, 12.5%, 15% lactose and 5% lactulose as sole carbon source

Lactobacillus plantarum 26	†	Biomass, g/L	pH
5% lactose	0	0	6.50
	6	2.72	4.74
	18	11.8	3.78
	24	13.7	3.56
	48	13.7	3.56
10% lactose	0	0.0	6.50
	6	1.5	5.42
	18	10.6	3.46
	24	11.8	3.36
	48	12.9	3.21
12.5% lactose	0	0.0	6.50
	6	1.8	4.85
	18	12.8	3.47
	24	15.3	3.39
	48	15.3	3.23
15% lactose	0	0.0	6.50
	6	1.7	5.14
	18	12.4	3.50
	24	13.9	3.40
	48	14.8	3.24
5% lactulose	0	0.0	6.50
	6	1.2	5.68
	18	13.8	3.62
	24	15.4	3.34
	48	15.9	3.22

L. plantarum S26 and S30 showed the highest α-galactosidase and β-galactosidase activities. The growth characteristics and enzyme activity of the strain *L. plantarum* S26 were studied for its potential to utilize of 10%, 12.5%, 15% lactose and 5% lactulose as a carbon sources (Table 2, Figures 1 and 2). Their growth kinetics evaluated at 600 nm during 48 hours show typical growth like when the strains are cultivated on different concentration of lactose.

The activity of the studied enzymes was evaluated during the growth of the strains. The maximum of β-galactosidase activity was found in the early log growth phase, in contrast the maximum of α-galactosidase activity was measured at 18 h of cultivation. It should be noted that the activity of β-galactosidase was highest when the strains are cultivated in 12.5% lactose. The maximum of α-galactosidase activity was found during the cultivation in 5% lactulose. The activity of α-galactosidase is 10 times lower than β-galactosidase during the cultivation in medium with lactose concentration 15%.

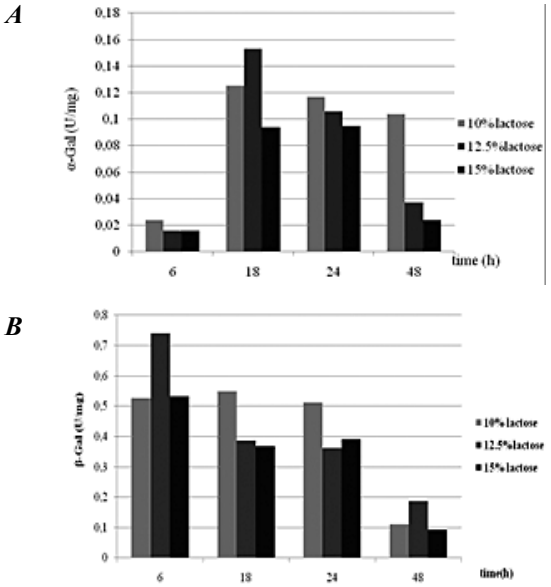

Fig. 1. Comparison of α-galactosidase and β-galactosidase activity by cultivation of *Lactobacillus plantarum* S26 in mMRS with different lactose concentracion
***A* – α-galactosidase activity**
***B* – β-galactosidase activity**

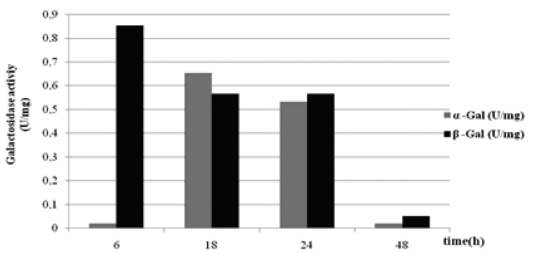

Fig. 2. α-Galactosidase and β-galactosidase activity of *Lactobacillus plantarum* S26, cultivated in medium mMRS 5% lactulose

Discussion

We have shown that 2 LAB strains identified as *Lactobacillus plantarum* S26 and *Lactobacillus plantarum* S30 isolated from home made sausages can be cultivated in media with lactose in concentration more than 10%. It is well known that while most lactobacilli can use 5 % lactose easily, we have shown that only a few strains from *Lactobacillus plantarum*, possess the ability to grow in media with 15% lactose. In this study, it has been demonstrated that in the presence of 12.5% lactose and 5% lactulose, the stud-

ied strain *Lactobacillus plantarum* S26 showed the highest α-galactosidase and β-galactosidase activities. Their specific growth rates differed significantly from one another, with the most important growth rate being obtained in the case of *L. plantarum* S26. Two factors that are especially important in this respect are the rate at which an organism can grow on a particular carbon source, as this will influence its ability to compete with other bacteria in the colon; the other is the extent to which the substrate is converted into bacterial cell mass. In this aspect, our preliminary results on the enzyme activity demonstrated *in vitro* the capacity of *L. plantarum* S26 to metabolize lactose in concentration 10% and 12.5%. Concentration of lactose 15% showed inhibitory effect at α-galactosidase and β-galactosidase activity.

Conclusion

We showed at first that the strain of *L. plantarum* S26 produce α-galactosidase and β-galactosidase during the cultivation in medium with lactose as a sole carbon source in concentration 10%, 12.5% and 15%. When the studied strain is cultivated in mMRS with 15% lactose the activity of the two studied enzymes decrease more than 30%. It could be propose that the studied β-galactosidase catalyzes transglycosylation reaction at concentration of lactose 12.5% and 15%. The activity of α-galactosidase is 10 times lower than β-galactosidase measured in mMRS with 12.5% lactose concentration. On media with 5% lactulose, α-galactosidase activity of the studied strain S26 is 20% lower than measured β-galactosidase activity. It could be concluded that lactulose is better inductor for α-galactosidase than lactose.

Acknowledgements

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