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Pentocin 31-1, an anti-*Listeria* bacteriocin produced by *Lactobacillus pentosus* 31-1 isolated from Xuan-Wei Ham, a traditional China fermented meat product

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Abstract

Strain 31-1 was isolated from the traditional China fermented Xuan-Wei Ham and identified as *Lactobacillus pentosus* 31-1, produced pentocin 31-1 at 640 arbitrary units (AU)/ml in MRS broth in the early stationary phase (i.e. after 24 h). After concentration by ammonium sulfate precipitation, followed by separation in SP-Sepharose fast flow cation exchange chromatography, the bacteriocin had a molecular weight of approximately 14.2 kDa when analyzed by Tricine–SDS–PAGE. Pentocin 31-1 showed a wide range of antimicrobial activity against *Listeria* spp., *Staphylococcus* spp., *Bacillus* spp., *Lactobacillus* spp., *Streptococcus* spp., *Pediococcus* spp. and *Escherichia* spp. All *Listeria* strains tested, including *Listeria monocytogenes*, were highly sensitive to the bacteriocin. Pentocin 31-1 was heat stable, pH resistant and protease sensitive. Tween 80, tween 20 and urea did not decrease the activity, however, SDS induced 75% activity loss. The bacteriocin exerted a bactericidal action on sensitive cells of *L. monocytogenes* 54002 in TSYE broth. It did not adhere to the surface of the producer cells. And 50% bacteriocin adsorption to the surface of *L. monocytogenes* 54002 cells was found at pH 6.0–7.5, whereas no bacteriocin adsorption was detected at pH 5.0–5.5.

Keywords: Xuan-Wei Ham; *Lactobacillus pentosus*; Pentocin 31-1

1. Introduction

The antimicrobial potential of lactic acid bacteria (LAB) is significant in the preservation, microbiological stability and development of starter cultures for fermented foods. It allows biological control of foodborne pathogens without resorting to more severe physical treatments (De-Vuyst & Vandamme, 1994; Ruiz-Barba, Piard, & Jimenez-Diaz, 1991). The antimicrobial effect may be due to acid production, competition for nutrients, formation of hydrogen peroxide, CO_2, diacetyl or antimicrobial peptides, i.e. bacteriocins (Klaenhammer, 1988).

Bacteriocins are defined as proteins or protein complexes antagonistic to bacteria genetically closely related to the producer organism (Klaenhammer, 1988). Numerous bacteriocins from Gram-positive bacteria, mainly LAB, have been isolated and characterized. *Lactobacillus pentosus* is a nonpathogenic Gram-positive LAB and widely used for production of fermented meat products all over the world. However, only a few bacteriocin-producing strains of *L. pentosus* have been reported, such as *L. pentosus* B96 isolated from fermenting green olives of "Azeiteira" cultivar, of Portuguese origin (Delgadoa, Britob, Peresb, Noe-Arroyoc, & Garrido-Fernandezc, 2005); a commercial starter culture of *L. pentosus* (two strains) (Nieto-Lozano, Reguera-Useros, Pelaez-Martinez, & Hardisson-dela, 2002); *L. pentosus* 191 and *L. pentosus* 204, isolated from sucuk (Con & Gokalp, 2000); and pentocin

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TV35b, produced by *L. pentosus* TV35b, isolated from posterior fornix vaginal secretions (Okkers, Dicks, Silvester, Joubert, & Odendaal, 1999); *L. pentosus* DK7 isolated from ogi (Olukoya, Tichaczek, Butsch, Vogel, & Hammes, 1993).

Xuan-Wei Ham, one of the most famous and popular traditional fermented meat products in China, contains a large population of LAB. Owing to particular geographical environment and climatic condition, traditional techniques in manufacturing, and free-range feeding, its excellent characteristic flavor is welcomed by consumers. As far as we could determine, nothing has been reported on bacteriocins produced by LAB isolated from Xuan-Wei Ham.

In this paper we reported on a novel bacteriocin with anti-*Listeria* activity, produced by *L. pentosus* 31-1 isolated from the traditional China fermented Xuan-Wei Ham.

