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INFLUENCE OF DIVERSE SUGARS ON BLIS PRODUCTION BY THREE DIFFERENT ENTEROCOCCUS STRAINS

Sv. Dimov, Sl. Peykov, D. Raykova, P. Ivanova

University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics

ABSTRACT

AIM: Non-pathogenic dairy *Enterococci* possessing probiotic properties represent a great potential for food industry as natural replacing agents for chemical food preservatives. The main objective of this research is to investigate the behaviour of three such BLIS producing strains in the presence of additional sugars in concentrations that can be found in many sweet "life" foods such as fruit yoghourts etc. METHODS: Cultivations in the presence of selected carbohydrates (sucrose, maltose, fructose, arabinose and lactose) to concentrations of 0-5 % was performed, and the bacteriocin titers were determined. RESULTS: It was found that some sugars can stimulate growth and bacteriocin synthesis. On one hand, stimulation depends on the medium used, but on the other hand in concentrations greater than 3% an inhibiting effect was observed for both phenomena. CONCLUSIONS: Results of potential practical use in food industry have been observed, allowing one to optimize the types of sugars and their concentrations in food preparation where conservation of the probiotic properties is sought.

Keywords: LAB (lactic acid bacteria) with probiotic properties, bacteriocins

INTRODUCTION

Enterococci are Gram-positive *cocci* which often occur in pairs and chains. Many of them are generally recognized as safe (1-3) non-pathogenic probiotic strains, and synthesize bacteriocins – membrane-depolarizing pore-forming toxins with protein nature that are ribosomally synthesized and excreted extracellularly (4-6).

The presence of various types of sugars in the culture media influences BLIS production (7-9). Oftentimes, in laboratory conditions dairy *Enterococci* are being grown both on M-17 and MRS media. M-17 and MRS contain different sugars as their carbon source (these components often being used for this purpose because of their easy assimilation by plethora of bacterial strains) – lactose and dextrose respectively. The addition of different types of carbohydrates such as sucrose, maltose, glucose, fructose, arabinose and lactose leads to the formation of complex two-component carbon sources (with exception to the case in which additional lactose is added to M-17, and glucose to MRS). This reflects bacterial growth and bacteriocin excretion, and may lead to the observation of the phenomenon of catabolic repression.

Corresponding author: *Svetoslav Dimov, Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria, e-mail: svetoslav@biofac.uni-sofia.bg; Phone: +359 (2) 8167342; fax: +359 (2) 8656641*

MATERIALS AND METHODS

Bacterial strains and media. All bacterial strains and their suppliers are enumerated in Table 1. All *Enterococcus* species were grown on M-17 or MRS broth, either agar or liquid media (Merck KGaA, Scharlau Chemie S.A.). If not mentioned otherwise, the incubation was performed at 30°C for 14-16 hours. All media were prepared as described by the suppliers.

Sugar influence on bacteriocin production. The different sugar influences on the three producer strains were observed after the inoculation of 5 µl of overnight cultures in 5 ml M-17 broth and 5 ml MRS broth with addition of selected carbohydrates (sucrose, maltose, fructose, arabinose and lactose) to concentrations of 0-5 %, with a step of 1%. Cultures were allowed to grow at 30°C in

thermostat. The OD_{600} (optical density at 600 nm wavelength) of all cultures was measured at the 24th hour by taking of aliquots. After each measurement of the OD_{600} 1 ml of a cell free culture supernatant was obtained by filtration and the bacteriocin activity was expressed in AU/ml.

Table 1*. Bacterial strains and suppliers*

Bacterial strain	Supplier
Enterococus durum M-3	1
Enterococcus faecium T81A	2
Enterococcus faecium 3587	1
Enterococcus faecalis 3668	1
Enterococcus faecalis 3915	1

1. National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia, Bulgaria
2. Department of General and Applied Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

Determination of bacteriocin activity. A well diffusion assay procedure was used (10). Pre-cooled at 30°C 0,7% agar media were contaminated with the tested bacterial strains, laid on Petri dishes and allowed to solidify. After 20-30 min the desired number of wells with a diameter of 5 mm was made with a sterile hollow. Aliquots of 100 µl from each bacteriocin probe were placed into the wells of the plates seeded with the bioassay strain. After 12-18 hours of incubation at optimal for the tested strain temperature, clear zones of inhibition appeared where the strain was sensitive.

