

Characterization of a bacteriocin produced by *Pediococcus pentosaceus* SB83 for vaginal application

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Introduction

Bacteriocins are ribosomally synthesized and they are mainly known to have effective antimicrobial activity against pathogenic microorganisms. They have a variety of action mechanism, including cytoplasmic membrane pore formation, cell wall interference and nuclease activity (Gillor *et al.* 2005). Bacteriocins can be used as antimicrobial agents for improving the safety of food products and for clinical therapeutics. These proteinaceous molecules appear to be capable of displacing or suppressing the growth of other bacteria, and as such, perhaps provides an advantage to microorganisms in fermenting ecosystems, including the female genital tract (Boris and Barbés 2000). As bacteriocins do not induce vaginal irritation they are suitable for human use. *Listeria monocytogenes*, the responsible agent for listeriosis, is one of several microorganisms which can be transmitted from mother to fetus/neonate by vertical transmission, transplacentally or during passage through the birth canal. This infection is acquired because some women carry *L. monocytogenes* in the vaginal tract. Vaginal infection during pregnancy represents many risks such as stillbirth, abortion, premature delivery or neonatal infection (Donati *et al.* 2010).

Since some lactic acid bacteria (LAB) exhibit antilisterial activity, these bacteria can be used in the prevention of neonatal listeriosis.

Pediococcus pentosaceus SB83 was selected because previous studies demonstrated that this strain is able to inhibit the growth of clinical isolates of *L. monocytogenes* (serotype 1/2a, 1/2b and 4b) by production of a bacteriocin, survives in simulated vaginal fluid at normal vaginal pH (pH of 4.2) and does not show the presence of virulence factors such as the production of gelatinase, lipase and DNase, hemolytic activity and presence of virulence genes (genes *esp*, *agg*, *gelE*, *efaAfm*, *efaAfs*, *cylA*, *cylB* and *cylM*).

The aim of this study was to characterize a bacteriocin produced by *P. pentosaceus* SB83 for potential vaginal application.

Methods

Characterization of bacteriocin SB83

Bacteriocin activity spectrum (against Gram- and Gram+ bacteria)

Bacteriocin production during growth

Effect of various physical and chemical parameters on bacteriocin activity:

- Enzymes (0.1 mg/mL and 1.0 mg/mL of trypsin, proteinase K, pepsin, papain, lipase and catalase)
- Detergents (1% of Tween 20, Tween 80, Triton X-100, sodium dodecyl sulphate (SDS) EDTA (0.1 mM, 2 mM and 5 mM))
- pH (range values between 2.0 and 12.0 (at increments of two pH units), also tested at pH of 4.2 (normal vaginal pH), 5.5 and 6.5 (pH values in case of infection)) (Pavletic, Hawes *et al.* 2004)
- Temperature (4, 25, 30, 37, 60, 80 and 100 °C for 1 h and 2 h. Bacteriocin activity was also tested after 15 min at 121 °C)
- Components of vaginal fluid (the composition of vaginal fluid was described by Owen and Katz (1999))

Mode of action

Adsorption study of the bacteriocin to the producer cells

Determination of approximate molecular weight of bacteriocin molecular size of the bacteriocin by SDS-PAGE

Antimicrobial activity (expressed as arbitrary units (AU) per mL) (Van Reenen *et al.* 1998)

Results and Discussion

Bacteriocin SB83 demonstrated inhibited *L. monocytogenes*, *Enterococcus faecium* DSM2 13590, *E. faecalis* ATCC 2912, *E. faecalis* DSM2 12956 and *Bacillus subtilis*. It should be noted that bacteriocin had no activity against all isolates of vaginal LAB tested

Bacteriocin production reached maximum levels against the three serotypes of *L. monocytogenes* between 24 h and 36 h of growth (6400 AU/mL against serotype 1/2b and 4b, 3200 AU/mL against serotype 1/2a). There was a large decrease of pH during the first 24 h of growth (pH decreased to 4.0)

The effect of various physical and chemical parameters on bacteriocin activity is represented in Table 1

The activity of bacteriocin decreased slightly (14-17%) in simulated vaginal fluid. The components of vaginal fluid that affected the antagonistic activity of bacteriocin were KOH and Ca(OH)₂

Addition of bacteriocin to a 3 h-old culture of *L. monocytogenes* (early exponential phase) repressed the growth for at least 9 h. After addition of bacteriocin a decrease in viable cells *L. monocytogenes* (approximately 4 log within 3 h) was observed

The bacteriocin did not adhere to the surface of the producer cells

The molecular weight of the bacteriocin produced by *P. pentosaceus* SB83, is between 3.5 and 6.5 KDa

Table 1 – Reduction of bacteriocin activity after incubation at different conditions (expressed in percentage values)

	<i>Listeria monocytogenes</i>	<i>Listeria monocytogenes</i>			
		2076 1/2b	2092 4b	2096 1/2a	
Temperature	4 °C	0	14	0	
	25 °C	0	14	0	
	30 °C	0	0	0	
	37 °C	0	0	0	
	60 °C	0	0	0	
	80 °C	0	0	0	
	100 °C	14	14	17	
	121 °C	43	29	17	
	pH	2	14	14	17
		4	0	0	0
4.2		0	0	0	
5.5		0	0	0	
6		0	0	0	
6.5		0	0	0	
8		14	14	17	
10		29	14	17	
12		57	43	100	
Enzymes		Proteinase K 0.1 and 1.0 mg/mL	100	100	100
	Papain 0.1 mg/mL	29	29	67	
	Papain 1 mg/mL	57	57	83	
	Pepsin 0.1 and 1.0 mg/mL	100	100	100	
	Trypsin 0.1 mg/mL	29	43	33	
	Trypsin 1 mg/mL	57	57	50	
	Catalase 0.1 and 1 mg/mL	0	0	0	
Detergents	Lipase 0.1 and 1 mg/mL	0	0	0	
	Tween 20	0	0	0	
	Tween 80	0	0	0	
	Triton X-100	0	0	0	
	SDS	0	0	0	
	EDTA 0.1mM	14	0	0	
	EDTA 2 mM	14	0	0	
EDTA 5 mM	14	0	0		

***Pediococcus pentosaceus* SB83 produces a bacteriocin that is stable in conditions of vaginal fluid (components and pH), that is resistant to different detergents and do not inhibit the vaginal lactobacilli. These characteristics are important to this bacteriocin be applied in vaginal tract**

References

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