2. Materials and methods

2.1. Bacterial culture and growth media

Strain 31-1 was isolated from Xuan-Wei Ham, which was manufactured in Hen-Da factory of Yun-Nan, China, and identified as *L. pentosus* by using the physiological and biochemical tests described by Müller (1990), Garver and Muriana (1993) and Atrih et al. (1993). Sugar fermentation reactions were obtained by using the API 50 CHL system (API System, BioMe'rieux, Montalieu Vercie, France) as the manufacturer's recommendations. The APILAB Plus computer-aided identification program version 4.0 (BioMe'rieux) was used to analyze the carbohydrate fermentation profiles obtained with the identification strips.

Strain 31-1 was grown in MRS medium (De-Man, Rogosa, & Sharpe, 1960) and incubated at 30 °C. *L. monocytogenes* 54002 was used as the indicator strain in the bacteriocin assay. They were stored at −80 °C in MRS and TSYE broth containing 15% (v/v) glycerol, respectively.

2.2. Bacteriocin activity assay

Bacteriocin screening was performed by using the agar-well diffusion method, as described by Mayr-Harting, Hedges, and Berkeley (1972) and Schillinger and Lücke (1989). To eliminate the antimicrobial effect of lactic acid, the pH of the supernatants was adjusted to 6.0 with sterile 1 M NaOH and the activity expressed in AU/ml. One AU was defined as the reciprocal of the highest serial 2-fold dilution showing a clear zone of growth inhibition of the indicator strain (Van-Reenen, Dicks, & Chikindas, 1998).

2.3. Bacteriocin production

Sterile MRS broth was inoculated with 1% (v/v) of a 24-h-old culture of *L. pentosus* 31-1. Incubation was at 30 °C with the pH of the culture not regulated. Samples were taken at different time intervals to determine the optical density (at 600 nm) of the culture, viable cell count and the antimicrobial activity (AU/ml) of the bacteriocin produced.

2.4. Bacteriocin purification to homogeneity

As described above, after 24-h-old cultivation of *L. pentosus* 31-1, cells were removed by centrifugation (9000*g*, 30 min). The bacteriocin present in the supernatant fraction was concentrated by ammonium sulfate precipitation (70% saturation). After the mixture had been stirred overnight at 4 °C, the precipitate was pelleted by centrifugation (12,000*g*, 30 min). The collected precipitate was then dissolved in 20 mM sodium phosphate buffer pH 5.8 and dialyzed using a 8–1.2 kDa cut-off membrane against the same buffer at 4 °C overnight (Fraction I).

Fraction I was applied to a SP-Sepharose fast flow (Pharmacia-LKB) column (1.6 cm × 20 cm) equilibrated with 0.02 M sodium acetate buffer pH 5.0 at a flow rate of 1 ml/min. The column was washed with 0.02 M sodium acetate buffer pH 5.5, until no absorbance was detected at 280 nm. Activity was eluted by a stepwise gradient-elution (0–0.5 M NaCl in 0.02 M sodium acetate buffer). Fractions of 3 ml were collected and the antimicrobial activity was determined. The active fractions were pooled and dialyzed against 20 mM sodium phosphate buffer pH 5.8 (Fraction II). Activity tests were performed by using the agar-well diffusion method as described before (Sahar, Deraz, Martin, Maria, & Bo-Mattiasson, 2005).

2.5. Determination of antimicrobial spectrum

Cell-free supernatant, Fraction I and Fraction II were used to determine the antimicrobial spectrum of activity, respectively. Cells were grown in MRS broth for 24 h at 30 °C and samples were prepared as described above (Noonpakdee, Santivarangkna, Jumriangrit, Sonomoto, & Panyim, 2003). The spectrum of activity of those samples was tested against a wide range of indicator strains comprising LAB and foodborne pathogens as shown in Table 2.

2.6. Sensitivity of bacteriocin to proteolytic enzymes, heat, pH and surfactants

The effect of various enzymes on bacteriocin activity was determined by incubating 200 μl of Fraction II with 20 μl of the following enzyme solutions at a final concentration of 1 mg/ml: proteinase K (pH 7.0; Sigma), trypsin (pH 7.0; Sigma), pepsin (pH 3.0; Merck), neutral proteinase (pH 7.0; Sigma), lysozyme (pH 7.0; Sigma), papain (pH 6.0; Sigma), a-amylase (pH 7.0; Sigma) and acid proteinase (pH 7.0; Sigma). After 2 h of incubation at 37 °C, enzyme activity was terminated by heating at 100 °C for 5 min. Untreated samples were used as control (Noonpakdee et al., 2003). The residual bacteriocin activity was assayed against indicator strain *L. monocytogenes* 54002.