Determination of bacteriocin titers. The titers of the produced bacteriocin were quantified by two-fold serial dilutions of the supernatants of the probes or the precipitates (11). Aliquots of 100 µl were placed in wells seeded with the bioassay strain. The antimicrobial activity was defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and was expressed in activity units per ml (AU/ml).

RESULTS AND DISCUSSION

Three *Enterococcus* stains showing BLIS activity were investigated. Two of them, *Enterococcus durans* M-3 and *Enterococcus faecium* 3587 are already described (12-13), while the third, *Enterococcus faecalis* 3915, is still under investigation. It was found that bacteriocin synthesis by the three Enterococcus strains is influenced by the presence of different sugars in the culture media. This impact is a subject of definite interest, because sugars are easily utilized, relatively cheap and readily accessible carbon sources with strictly defined contents, oftentimes used as a component of media for microorganism cultivation. In this research we introduce data showing the influence of sucrose, maltose, glucose, fructose, arabinose, etc., some of which stimulate BLIS production, while others suppress it.

Impact of the additional sugars on bacterial growth. The OD_{600} at the 24^{th} hour, which in general corresponds to the beginning of the stationary phase of development at 30 ºC, was used for comparison of the impact of the additional carbon source on the strain expansion. The results are summarised in Table 2. From the data obtained it can be concluded that in most cases the addition of a sugar to the medium at concentrations of 1% does not have tangible effect, or it has only a slight stimulating consequence on bacterial growth. This tendency was more expressed when cultivation was performed on M-17. In contrast, at concentrations of 3-5 % an inhibiting effect on growth was observed, probably due to some extent to the osmotic stress. Nevertheless, it can be concluded that the effect of the sugars added at the concentrations used had only slight effect on

growth.

Impact of the additional sugars on bacteriocin production. Even though the obtained results are somewhat dubious, several tendencies could be followed up.

The histograms in Figure 1 represent the bacteriocin titers of *Enterococcus durans* M-3. For this strain two of the sugars, glucose and sucrose, do not affect bacteriocin production at the concentrations investigated. For the other four carbohydrates, more or less expressed inhibiting effect was observed on both media except for the stimulating effect of the fructose and maltose at 1% when the cultivation was performed on M-17 broth. The impact of the additional sugars was more professed on MRS broth rather than on M-17. A more obvious effect on bacteriocin synthesis was observed for the strain *Enterococcus faecium* 3587 (Figure 2). Very evident stimulation was monitored for lactose at 1% and for maltose at 2-3% when the cultivation was performed on M-17 broth, and for glucose at 1-3% on MRS broth. Again, the additional sucrose did not affect the BLIS titers on neither media. A noticeable inhibition, even at 1% concentration, was discovered for fructose on both media.

