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ORIGINAL PAPER

Purification, characterization, and action mechanism of plantaricin DL3, a novel bacteriocin against *Pseudomonas aeruginosa* produced by *Lactobacillus plantarum* DL3 from Chinese Suan-Tsai

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Abstract A novel bacteriocin produced by *Lactobacillus plantarum* DL3 isolated from Suan-Tsai, a traditional Chinese fermented cabbage, was designated as plantaricin DL3. It was purified by ethyl acetate extraction followed by gel filtration and high-performance liquid chromatography. The molecular weight of plantaricin DL3 was determined as 2149 Da by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF–MS) analysis. The amino acid sequence of plantaricin DL3 was predicted to be VGPGAINAGTYLVSRELFER by MALDI-TOF–MS/MS. This bacteriocin exhibited broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria, especially *Pseudomonas aeruginosa*, high thermal stability (15 min, 121 °C) and narrow pH stability (pH 2.5–5.5). The mechanism of action of this bacteriocin was responsible for the disruption of cell wall, accompanied with the leakage of proteins. These results suggested that plantaricin DL3 has potential applications in the control of *Pseudomonas* spp. in aquatic products.

Keywords *Lactobacillus plantarum* · Bacteriocin · Purification · Characterization · Action mechanism · *Pseudomonas aeruginosa*

Introduction

Psychrotrophic microorganisms are a concern to the fishery industry due to their ability to cause spoilage in chilled fresh fish under aerobic storage conditions [1]. The ability of psychrophilic bacteria to grow rapidly at refrigerated temperature increases the risk of the spoilage and pathogen bacteria growth during processing and storage [2]. *Pseudomonas* spp., Gram-negative rods, are considered to be very detrimental organisms because they produce large amounts of extracellular hydrolytic enzymes and unacceptable organic compounds [1, 3]. In the presence of oxygen under refrigerated conditions, contaminating *Pseudomonas* can cause fish spoilage resulting in off-odors, discolouration, and slime production that affect product quality and shorten shelf life [4].

Numerous studies have been carried out to develop effective processing treatments to control the growth of *Pseudomonas* spp. in fish products. In this context, physical treatments, such as refrigeration, ionizing radiation, modified atmosphere packaging (MAP), high hydrostatic pressure (HHP) [5], as well as chemical preservatives as ozone, chlorinated products, and electrolyzed oxidizing water [6], have been applied into aquatic products to inhibit the growth of undesirable bacteria. However, the drawback of both physical and chemical treatments is that the sensory quality and nutritional properties of fish products may be destroyed [7]. Additionally, consumers are more concerned about the overuse of chemicals in food. Therefore, there is an ever-growing interest in developing

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novel bio-preservation strategies which will be used to the improvement of fish products.

Bacteriocins are a class of ribosomally synthesized peptides with inhibitory activity produced by various bacteria [8, 9]. Bacteriocins produced by lactic acid bacteria (LAB) have attracted significant attention because of their potential use as natural bio-preservatives for improving the safety of foods [5, 10]. For instance, nisin produced by *Lactococcus lactis*, the first bacteriocin discovered, has been used as a natural food antiseptic agent in the food industry in dozens of countries [11, 12].

Bacteriocin-producing LAB exist widely in Chinese fermented vegetables. Suan-Tsai is the most popular traditional fermented vegetable in the northeast region of China. Meanwhile, LAB are found to be the dominant microorganisms in Suan-Tsai and they are derived from raw Chinese cabbages, the air, and the fermentation containers. Although numerous plantaricins produced by *Lactobacillus plantarum* isolated from fermented vegetables have been reported, including plantaricin JLA-9, plantaricin 163 [13], plantaricin C8 [14], and plantaricin ZJ5 [15], few plantaricins with inhibitory activity to *Pseudomonas* spp. have been reported.

The aims of the present paper were to obtain a producing-bacteriocin LAB strain from Suan-Tsai with a broad inhibitory spectrum against *Pseudomonas aeruginosa*, then to identify the structure and properties of a novel bacteriocin, and to preliminarily reveal its mechanism of action against *P. aeruginosa*, in order to develop a natural and highly efficient food preservative to extend the shelf-life of aquatic products.

Materials and methods

Bacterial strains and growth conditions

The bacteriocin-producing strain *L. plantarum* DL3 (Genbank: KU535872) was isolated from traditionally fermented Suan-Tsai in the northeast region of China (Jinzhou, Liaoning province, China). This strain DL3 was identified by 16S rRNA gene sequencing with forward primer 5′-AGAGTT TGATCCTGGCTCAG-3′ and reverse primer: 5′-TACGGT TACCTTTGTTACGACTT-3′ [16]. Homology searches of 16S rRNA sequences were performed in GenBank by the Blast program. *L. plantarum* DL3 was cultured in de Mann Rogosa Sharpe (MRS, Aoboxing, Beijing, China) broth at 37 °C for 24 h. Cell free supernatant (CFS) produced by *L. plantarum* DL3 was obtained by centrifugation of overnight cultures (8000*g* at 4 °C for 10 min) and filtered through 0.45-μm filters.

Indicator bacteria *P. aeruginosa* ATCC9027 were purchased from Shanghai Beinuo Bnbiotech Co., Ltd. and grown in Luria–Bertani (LB, Aoboxing, Beijing, China) broth at 37 °C for 12 h. All strains were stored at −80 °C in the appropriate cultivation broth with 20% (v/v) glycerol.

Determination of bacteriocin activity

The antimicrobial activity of the bacteriocin was determined by using the agar-well diffusion method [17]. The activity expressed in arbitrary unit (AU)/ml and 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 [18]. Briefly, the bacteriocin was serially diluted two-fold with sterile deionized water and 180 μl of each dilution was added into the wells. The arbitrary unit (AU) of antibacterial activity per milliliter was defined as $2^n \times 1000\ \mu l \times 180\ \mu l^{-1}$.

Purification of bacteriocin

Proteins were firstly extracted from CFS using equal volume of ethyl acetate. Subsequently, the proteins extracted were purified by Sephadex G50 column (1.6 cm × 60 cm) chromatography system. The column was equilibrated with phosphate buffer solution (PBS, 0.02 mol/l, pH 6.0). The bacteriocin was eluted at a flow rate of 0.5 ml/min. The eluted peaks were collected and detected at 280 nm by UV–visible spectrophotometer (UV2550, Shimadzu, Japan). The collected fractions were tested for antimicrobial activity and concentrated using vacuum freeze-drying (Free Zone 2.5 L, Labconco, USA), and the active fractions were further purified by high-performance liquid chromatography (HPLC1200, Agilent, US). The elution solvents were ultrapure water containing 0.1% trifluoroacetic acid (TFA) (solvent A) and methanol containing 0.1% TFA (solvent B). One hundred microliters of concentrated sample was injected into an analytical Eclipse Plus C18 column (4.6 × 250 mm, 5 μm) and was performed at a flow rate of 0.8 ml/min with a linear gradient elution as follows: 0–4 min, 0% solvent B; 4–31 min, 0–100% solvent B; 31–40 min, 100–0% solvent B. The eluted peaks were monitored spectrophotometrically at 280 nm and were manually collected. The collected fractions were tested for antimicrobial activity and the active fractions were concentrated by vacuum freeze-drying.

The antibacterial activity was measured as previously described, using *P. aeruginosa* as indicator strain. The protein concentration for each sample at different steps of the purification was determined using Bradford's assay [19].