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NaCL SUPPRESSION OF BACTERIOCIN PRODUCTION BY THREE ENTEROCOCCUS STRAINS

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ABSTRACT

AIM: The goal of this study was to investigate the influence of sodium chloride – the most commonly used preservative in many foods – on bacteriocin production by three different probiotic *Enterococcus* strains naturally occurring in the Bulgarian Yellow cheese "Kashkaval". METHODS: Cultivation of the BLIS producer strains were performed in presence of different concentrations of NaCl, and cell number and the BLIS activities were determined. RESULTS: It was found that at concentrations at which the strains continued growing, NaCl strongly suppressed bacteriocin synthesis. CONCLUSIONS: 1) High NaCl concentration significantly reduced the BLIS activity of the three strains investigated, *Enterococcus durans* M-3, *Enterococcus faecium* 3587 and *Enterococcus faecalis* 3915. 2) The NaCl inhibition of bacteriocin production was stronger when the cultivation was performed on MRS broth for *Enterococcus durans* M-3 and *Enterococcus faecium* 3587, and on M-17 for *Enterococcus faecalis* 3915. 3) The three strains showed the typical growth for *Enterococci* in conditions of high NaCl concentrations. Keeping this in mind, in order to preserve the probiotic properties of bacteriocin-producing strains isolated from dairy products such as ours, NaCl concentrations should be kept as low as possible.

Key words: *Enterococci*, Lactic acid bacteria, probiotics, bacteriocins

INTRODUCTION

Enterococci are Gram-positive *cocci* which often occur in pairs and chains. Two species are common commensal organisms in the intestines of humans: *Enterococcus faecalis* and *Enterococcus faecium*. *Enterococci* are facultative anaerobic organisms which are able to grow at concentrations of 6,5% NaCl(1). The three producer strains (which are among the lactic acid bacteria) synthesize bacteriocins – membrane-depolarizing pore-forming toxins with protein nature that are ribosomally synthesized and excreted extracellularly(2,3).

Bacteriocins play an important role in food safety (4). However, BLIS production depends on various factors such as presence of salts in the culture media. Although various salts could display similar effects on bacteriocin synthesis, sodium chloride is in the focus of this research because of its wide use in dairy products (e.g. cheese) and its high concentrations in natural conditions. NaCl has also strong impact on the osmotic properties of the medium and it directly influences bacterial growth.

Recently, a collection of 13 different *Enterococcus* strains isolated from diverse "Kashkaval"-type cheeses was screened for probiotic properties, and 3 strains were found to secrete three different bacteriocins. Two of them were already described (5,6), while the third one is still under investigation.

NaCl concentrations in this project were chosen such as to exert least impact on the growth characteristics of the three strains of interest, and yet to clearly display the significance of the addition of salt on bacteriocin excretion.

MATERIALS AND METHODS

Bacterial strains and media. All bacterial

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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.

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

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NaCl influence. NaCl influence on the three producer strains was obtained by inoculating 5 ml of overnight cultures in 5 ml M-17 broth and 5 ml MRS broth with addition of NaCl (Merck) to concentrations of 0-10 % with a step of 1% (a total of 20). Cultures were allowed to grow at 37°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.

Determination of bacteriocin titers. The titers of the produced bacteriocin were quantified by two-fold serial dilutions of the supernatant probes or precipitates. Aliquots of 100 ml 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) (7).

Determination of bacteriocin activity. A well diffusion assay procedure was used (8). 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 ml from each bacteriocin probe were pipetted 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.

RESULTS AND DISCUSSION

It was discovered that BLIS production by three different *Enterococcus* strains is influenced by the presence of salts in the culture media. Figure 1 represents the correlation between the growth and NaCl concentration, and on the other hand the BLIS activity and NaCl concentration. As it can be seen independently of the broth used, for all strains concentrations higher than 2% lead to at least 2-fold decrease. As also evident from the data presented, NaCl concentrations of 3% do not affect the growth of the producer strains significantly. However, several observations emerge: 1) Most affected by the presence of NaCl in the medium is *Enterococcus durans* M-3, where 2-fold reduction of the BLIS activity is observed at a concentration of 1% when the cultivation was performed on MRS broth, and 4-fold reduction on M-17 broth; 2) for all the three bacteriocins the decrease of their production appears at higher NaCl concentrations in MRS broth than on M-17

broth; 3) for all the three strains bacteriocin biosynthesis is almost completely inhibited at concentrations higher than 6% even in the cases when significant culture growth is present.

Interesting bacteriocin production kinetics is observed for *Enterococcus faecalis* 3915. For this strain the BLIS titers are hightened four times in MRS broth in comparison to M-17 broth at 0% - 1% NaCl concentrations, but the activity practically disappears in MRS broth containing 3% NaCl, while some BLIS activity is observed at 5% NaCl concentration when the cultivation is on M-17 broth.