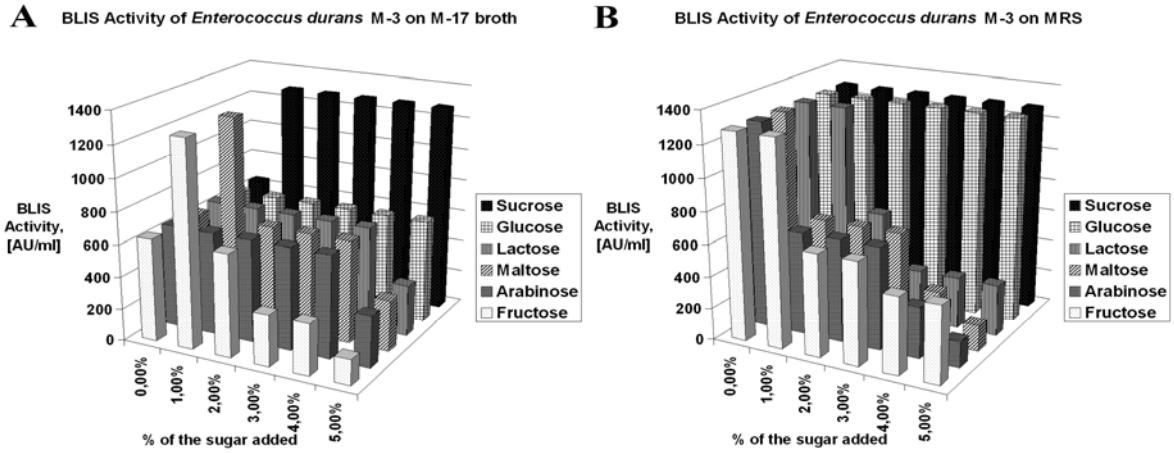

Figure 1. *BLIS activity of Enterococcus durans M-3 in the presence of additional sugars. The strain was grown on M-17 broth (A), and MRS broth (B).*

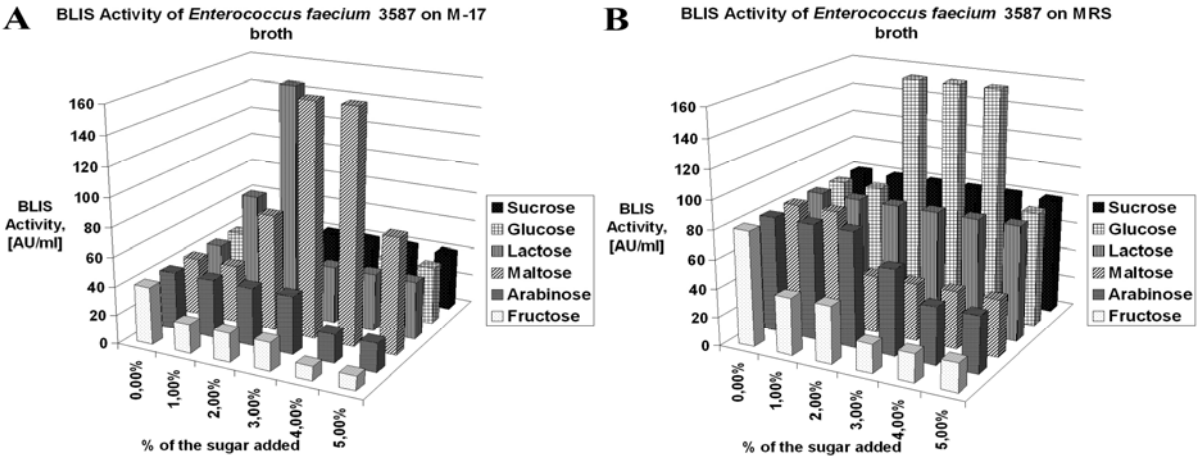

Figure 2. *BLIS activity of Enterococcus faecium 3587 in the presence of additional sugars. The strain was grown on M-17 broth (A), and MRS broth (B).*

The third bacteriocinogenic strain, *Enterococcus faecalis* 3915, appears to have the least influenced by the additional sugars bacteriocin synthesis (Figure 3). For this strain no effects were observed for sucrose, arabinose and fructose on M-17, and for sucrose, glucose, maltose on MRS. A very strong, four-fold stimulating effect was observed for lactose on M-17 at 3%, followed by a clear inhibition at 5 %. Again, the effects of the sugars added were less expressed on MRS.

