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Original article

Genetic characterization and expression of leucocin B, a class IId bacteriocin from *Leuconostoc carnosum* 4010

Xing Wan, Per E.J. Saris, Timo M. Takala*

Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 56, Helsinki, Finland

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Abstract

Leuconostoc carnosum 4010 is an antimicrobial strain used as a protective culture in vacuum-packed meats. In this study, we showed that, in addition to antilisterial class IIa bacteriocins leucocin A and C, the strain also produces class IId bacteriocin leucocin B, the antimicrobial activity of which is limited to the genera *Leuconostoc* and *Weissella*. Two novel genes, *lebBI* encoding the leucocin B precursor with a double-glycine-type leader and putative immunity protein LebI, were identified on *L. carnosum* 4010 plasmid pLC4010-1. LebI contains three transmembrane spans and shares 55% identity with the mesentericin B105 immunity protein. Genes *lebBI* were shown to be transcribed in 4010 by RT-PCR analysis. The secretion of leucocin B in *L. carnosum* 4010 was shown by spot-on-lawn and SDS-gel overlay methods with a *Leuconostoc* strain sensitive to leucocin B but resistant to leucocins A and C. In addition, leucocins A and B from *L. carnosum* 4010 were cloned as *SSusp45* fusions in heterologous host *Lactococcus lactis* and the secretion of active bacteriocins was detected on indicator plates.

Keywords: *Leuconostoc*; Bacteriocin; Leucocin B; Heterologous expression

1. Introduction

Lactic acid bacteria (LAB) are a prolific source of ribosomally synthesized antimicrobial peptides [1]. These peptides, also known as bacteriocins, generally kill target bacteria by forming pores in the cell membrane or by inhibiting cell wall synthesis. Many bacteriocin-producing LAB, e.g. *Lactobacillus*, *Lactococcus* and *Leuconostoc*, are natural preservatives potentially of use in extending the shelf-life and safety of food products [1,2].

LAB bacteriocins can be sorted into two main groups: class I post-translationally modified bacteriocins, which are mainly lantibiotics, and class II unmodified bacteriocins [3,4]. Class II bacteriocins are small (<10 kDa), heat-stable, non-lanthionine-containing peptides. Because of variation in their structural properties, class II bacteriocins are divided into different subclasses: IIa pediocin-like, IIb two-peptide, IIc cyclic bacteriocins and IId single linear peptides [3,5]. Class IId represents various heterogeneous one-peptide linear non-pediocin-like bacteriocins, which do not fall into the above-mentioned three subgroupings [3]. In the proposal by Iwatani et al. [6], class IId bacteriocins were suggested to be further divided into *sec*-dependent leaderless and unclassified double-glycine types according to the type of signal peptide.

Leuconostoc spp. are widely used in the food industry as starters and protective cultures. Many *Leuconostoc* strains produce bacteriocins, among which class IIa bacteriocins leucocin A (LcnA) and leucocin C (LecC) are among the most frequently studied [7,8]. Additionally, other *Leuconostoc* bacteriocins have been found, such as the class IIb two-peptide bacteriocin leucocin H from *Leuconostoc* MF215B [9] and the class IIc cyclic bacteriocin leucocyclicin Q from *Leuconostoc mesenteroides* TK41401 [10]. Many *Leuconostoc* species produce multiple bacteriocins. For example, *Leuconostoc pseudomesenteroides* QU 15 produces leucocin A and two

* Corresponding author.
E-mail addresses: xing.wan@helsinki.fi (X. Wan), per.saris@helsinki.fi (P.E.J. Saris), timo.takala@helsinki.fi (T.M. Takala).

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class IId bacteriocins leucocin Q and N [11]. *L. mesenteroides* TA33a has been reported to produce three bacteriocins, i.e., class IIa leucocins A and C and class IId leucocin B [12]. In fact, *L. mesenteroides* TA33a is thus far the only strain found to produce leucocin B.

Leucocin B is a 31-aa peptide with 7 positively charged residues [13]. It has a narrow spectrum of activity against some *Leuconostoc* and *Weissella* strains [14]. In general, the knowledge of leucocin B is limited; for example, no gene sequence of the bacteriocin has been published.

