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Characterization of a novel bacteriocin produced by *Lactobacillus sakei* LSJ618 isolated from traditional Chinese fermented radish

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ABSTRACT

A novel bacteriocin, sakacin LSJ618, produced by the strain *Lactobacillus sakei* LSJ618 isolated from traditional Chinese fermented radish, was studied. *L. sakei* LSJ618 was identified by both phenotypical and physiological tests combined with 16S rDNA sequence analysis. Sakacin LSJ618 is sensitive to hydrolytic enzymes including lipase, is stable between pH 2–8, and is heat resistant (30 min at 121 °C). Sakacin LSJ618 exhibits inhibitory activity against food-spoiling bacteria and food-borne pathogens, including the Gram-positive *Listeria monocytogenes*, *Staphylococcus aureus*, *Sarcina* sp., *Micrococcus luteus*, and the Gram-negative *Proteus* sp. and *Escherichia coli*, but not against most of the lactic acid bacteria tested. Maximal production of bacteriocin was reached in the late stationary phase, and inhibitory activity declined within 26 h. The mode of action of sakacin LSJ618 was determined to be bactericidal, as evidenced by its action upon *Micrococcus tetragenus*. After partial purification by ammonium sulfate precipitation and Sephadex G-25 chromatography, the molecular weight of sakacin LSJ618 was determined to be 5.2 kDa by Tricine-SDS-PAGE. The identified properties of sakacin LSJ618 indicate that it is a novel bacteriocin with potential application as a biopreservative in the food industry.
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1. Introduction

Food spoilage and food-borne pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* not only endanger human health, but also lead to severe losses of foodstuff. Although chemical additives can, to some extent, effectively inhibit the growth of bacteria, some of them have been proven harmful to human health as well as the environment. Fortunately, many lactic acid bacteria (LAB) can produce inhibitory compounds such as organic acids, hydrogen peroxide, diacetyl, enzymes and bacteriocins (Deegan, Cotter, Hilla, & Ross, 2006) that are effective against spoilage and pathogenic bacteria. Among these antibacterial compounds, bacteriocins represent a potentially safe class of biopreservatives for use in the food industry because of their sensitivity to proteolytic enzymes. For example, Nisin, a bacteriocin produced by *Lactococcus lactis*, has been used in many countries to extend the shelf-life of food (Deegan et al., 2006).

Lactobacillus sakei is widely observed in diverse habitats such as meat, vegetables, dairy and bakery products; they are widely used as starter cultures in dry sausage production, and contribute to their characteristic color, texture and flavor. Since the *L. sakei* Lb 706 product sakacin A (Schillinger & Lücke, 1989) was identified, more than ten other bacteriocins have been well studied, including sakacin P, sakacin K, sakacin 23K, sakacin Q, sakacin 2a, sakacin C2, bacteriocin AMA-K and bacteriocin ST8KF (Gao, Jia, Gao, & Tan, 2010; Hugas, Garriga, Aymerich, & Monfort, 1995; Kjos, Snipen, Salehian, Nes, & Diep, 2010; Mathiesen, Huehne, Kroeckel, Axelsson, & Eijsink, 2005; Powell, Witthuhn, Todorov, & Dicks, 2007; Tichaczek, Vogel, & Hammes, 1994; Todorov, Nyati, Meincken, & Dicks, 2007). The most important characteristic of bacteriocins produced by *L. sakei* is their activity against *Listeria* sp. (Schillinger & Lücke, 1989; Todorov et al., 2010; Trinetta, Floros, & Cutter, 2010). Some antilisterial bacteriocins and their producing strains can control a number of viable *L. monocytogenes* cells in different meat productions for 3–4 weeks (Katla et al., 2002; Schillinger, Kaya, & Lücke, 1991). In addition, the individual 50% inhibitory concentrations of sakacin P and sakacin A are less than that of nisin, and thus are considered to be safe (Dortu, Huch, Holzapfel, Franz, & Thonart, 2008; Jones, Zagorec, Brightwell, & Tagg, 2009; Katla, Naterstad, Vancanneyt, Swings, & Axelsson, 2003; Trinetta et al., 2010). The aforementioned studies have revealed that these bacteriocins are very promising biological food preservatives, especially in meat products. In fact, most bacteriocin-producing strains of *L. sakei* have been isolated from meat products; only two strains, *L. sakei* C2 (Gao

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et al., 2010) and *L. sakei* 5 (Vaughan, Eijsink, O'Sullivan, O'Hanlon, & Van Sinderen, 2001) were isolated from vegetable sources (traditional Chinese fermented cabbage and malted barley, respectively).

In an effort to find a bacteriocin that can be used as a food preservative, a strain producing a novel bacteriocin, termed sakacin LSJ618, was isolated from the traditional Chinese fermented radish. The strain was identified as *L. sakei* strain LSJ618. Furthermore, this study reports the characteristics and mode of action of sakacin LSJ618.

2. Materials and methods

2.1. Strains, media and culture conditions

The 24 types of traditional Chinese fermented foods used in this study were purchased from Hubei Province, China. Bacterial strains used in this study are shown in Table 1.

The bacteriocin-producing strain *L. sakei* LSJ618, isolated from traditional Chinese fermented radish, as well as the indicator strains of LAB, were cultured in MRS medium (Generay Biotech Co. Ltd., Shanghai, China (Sangon)); other indicator strains were cultured in LB medium (Sangon). The strains were stored at −70 °C in MRS or LB broth supplemented with DMSO (7.5%, v/v) (Sigma–Aldrich, St. Louis, MO), and were propagated twice in MRS or LB medium before use. Seed cultures of *L. sakei* LSJ618 were prepared by cultivating the cells in MRS broth with shaking (120 r/min), and maintained at 37 °C for 12 h. For fermentation, 4% of the seed culture was inoculated into 1000 ml of MRS broth and maintained at 37 °C for 24 h.