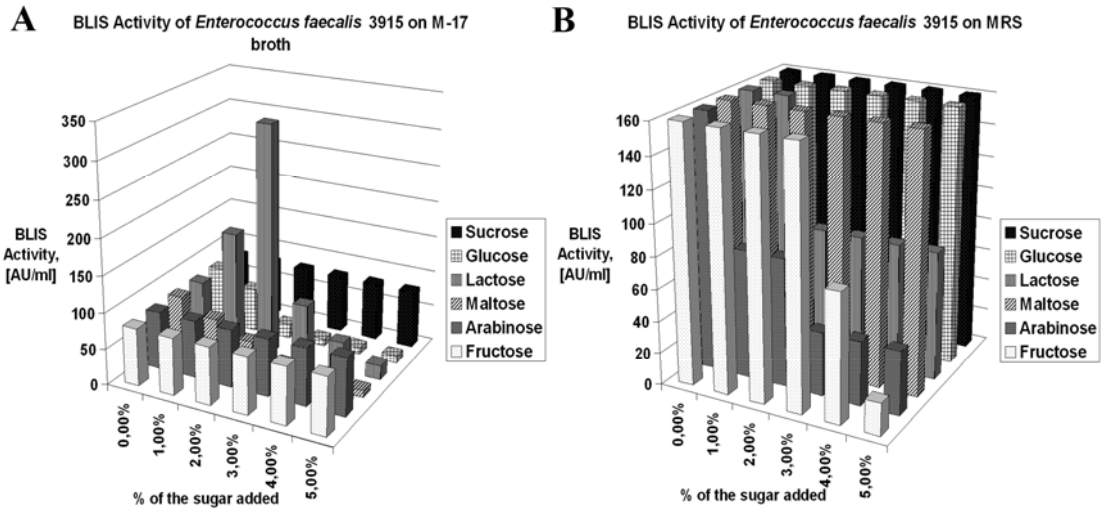

Figure 3. *BLIS activity of Enterococcus faecalis 3915 in the presence of additional sugars. The strain was grown on M-17 broth (A), and MRS broth (B).*

If the results obtained are summarized, it is apparent that some sugars such as sucrose, and a less extent the glucose and lactose, have a weak, or do not have effect on the production of bacteriocins, especially on MRS broth, while others influence their synthesis more apparently. Concerning the broth used, the synthesis was more "stable" on MRS rather M-17 broth.

Sugars added to the culture media have impact both on bacteriocin synthesis and the growth parameters of the three strains. Their concentrations were chosen so that they have minimal influence on growth while at the same time they clearly demonstrate the influence of sugars on BLIS production.

In many cases the addition of sugars led to decrease of BLIS production which can be explained with the favourable conditions for growth, and to some extent with the osmotic stress leading to a decreased growth rate in principle. Generally, the above mentioned phenomenon was more expressed when the cultivation was performed on M-17 broth.

CONCLUSIONS

The experimental results are oracular, yet in most cases sugar addition tends to increase the observed asymptotic value (due to the presence of higher quantities of the carbon source components). BLIS synthesis shows increase under conditions of low sugar concentrations; high concentrations inhibit production of bacteriocins, but not completely (in the concentration interval used in the experiments).

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REFERENCES:

1. Giraffa, G. *Enterococci* from foods. FEMS Microbiol Rev 26(2):163-171, 2002
2. Giraffa, G. Functionality of *enterococci* in dairy products. Int J Food Microbiol 88(2-3):215-222, 2003
3. Foulquié Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E., De Vuyst, L. The role and application of *enterococci* in food and health. Int J Food Microbiol 106(1):1-24, 2006
4. Fimland, G., Johnsen, L., Dalhus, B., Nissen-Meyer, J. Pediocin-like antimicrobial peptides (class IIa bacteriocins) and their immunity proteins: biosynthesis, structure, and mode of action. J Pept Sci 11(11):688-696, 2005
5. Drider, D., Fimland, G., Héchard, Y., McMullen, L.M., Prévost, H. The continuing story of class IIa bacteriocins. Microbiol Mol Biol Rev 70(2):564-582, 2006

