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Pediocin SA-1, an antimicrobial peptide from *Pediococcus acidilactici* NRRL B5627: Production conditions, purification and characterization

Sofia Anastasiadou, Maria Papagianni *, George Filiousis, Ioannis Ambrosiadis, Pavlos Koidis

Department of Hygiene and Technology of Food of Animal Origin, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece

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Abstract

Fermentation broths of *Pediococcus acidilactici* NRRL B5627 exhibited a certain antimicrobial activity due to a bacteriocin produced during early growth and until the stationary phase of growth was reached (at optimum of 60% dissolved oxygen saturation). Its size was determined by electrospray ionization mass spectrometric analysis as 3.660 kDa. N-terminal sequencing showed that the bacteriocin had 19 amino acid residues in the order KYYGXNGVXTXGKHSXVDX. The purified bacteriocin is similar to pediocins isolated by various *Pediococci* and therefore, it belongs to the class IIa of bacteriocins and is thus designated pediocin SA-1. Sensitivity of the purified pediocin to various storage temperatures and enzyme treatments was examined. Purified pediocin SA-1 is heat stable for up to 60 min at 121 °C. Pediocin SA-1 is inhibitory to several food-borne pathogens and food spoilage bacteria. It appears to be significantly more effective against *Listeria* spp. compared to pediocin PD-1 produced by *P. damnosus*. The mode of action of the purified bacteriocin appears to be bactericidal.

Keywords: *Pediococcus acidilactici*; Pediocin; Bacteriocin; Biopreservative

1. Introduction

Lactic acid bacteria (LAB) are well known for their production of peptides and proteins with antimicrobial properties, known as bacteriocins. The potential applications of bacteriocins from LAB in the food and health care sectors have attracted the strong interest of academia and the industry resulting in an impressive amount of published research on their production, purification, genetics and applications (Papagianni, 2003). So far, only nisin produced by *L. lactis*, is a commercial product and an approved food additive in most major food producing countries. Another bacteriocin that attracts research interest and will likely be the next to be used in the food industry is pediocin (Ray, 1992; Turcotte et al., 2004), which is an antilisterial bacteriocin (Guyonnet et al., 2000; Simon et al., 2002) produced by several *Pediococcus* strains. However, unlike nisin and *L. lactis*, studies on the physiology and genetics of pediocin producing *Pediococcus*, are still rather limited.

Most commonly, strains of *Pediococcus acidilactici* and *P. pentosaceus* have been reported to produce bacteriocins (Gonzales and Kunka, 1987; Biswas et al., 1991; Kim et al., 1992; Elegado et al., 1997; Gurira and Buys, 2005). Some of these are designated as pediocin AcH by *P. acidilactici* H, E, F, and M (Bhunia et al., 1987; Ray et al., 1989; Kim et al., 1992), pediocin PA-1 by *P. acidilactici* PAC 1.0 (Gonzales and Kunka, 1987), pediocin JD by *P. acidilactici* SJ-1 (Schved et al., 1993), pediocin 5 by *P. acidilactici* UL5 (Huang et al., 1996), pediocin A by *P. pentosaceus* FBB-61 (Etchells et al., 1964; Flemming et al., 1975), pediocin N5p by *P. pentosaceus* (Strässer de Saad et al., 1995), and pediocin ST18 by *P. pentosaceus* (Todorov and Dicks,

* Corresponding author. Tel.: +30 2310 999804; fax: +30 2310 999829.
E-mail address: mp2000@vet.auth.gr (M. Papagianni).

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2005). *P. damnosus* has also been reported to produce a pediocin, designated as pediocin PD-1 (Green et al., 1997). It is now known for most studied pediocins that they are plasmid encoded (Ray, 1995; Le Marrec et al., 2000) and posttranslationally modified hydrophobic molecules (Green et al., 1997; Yin et al., 2003; Wu et al., 2004), which also share a similar N-terminal sequence (Henderson et al., 1992; Wu et al., 2004).

Pediocins form a group of bacteriocins belonging to the class IIa of bacteriocins, characterized as "antilisterial" (Papagianni, 2003). They inhibit several gram-positive spoilage and pathogenic bacteria. The spectra of antimicrobial activity of pediocins produced by strains of *P. acidilactici* and *P. pentosaceus* have been found to be similar and this has been attributed to the phylogenetically close relation of the producer organisms (Collins et al., 1991). Pediocin PD-1 by *P. damnosus* however, has been found to be inactive against other pediococci, a characteristic that makes it different from the known pediocins produced by *P. acidilactici* and *P. pentosaceus* strains (Green et al., 1997).