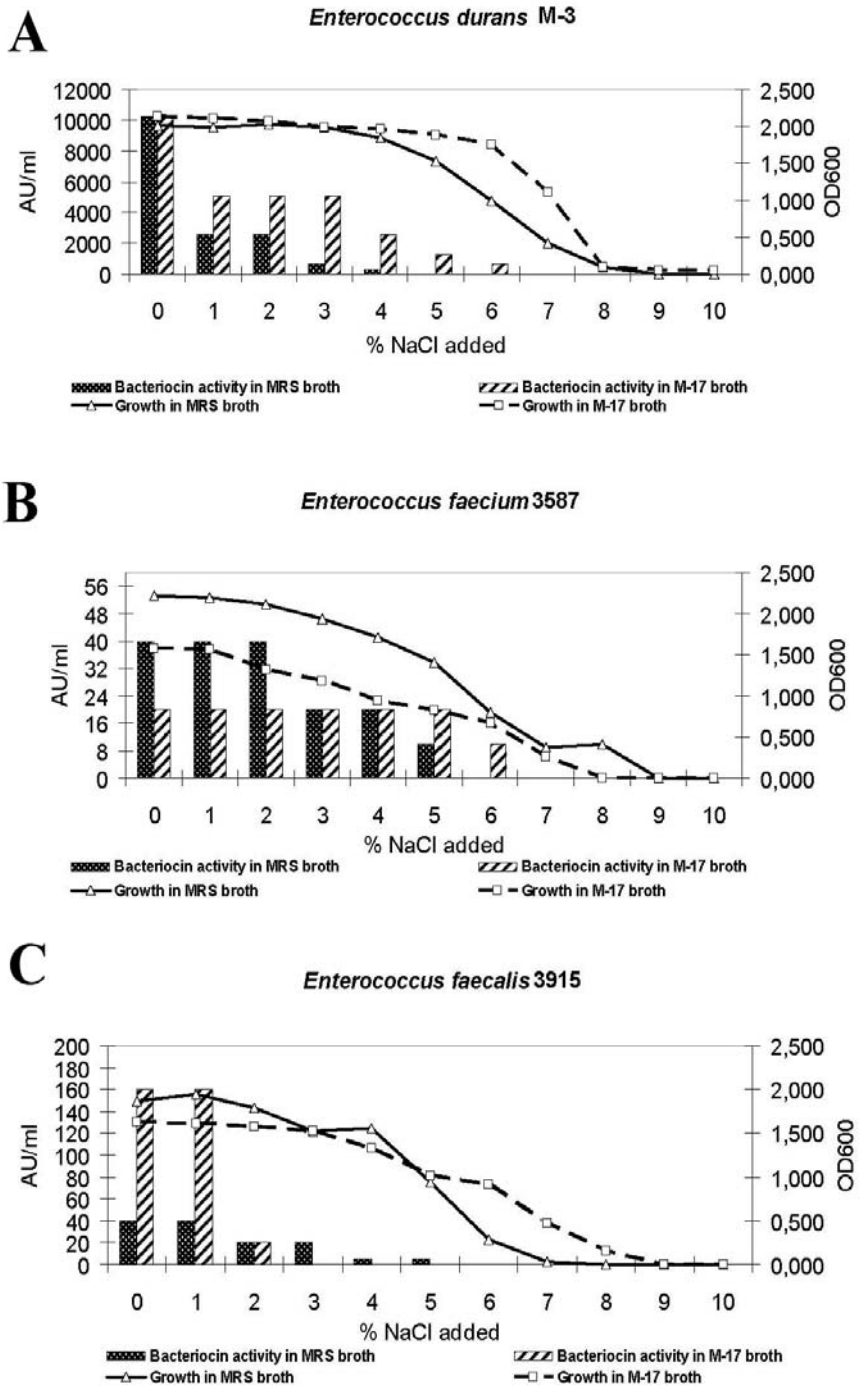

Figure 1. Influence of NaCl concentrations on the maximum growth and bacteriocin production by *Enterococcus durans* M-3 (A), *Enterococcus faecium* 3587 (B), and *Enterococcus faecalis* 3915(C).

The possibilities of bacteriocin synthesis suppression by sodium chloride are a point of particular interest, because this substance is widespread and often used in a number of dairy products (e.g. cheese). In this study data are introduced, illustrating the decreasing amount of bacteriocin synthesized under conditions of increasing NaCl concentrations. Those were chosen such as to exert least impact on the growth characteristics of the three strains of interest, and yet to clearly demonstrate the influence of salt on BLIS production.

The data derived in this study show that sodium chloride has suppressing activity upon BLIS production, a fact which is more clearly observed in conditions of higher salt concentrations.

In conclusion, the effect of sodium chloride on BLIS synthesis has to be taken in account in food industry because of the possibilities of the future three *Enterococcus* bacteriocins application.

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