

Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria Comparison of the bacteriocins

S.D. Todorov, L.M.T. Dicks *

Department of Microbiology, University of Stellenbosch, 7600 Stellenbosch, South Africa

Received 28 October 2004; accepted 24 January 2005

Abstract

Lactobacillus plantarum (strains ST194BZ, ST414BZ and ST664BZ), *Lactobacillus pentosus* (strain ST712BZ), *Lactobacillus rhamnosus* (strains ST461BZ and ST462BZ) and *Lactobacillus paracasei* (strains ST242BZ and ST284BZ), isolated from boza, produced bacteriocins active against *Lactobacillus casei*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Thus far, only a few bacteriocins with activity against Gram-negative bacteria have been reported. Complete inactivation of antimicrobial activity was observed after treatment of the bacteriocins with proteinase K, pronase, papain, chymotrypsin, trypsin, pepsin and Qiagen protease, confirming their proteinaceous nature. Treatment with catalase and α -amylase did not result in any changes of antimicrobial activity, indicating that the inhibition recorded was not hydrogen peroxide and that carbohydrate moieties were not required for antimicrobial activity. The bacteriocins remained stable after 2 h of incubation at pH values between 2.0 and 10.0, and for 120 min at 100 °C. The bacteriocins were resistant to treatment with SDS, Tween 20, urea and EDTA, but sensitive to Tween 80, Triton X-100 and Triton X-114. All bacteriocins acted bactericidal. The bacteriocins did not adhere to the surface of the producer cells. Production occurred throughout logarithmic growth, with the highest activity recorded at the end of logarithmic and during stationary growth. Based on tricine-SDS-PAGE, the bacteriocins ranged from 2.8 to 14.0 kDa in size. No plasmids were recorded, suggesting that the genes encoding the bacteriocins are located on the genomes.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Bacteriocin; Boza; Lactic acid bacteria

1. Introduction

Lactic acid bacteria are well known for their production of bacteriocins, i.e. peptides inhibitory to genetically closely related species [1,2]. A large variety of foods and beverages have been screened for strains with antibacterial properties.

Countries of the Balkan region in Europe are famous for the production of food and beverages fermented with lactic acid bacteria. Boza is one such traditional drink, produced through the fermentation of different cereals by yeast and lactic acid bacteria. However, only a few papers have been published on the microbial composition of Boza and most of the lactic acid bacteria that have been isolated belong to the genera

Lactobacillus, *Lactococcus* and *Leuconostoc* [3–6]. Kabadjova et al. [5] and Ivanova et al. [7] reported on strains isolated from Boza with activity against Gram-positive bacteria, including *Listeria innocua*, and Gram-negative bacteria, including *Escherichia coli*. A bacteriocin produced by *Lactococcus lactis* subsp. *lactis* 14, isolated from boza, was partially characterized [7]. In previous papers [8,9], we reported on the characterization of pediocin ST18 and mesentericin ST99, produced by *Pediococcus pentosaceus* ST18 and *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 isolated from boza “Belogratchik”. Pediocin ST18 and mesentericin ST99 revealed good anti-*Listeria* activity [8,9].

This paper is a comparative study of bacteriocins ST194BZ, ST241BZ, ST284BZ, ST414BZ, ST461BZ, ST462BZ, ST664BZ and ST712BZ produced by lactic acid bacteria isolated from boza.

* Corresponding author. Tel.: +27 21 8085849; fax: +27 21 8085846.
E-mail address: lmdt@sun.ac.za (L.M.T. Dicks).

2. Materials and methods

2.1. Isolation and identification of bacteriocin-producing lactic acid bacteria

Two different samples of boza from Belogratchik in the North-West of Bulgaria were screened for lactic acid bacteria that produce bacteriocins. Isolation of bacteriocinogenic lactic acid bacteria was as described by Todorov and Dicks [10]. Serial dilutions of the samples were made and plated onto MRS agar [11] supplemented with 50 mg l⁻¹ Delvocid (Gist-brocades, B.V., Delft, The Netherlands). The colonies were covered with a second layer of MRS agar containing the same concentration Delvocid. The plates were incubated anaerobically (OXOID, Gas Generation Kit, Hampshire, England) at 30 °C for 48 h. Plates with 50 or less colonies were covered with semi-solid BHI (Merck, Darmstadt, Germany), inoculated with *Lactobacillus casei* LHS. The plates were then incubated for a further 24 h at 37 °C. Colonies producing inhibition zones were isolated, cultured in MRS broth and tested for antimicrobial activity by using the agar spot-test and the diffusion method, described by Schillinger and Lücke [12] and Tagg and McGiven [13]. The antimicrobial effect of lactic acid was eliminated by adjusting the pH of the supernatants to 6.0 with sterile 1N NaOH. Activity was expressed as arbitrary units (AU) ml⁻¹. One AU was defined as the reciprocal of the highest

serial two-fold dilution showing a clear zone of growth inhibition of the indicator strain [14].