We have undertaken studies on the physiology of the meat isolated, bacteriocin producer, *P. acidilactici* NRRL B5627, grown in stirred tank bioreactor cultures. In this work, we report information on production, isolation, purification, antimicrobial activity and mechanism of activity of the bacteriocin produced by this microorganism.

2. Methods

2.1. Bacterial strains and growth conditions

The microorganism used in this study was *Pediococcus acidilactici* NRRL B5627. This was maintained in MRS agar from which was transferred in 250 ml Erlenmeyer flasks, containing 100 ml of MRS broth. Following growth until the mid-logarithmic phase ($OD_{600} = 1.4$), the culture was transferred (2% v/v) in a stirred tank bioreactor – BIOFLO 110, New Brunswick Scientific – with a working volume of 2 l. The reactor was equipped with baffles. The agitation system consisted of two 6-bladed Rushton-type impellers (52 mm), operating at the stirrer speed of 150 rpm. Process temperature was maintained at 30 °C. The culture pH was left uncontrolled (pH at inoculation time was around 6.0). The reactor was equipped with a polarographic oxygen sensor (Mettler Toledo, Urdorf, Switzerland). The oxygen electrode was calibrated by sparging the medium with air (dissolved oxygen tension, DOT, 100%) and N_2 (DOT, 0%); the 100% saturation value was based on air. Experiments were carried out under semi-aerobic conditions, with the DOT kept at 60% by sparging the reactor with a mixture of N_2 and atmospheric air, adjusted by using two mass flow controllers, and the DOT was kept constant by feedback regulation of the ratio. Samples were taken every two hours for biomass, lactic acid, and bacteriocin activity determination. All runs were carried out in triplicate and repeated if experimental variation exceeded 10%.

2.2. Determination of biomass, lactate, and glucose concentrations

Biomass concentration was monitored spectrophotometrically by measuring the optical density at 600 nm and correlating the optical density with cell dry weight measurements. One unit of optical density at 600 nm was shown to be equivalent to 0.25 g (dry weight) of cells per liter. Cell dry weight was determined by filtering 10 ml of broth through nitrocellulose filters (pore size, 0.45 μm). The filters were tared after having been dried in a microwave oven at 150 W for 15 min. The biomass collected on the filter was washed twice with 10 ml distilled water before being dried and tared as indicated above.

Lactic acid concentration was determined with the EnzyPlus D/L Lactic Acid kit by Diffchamb AB (Diffchamb, Sweden). Glucose was determined using the glucose oxidase/peroxidase method of Kunst et al. (1986).

2.3. Determination of antimicrobial activity

Antimicrobial activity was assessed by the agar diffusion assay (Tramer and Fowler, 1964). For each indicator organism the appropriate solid medium was used. Solid media were prepared by adding 1.5% wt/vol technical agar to the broth media. Anaerobic conditions for plates were generated in a GasPak anaerobic jar. Anaerobic and microaerophilic growth for liquid cultures was ensured by growing the cultures in an incubator with controlled CO_2 partial pressure. An aliquot of 10 μl of cell-free culture supernatant fluid (pH adjusted at 6.0) was spotted on the appropriate solid media (1.5% w/v agar) seeded with a fresh culture of test cells ($\approx 10^6$ cells/ml). Plates were incubated at the optimum conditions for each test microorganism and examined for the presence of 2 mm or larger clear zones of inhibition.

2.4. Determination of bacteriocin activity

Production of bacteriocin in *P. acidilactici* cultures was examined in cell-free samples and the arbitrary units (AU) of activity (reciprocal of the highest dilution at which activity was still obtained) were determined (Wu et al., 2004). To eliminate the antimicrobial effect of lactic acid, the pH of the samples was adjusted to 6.0 with 1 N NaOH. In order to find a suitable indicator microorganism to be used in bacteriocin activity assays, ten microorganisms were examined for their sensitivity levels and linearity of response to bacteriocin according to Papagianni et al. (2006). Based on these criteria, *Micrococcus luteus* CECT 241 was chosen among the tested *P. acidilactici* ATCC 25740, *P. pentosaceus* ATCC 33316, *Lactococcus lactis* 11454, *Lactobacillus curvatus* ATCC 51436, *Lb. sakei* CECT 906 T, *M. luteus* CECT 241, *Lb. plantarum* NCAIM B 01133, *Lb. plantarum* NCAIM B 1074, *Lb. plantarum* CECT 220, and *Lb. plantarum* ATH.