Table 1
Inhibition of various bacteria by the supernatant powder (SP) of *Lactobacillus sakei* LSJ618 as determined by the well diffusion agar test.

Indicator strains	Source[a]	Inhibitory activity[b]
Escherichia coli ECX1	This Lab	−
Escherichia coli ECX2	This Lab	−
Escherichia coli ECX3	This Lab	−
Escherichia coli ECX4	This Lab	+
Proteus sp.	This Lab	+++
Pseudomonas aeruginosa	This Lab	−
Bacillus subtilis	This Lab	−
Bacillus thuringiensis	This Lab	−
Salmonella pullorum	CIVDC	−
Listeria monocytogenes	GIM	+++
Listeria innocua GIM1.230	GIM	+
Micrococcus tetragenus	This Lab	+
Staphyloccocus aureus	This Lab	+
Sarcina sp.	This Lab	++
Micrococcus luteus GIM1.226	GIM	+++
Pediococcus acidilactici	This Lab	−
Streptococcus thermophilus	This Lab	−
Enterococcus faecalis	This Lab	−
Lactobacillus sp. LSX801	This Lab	−
Lactobacillus plantarum	This Lab	−
Lactobacillus lactis cremoris	This Lab	−
Lactobacillus brevis	This Lab	+
Lactobacillus pentosaceus	This Lab	−
Lactobacillus fructivorans	This Lab	−
Lactobacillus helveticus	This Lab	−
Lactobacillus casei	This Lab	−
Lactobacillus fermentum	This Lab	−
Lactobacillus bulgaricus	This Lab	−
Lactobacillus sakei LSJ618	This Lab	−
Lactobacillus sakei K14-1	This Lab	−
Lactobacillus sakei L5-1	This Lab	−
Lactococcus lactis K303	This Lab	−

[a] CIVDC (China Institute of Veterinary Drug Control, Beijin, PR China); GIM (Guangdong Institute of Microbiology, Kuanzhou, PR China).
[b] Wells (8 mm in diameter) were filled with 200 μl SP, Mean counts of three duplications. +, Diameter of inhibition zone: 8.00–11.00 mm; ++, 11.00–15.00 mm; +++, more than 15.00 mm; −, no inhibition zone.

2.2. Isolation and identification of bacteriocin-producing strains

The traditional Chinese fermented radish was added to a bottle containing 50 ml of sterilized water and was mixed by shaking. The suspension was diluted, and a 0.1 ml sample was plated on MRS agar with 0.01% (w/v) bromocresol purple (Chemapol Asia Pte Ltd). After the plates were incubated at 37 °C for 48 h, colonies that changed the color of the medium from purple to yellow were restreaked onto MRS plates. The bacteriocin activities of the strains were determined by the agar well diffusion test (Ennahar, Sashihara, Sonomoto, & Ishizaki, 2000). Strains that inhibited the indicator strains were selected for further studies.

Strain identification was based initially on phenotypical and physiological tests (Kandler & Weiss, 1986). Further identification was carried out by sequence analysis of 16S rDNA. Bacterial DNA was isolated with a UNIQ-10 Column Bacterial Genomic DNA Isolation Kit (Sangon); 16S rDNA was amplified by PCR (94 °C, 5 min, for predegeneration, then 94 °C for 45 s, 55 °C for 45 s and 72 °C for 90 s, 30 cycles) with primers P1 (5′-AGAGTTTGATCCTGGCTCAG-3′), and P2 (5′-TACGGTTACCTTGTTACGACTT-3′) using a PCR amplification kit (Sangon). The PCR products were sequenced and sequence homologies were examined by comparing DNA databases (http://www.ncbi.nlm.nih.gov/BLAST). A multiple sequence alignment was carried out using CLUSTAL X, and a bootstrap consensus tree (1000 copies) was constructed by the Neighbor-Joining (NJ) method using Mega software, version 4.1 (Kumar, Tamura, & Nei, 2004; Thompson, Higgins, & Gibson, 1994).

2.3. Preparation of the crude bacteriocin

The cell-free supernatant of the *L. sakei* LSJ618 culture was prepared by centrifugation (9000 g for 15 min) and filter-sterilization (0.45 μm pore-size membrane). The cell-free supernatant was then dialyzed (MW cutoff 500 Da) against double-distilled (dd) H_2O (pH 6.0) for 6 h at 4 °C to remove organic acids, H_2O_2 and other small antibacterial materials; the solution was then lyophilized. The resulting supernatant powder (SP) was used as the sample for further analysis of the characteristics of the bacteriocin.

2.4. Assay of bacteriocin activity and antibacterial spectrum

Bacteriocin activity was measured by the agar well diffusion test (Ennahar et al., 2000) using *Micrococcus tetragenus* as the indicator strain. The bacteriocin titer was determined by measuring the diameter of the inhibition zone around the wells, and expressed as arbitrary units (AU) per ml; one AU was defined as the reciprocal of the highest serial two-fold dilution producing distinct inhibition of the indicator lawn (Todorov & Dicks, 2005). All of the indicators for the antibacterial spectrum are listed in Table 1.

2.5. Preliminary characterization of sakacin LSJ618

To avoid artifacts caused by the presence of hydrogen peroxide, organic acids and other small molecular antibacterial compounds in the cell-free supernatant, SP was used in the agar well diffusion test (Ennahar et al., 2000). All experiments were conducted in triplicate.

To test thermostability, SP samples were heated to 60 °C, 80 °C, 100 °C and 121 °C for 30 min, and then placed in an ice bath for 10 min. Residual bacteriocin activity was then determined; unheated SP served as the control. To determine pH stability, SP

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