6. Oppegård, C., Rogne, P., Emanuelsen, L., Kristiansen, P.E., Fimland, G., Nissen-Meyer, J. The two-peptide class II bacteriocins: structure, production, and mode of action. J Mol Microbiol Biotechnol 13(4):210-219, 2007
7. Guerra, N.P., Pastrana, L. Nisin and pediocin production on mussel-processing waste supplemented with glucose and five nitrogen sources. Lett Appl Microbiol 34(2):114-118, 2002
8. Mataragas, M., Drosinos, E.H., Tsakalidou, E., Metaxopoulos, J. Influence of nutrients on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. Antonie Van Leeuwenhoek 85(3):191-198, 2004
9. Leroy, F., De Winter, T., Adriany, T., Neysens, P., De Vuyst, L. Sugars relevant for sourdough fermentation stimulate growth of and bacteriocin production by *Lactobacillus amylovorus* DCE 471. Int J Food Microbiol 112(2):102-111, 2006
10. Schillinger V, Lucke F.K. Antimicrobial activity of Lactobacillus sake isolated from meat. Appl Environ Microbiol 39:189-195, 1989
11. Graciela M, Vignolo M, de Kairuz N et al. Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *Lactobacillus casei* CRL 705. J Appl Bacteriol 78:5-10, 1995.
12. Peeva, V.M., Ivanova, P.M., Harizanova, N.T., and S.G. Dimov. A preliminary characterization of a novel bacteriocin-like substance from *Enterococcus durum* M-3. Biotechnol Biotechnol Eq 20(3):120-127, 2006.
13. Dimov, S.G. A novel bacteriocin-like substance produced by *Enterococcus faecium* 3587. Curr Microbiol 55(4):323-327, 2007.

Table 2. *OD_{600} at the 24th hour of development of the cultures in the presence of additional sugars added to the medium*

		Sucrose		Glucose		Lactose		Maltose		Arabinose		Fructose	
		M-17	***MRS***	***M-17***	***MRS***	***M-17***	***MRS***	***M-17***	***MRS***	***M-17***	***MRS***	***M-17***	***MRS***
***Enteroccus durans* M-3**	**0,00%**	2,207	2,001	2,231	2,005	2,230	1,999	1,778	2,079	2,140	2,048	2,130	2,061
	1,00%	2,235	1,966	2,400	2,222	2,325	1,945	1,983	1,962	2,058	1,969	2,235	1,984
	2,00%	2,203	1,659	2,365	2,040	2,249	1,833	1,830	1,907	1,999	0,906	2,094	1,910
	3,00%	2,190	1,936	2,365	1,996	2,147	1,796	1,733	1,847	1,900	1,799	1,891	1,799
	4,00%	2,161	1,910	2,330	2,008	2,064	1,732	1,656	1,731	1,735	1,635	1,715	1,700
	5,00%	2,142	1,917	2,125	1,987	1,964	1,364	1,537	1,683	1,540	1,516	1,613	1,583
***Enteroccus faecium* 3587**	**0,00%**	1,427	2,177	1,780	2,185	1,594	2,176	0,997	2,142	1,633	2,129	1,750	2,162
	1,00%	2,227	2,115	2,190	2,197	1,967	2,206	1,687	2,082	1,560	2,043	2,099	2,098
	2,00%	2,221	2,088	2,240	2,166	2,121	2,101	1,733	2,027	1,444	1,971	2,059	2,046
	3,00%	2,190	2,075	2,217	2,140	2,105	1,978	1,695	1,945	1,321	1,878	1,934	1,925
	4,00%	2,175	2,036	2,190	2,127	2,064	1,938	1,585	1,857	1,239	1,717	1,693	1,836
	5,00%	2,153	1,999	2,126	2,118	1,969	1,860	1,471	1,721	1,103	1,460	1,463	1,743
***Enteroccus faecalis* 3915**	**0,00%**	1,483	1,785	1,496	1,900	1,626	1,868	1,089	1,824	1,525	1,705	1,341	1,805
	1,00%	2,275	1,760	2,020	1,880	1,482	1,769	1,721	1,744	1,541	1,661	2,176	1,743
	2,00%	2,255	1,704	2,245	1,845	1,396	1,706	1,596	1,667	1,393	1,560	2,033	1,663
	3,00%	2,230	1,669	2,220	1,832	1,187	1,593	1,505	1,527	1,289	1,374	1,887	1,478
	4,00%	2,217	1,634	2,200	1,808	1,021	1,477	1,365	1,419	1,158	1,220	1,669	1,342
	5,00%	2,190	1,590	1,968	1,776	0,871	1,322	1,223	1,318	1,022	1,035	1,310	1,231