Strains were identified based on carbohydrate fermentation reactions recorded with API 50 CHL (bio Merieux, France) and phenotypic characteristics described by Schillinger and Lücke [15] and Stiles and Holzapfel [16]. Further identification was by PCR-generated DNA banding patterns obtained with species-specific primers for *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus pentosus* and *Lactobacillus rhamnosus*. The methods of Torriani et al. [17] and Ward and Timmins [18] were used. The spectrum of antimicrobial activity was recorded by using the agar-spot method, as described elsewhere. The indicator strains are listed in Table 1.

2.2. Effect of enzymes, pH, temperature, detergents and protease inhibitors on bacteriocin activity

The proteinaceous nature of the inhibitory compounds was confirmed by testing their sensitivity to proteolytic enzymes. All active isolates were grown in MRS broth at 30 °C for 24 h, the cells harvested by centrifugation (8000 × g, 10 min, 4 °C) and the pH of the cell-free supernatant adjusted to 6.0 with sterile 1N NaOH. Aliquots of these samples were treated with the following enzymes (1 mg ml⁻¹) and incubated for 2 h at 30 °C: Proteinase K (Roche, Indianapolis, IN, USA), Pronase (Boehringer Mannheim GmbH, Germany), Papain (Boehringer Man-

Table 1
Inhibitory spectrum of bacteriocins

Indicator organism	Culture medium and incubation temperature	Bacteriocin produced by strain							
		ST194BZ	ST242BZ	ST284BZ	ST414BZ	ST461BZ	ST462BZ	ST664BZ	ST712BZ
<i>Acinetobacter baumannii</i> 19	BHI, 37 °C	–	+	–	–	–	–	–	–
<i>Echerichia coli</i> 40	BHI, 37 °C	–	–	+	–	–	–	–	–
<i>E. coli</i> 8	BHI, 37 °C	+	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i> 24	BHI, 37 °C	–	–	–	–	–	–	–	–
<i>E. cloacae</i> 15	BHI, 37 °C	+	+	+	+	–	–	–	–
<i>Klebsiella pneumoniae</i> 31	BHI, 37 °C	–	+	+	+	+	–	–	+
<i>Lactobacillus casei</i> LHS	BHI, 30 °C	+	+	+	+	+	+	+	+
<i>Lactobacillus curvatus</i> DF38	MRS, 30 °C	–	–	–	+	+	–	–	+
<i>Lactobacillus delbruekii</i> subsp. <i>bulgaricus</i>	MRS, 30 °C	+	+	+	–	–	+	+	–
<i>Lactobacillus plantarum</i> 423	MRS, 30 °C	–	–	–	–	–	–	–	–
<i>Lactobacillus sakei</i> DSM 20017	MRS, 30 °C	+	+	+	–	–	+	+	–
<i>Lactobacillus salivarius</i> 241	MRS, 30 °C	–	–	–	–	–	–	–	–
<i>Pseudomonas aeruginosa</i> 7	BHI, 37 °C	+	+	+	+	+	+	+	+
<i>P. aeruginosa</i> 22	BHI, 37 °C	–	+	–	–	–	+	–	–
<i>Pseudomonas</i> sp. 25, 28	BHI, 37 °C	–	–	+	–	+	–	–	–
<i>Staphylococcus aureus</i> 2, 33, 34, 35, 36, 37, 38	BHI, 37 °C	–	–	–	–	–	–	–	–
<i>S. aureus</i> 5	BHI, 37 °C	–	+	–	–	–	–	–	–
<i>Streptococcus agalactiae</i> 9	BHI, 37 °C	–	–	–	–	–	–	–	–
<i>Enterococcus faecalis</i> 20	BHI, 37 °C	+	+	+	+	+	+	+	+
<i>E. faecalis</i> 21	BHI, 37 °C	+	+	+	–	+	+	+	–
<i>Streptococcus pneumoniae</i> 4, 27	BHI, 37 °C	–	–	–	–	–	–	–	–
<i>S. pneumoniae</i> 29	BHI, 37 °C	–	–	+	–	+	–	–	–
<i>Streptococcus</i> sp. TL1, TL2R, TL2W	MRS, 30 °C	–	–	–	–	–	–	–	–

(+) bacteriocin activity; (–) no bacteriocin activity.

Download English Version:

<https://daneshyari.com/en/article/36273>

Download Persian Version:

<https://daneshyari.com/article/36273>

[Daneshyari.com](https://daneshyari.com)