In addition to leucocin B from *L. mesenteroides* TA33a, three leucocin B-like bacteriocins have been identified from *Leuconostocaceae*. The closest relative is weissellicin L from *Weissella hellenica* 4–7. The 17-aa determined N-terminal sequence of weissellicin L shares 88% identity with leucocin B [15]. *L. mesenteroides* subsp. *mesenteroides* FR52 produces mesenterocin 52B, which displays 52% identity to leucocin B [14]. According to known 19 N-terminal amino acids, dextranicin 24 from *L. mesenteroides* subsp. *dextranicum* J24 appears to be identical to mesenterocin 52B [16]. Antimicrobial *Leuconostoc kimchii* strain IMSNU 11154 carries gene LKI_04080, whose deduced amino acid sequence matches 74% the leucocin B aa sequence. However, the actual bacteriocin has not been identified from the strain.

Leuconostoc carnosum 4010 is used to protect vacuum-packed meats against the foodborne pathogen *Listeria monocytogenes* [17]. As strain 4010 is generally regarded as safe for food, understanding its antimicrobial properties is important. The strain has been shown to produce antilisterial leucocins A and C. In our previous study, we characterized the gene clusters required for production of leucocins A and C in *L. carnosum* 4010 [18]. In this study, we identified a novel bacteriocin operon for leucocin B expression in *L. carnosum* 4010 and further studied bacteriocin production in native and heterologous hosts.

2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

Strains and plasmids used in this study are listed in Table 1. *Leuconostoc* and *Weissella* strains were grown in calcium-citrate medium (tryptone 20 g/l, yeast extract 5 g/l, NaCl 4 g/l, sodium citrate 2 g/l, calcium lactate 8 g/l, $MnSO_4$ 0.05 g/l) supplemented with sucrose 10 g/l (CCS) at 28 °C. *Lactococcus lactis* and *Carnobacterium* strains were grown in M17 (Oxoid Ltd. Basingstoke, UK) supplemented with 0.5% (w/v) glucose (M17G) at 30 °C. One μg nisin/ml was used for *L. lactis* transformant selection and 0.5 μg nisin/ml for plasmid maintenance and nisin induction. *L. monocytogenes* strains were grown in brain heart infusion (Lab M, Lancashire, UK) agar at 30 °C or in broth with shaking at 28 °C.

2.2. Nucleic acid techniques

L. carnosum 4010 plasmids were isolated according to Anderson and McKay [19] with modifications described in our previous study [18]. Vectors and constructed plasmids from *L.*

Table 1
Strains and plasmids used in this study.

Bacterial strain or plasmid	Relevant properties	Reference/source
Strains		
L. carnosum 4010	Wild-type leucocin producer, DMRICC 4010[a]	Chr. Hansen A/S, Hørsholm, Denmark; Budde et al. [17]
L. pseudomesenteroides CIP103316	Indicator strain sensitive to leucocins A, B and C	A gift from Dr. Anne-Marie Revol-Junelles, University of Lorraine, Nancy, France
Weissella paramesenteroides LMA19	Indicator strain sensitive to leucocins A, B and C	A gift from Dr. Anne-Marie Revol-Junelles, University of Lorraine, Nancy, France
L. pseudomesenteroides AC^R	Spontaneous mutant of CIP103316, resistant to leucocins A and C	This study
L. lactis MG1614	Transformation host	Gasson [41]
L. lactis NZ9000	Transformation host for nisin-induced gene expression with *nisRK* *nisRK* integrated into the chromosome	Kuipers et al. [42]
L. lactis LAC360	NZ9000 carrying the secretion vector pLEB690	Li et al. [21]
L. lactis LAC404	MG1614 carrying *lebB*-plasmid pLEB727	This study
L. lactis LAC409	NZ9000 carrying *lecC*-plasmid pLEB728	Wan et al. [18]
L. lactis LAC410	NZ9000 carrying *lebB*-plasmid pLEB727	This study
L. lactis LAC420	MG1614 carrying *lcnA*-plasmid pLEB756	This study
L. lactis LAC428	NZ9000 carrying *lcnA*-plasmid pLEB756	This study
Listeria monocytogenes WSLC 1018	Indicator strain sensitive to leucocins A and C, ATCC 19118	A gift from prof. Martin Loessner, ETH Zürich, Switzerland
Plasmids		
pLEB690	*L. lactis* secretion vector harboring lactococcal promoters P45 and P_{nisZ} and signal sequence *SSusp45*. 3746 bp, Nis^R	Li et al. [21]
pLEB727	Leucocin B gene *lebB* fused to *SSusp45* in pLEB690 (*Nae*I)	This study
pLEB728	Leucocin C gene *lecC* fused to *SSusp45* in pLEB690 (*Nae*I)	Wan et al. [18]
pLEB756	Leucocin A gene *lcnA* fused to *SSusp45* in pLEB690 (*Nae*I)	This study

[a] DMRICC, Danish Meat Research Institute Culture Collection, Roskilde, Denmark.

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