Anaerobe 17 (2011) 23–31

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Food Microbiology

Characterization of a bacteriocin produced by *Lactobacillus sakei* R1333 isolated from smoked salmon

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ARTICLE INFO

Article history:
Received 25 February 2009
Received in revised form
26 November 2009
Accepted 29 January 2010
Available online 10 February 2010

Keywords:
Salmon
Lactobacillus sakei
Sakacin G

ABSTRACT

Strain R1333, isolated from commercially available smoked salmon, was identified as *Lactobacillus sakei* based on biochemical tests, sugar fermentation reactions (API 50 CHL), PCR with species-specific primers and sequencing of the 16S rRNA gene. Strain R1333 produces a 3811 kDa class IIa bacteriocin, active against *Streptococcus caprinus*, *Streptococcus macedonicus*, *Streptococcus* spp., *L. sakei*, *Lactococcus lactis* subsp. *lactis*, *Listeria innocua*, *Listeria ivanovii* subsp. *ivanovii* and *Listeria monocytogenes*. The mode of activity against *L. innocua* 2030C and *L. ivanovii* subsp. *ivanovii* ATCC 19119 was bactericidal, resulting in cell lysis and enzyme- and DNA-leakage. The highest level of activity (1600 AU/mL) was recorded when cells were grown at 30 °C in MRS broth (initial pH 6.5). Only 800 AU/mL was recorded when strain R1333 was grown in MRS without Tween 80. Lower levels of bacteriocin production were recorded when strain R1333 was grown in MRS at 20 °C. Peptide R1333 adsorbs at low levels (200 AU/mL) to producer cells.

Purification of bacteriocin R1333 was performed by 60% ammonium sulfate precipitation, followed by separation on a SepPak C_{18} column and reverse-phase HPLC on a Nucleosil C_{18} column with a linear gradient from 0.1% TFA to 90% acetonitryl. A molecular mass of 3811 kDa was determined by mass spectrometry. Based on mass spectrometry and sequencing of the PCR amplified fragment targeting the *sak*G gene, *L. sakei* R1333 is a potential producer of sakacin G. This is the first report of the identification of sakacin G produced by *L. sakei* isolated from smoked salmon.

1. Introduction

Lactic acid bacteria (LAB) are known for their production of antimicrobial compounds, including bacteriocins and bacteriocin-like peptides [1]. Bacteriocins of LAB are defined as ribosomally synthesized proteins or protein complexes usually antagonistic to genetically closely related organisms [1,2]. They are generally low molecular weight proteins that gain entry into target cells by binding to cell surface receptors. Their bactericidal mechanisms vary and may include pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rRNA, and inhibition of peptidoglycan synthesis [1,3,4].

These peptides are sorted into four classes, with most bacteriocins grouped in classes I and II [5]. Class I bacteriocins, known as lantibiotics (<5 kDa), are post-translationally modified and contain lanthionine and β-methyl-lanthionine [6]. Class II bacteriocins are small (<10 kDa), heat-stable, cationic, hydrophobic and membrane-active [1,5]. Bacteriocins with a highly conserved N-terminal amino acid sequence (YGNGVXaaC), non-polar amino acids, one or more disulphide bridges, and activity against *Listeria* spp. are grouped in class IIa [7]. Bacteriocins that function in pairs, usually as two distinct peptides, are grouped in class IIb [8]. Thiol-activated bacteriocins that rely on a *sec*-dependent secretion mechanism are grouped in class IIc [9].

Recently, according to a complete genome analysis, it was proposed that *Lactobacillus sakei* can be used to control pathogens in meat because its metabolism is particularly well adapted to a meat medium [10]. The *Lactobacillus* species represents the most common LAB strains currently found in meat starter cultures [11]. Moreover, *L. sakei* and *Lactobacillus curvatus* isolates from meat often contain bacteriocinogenic strains. Several bacteriocins have

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doi:10.1016/j.anaerobe.2010.01.004

been described for *L. sakei* and their potential application in meat preservation has been studied: sakacin A [12], sakacin P [13–15], sakacin 674 [16], sakacin B [17], sakacin K [18,19], sakacin V18 [20], sakacin M [21], sakacin T [22], sakacin G [23], sakacin X [24], sakacin Q [25], sakacin 1 [26,27] and sakacin 29 [28]. All sakacins possess strong antilisterial activity.

The preservation of meat products is increasingly directed towards biocontrol using bacteriocinogenic *Lactobacillus* species as protective microflora to inhibit the growth of *Listeria monocytogenes* and other undesirable microorganisms [29–31].

Bacteriocin production does not always correlate with an increase in cell mass or growth rate of the producer strain [32]. Higher bacteriocin levels are often recorded in the absence of growth-stimulating nutrients, or at temperatures and pH conditions lower than required for optimal growth [33,34]. Optimal bacteriocin production is often detected in a medium with limiting concentrations of sugars, nitrogen sources, vitamins and potassium phosphate, or when the medium pH is regulated [35].

The objective of this study was to characterize bacteriocin R1333, produced by *L. sakei* strain R1333 isolated from smoked salmon, with the aim of using this strain as a bio-preservative culture in fish and other seafood.

To our knowledge, this is the first report about a bacteriocinogenic strain of *L. sakei* isolated from smoked salmon.

2. Materials and methods

2.1. Screening for bacteriocin producer

One-hundred and fifteen isolates from smoked salmon (bacterial collection of ENITIAA, Nantes, France) were screened for antibacterial activity against strains *Listeria innocua* F and CIP 80.11T to select the most active bacteriocin producer.

Antimicrobial activity was determined by the agar-spot-test method [36]. Activity was expressed as arbitrary units (AU) per mL, with one AU defined as the reciprocal of the highest dilution showing a clear zone of inhibition [36]. In addition to *L. innocua* F and *L. innocua* CIP 80.11T, *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 and *Enterococcus faecium* HKLHS (as resistant control) were used as test strains. The spectrum of activity of the selected bacteriocin producer (strain R1333) was determined against several Gram-positive bacteria (Table 1). Pure cultures were obtained by streaking onto MRS agar (Biolab, Biolab Diagnostics, Midrand, SA), grown on MRS broth at 30 °C and stored at −80 °C in the presence of 15% (v/v) glycerol.

2.2. Identification of strain R1333

The morphology of strain R1333 was determined using Atomic Force Microscopy (AFM). Cells were harvested by centrifugation

Table 1
Inhibitory spectrum of bacteriocin R1333.

Test strain	Culture collection	Growth medium	Incubation temperature (°C)	Bacteriocin R1333 activity
Enterococcus faecalis 1071	SU	MRS	37	−
Enterococcus faecalis FA2	SU	MRS	37	−
Enterococcus faecalis FAIR E88	SU	MRS	37	+
Enterococcus faecalis FAIR E90	SU	MRS	37	−
Enterococcus faecalis FAIR E92	SU	MRS	37	−
Enterococcus faecium ET06	UC	MRS	37	−
Enterococcus faecium ET88	UC	MRS	37	−
Enterococcus faecium HKLHS	SU	BHI	37	−
Enterococcus mundtii ST4SA	SU	MRS	30	−
Lactobacillus curvatus DF38	SU	MRS	30	−
Lactobacillus curvatus ET31	UC	MRS	37	−
Lactobacillus delbrueckii ET32	UC	MRS	37	−
Lactobacillus fermentum ET35	UC	MRS	30	−
Lactobacillus johnsonii La1	SU	MRS	30	−
Lactobacillus paracasei ST284BZ	SU	MRS	30	−
Lactobacillus plantarum 423	SU	MRS	30	−
Lactobacillus plantarum ATCC 14917^T	ATCC	MRS	30	−
Lactobacillus plantarum ST23LD	SU	MRS	30	−
Lactobacillus plantarum ST26MS	SU	MRS	30	−
Lactobacillus plantarum ST28MS	SU	MRS	30	−
Lactobacillus rhamnosus Lgg	SU	MRS	30	−
Lactobacillus sakei DSM 20017	DSM	MRS	30	+
Lactococcus lactis subsp. *lactis* HV219	BAS	MRS	37	+
Lactobacillus plantarum ST194BZ	SU	MRS	30	−
Leuconostoc lactis ST612BZ	SU	MRS	30	−
Leuconostoc lactis ST63BZ	SU	MRS	30	−
Listeria innocua 2030C	ESB	BHI	37	+
Listeria innocua CIP 80.11T	IP	Elliker	37	+
Listeria innocua F	ENITIAA	Elliker	37	+
Listeria innocua LMG13568	LMG	BHI	37	+
Listeria ivanovii subsp. *ivanovii* ATCC 19119	ATCC	BHI	37	+
Listeria monocytogenes Scott A	UWC	BHI	37	+
Pediococcus acidilactici ET34	UC	MRS	30	−
Streptococcus caprinus ATCC 700065	ATCC	BHI	30	+
Streptococcus gallolyticus subsp. *macedonicus* BAA-249™	ATCC	BHI	30	+
Streptococcus spp. TL1	SU	BHI	30	+
Streptococcus spp. TL2R	SU	BHI	30	+
Streptococcus spp. TL2W	SU	BHI	30	+

SU: Department of Microbiology, Stellenbosch University, Stellenbosch, South Africa; UC: Instituto de Ciencia y Tecnología de Alimentos, Universidad Central de Venezuela, Caracas, Venezuela; ATCC: American Type Culture Collection; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; BAS: Department of Microbial Genetics, Institute of Microbiology "Stephan Angeloff", Bulgarian Academy of Sciences, Sofia, Bulgaria; ESB: Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal; IP: Institute Pasteur, Paris, France; ENITIAA: UMR INRA Secalim, ENITIAA ENVN, Nantes, France; LMG: Laboratorium voor Mikrobiologie, Ghent, Belgium; UWC: Department of Microbiology, University of Western Cape, Cape Town, South Africa.