Assessment of characteristics of *Pediococcus pentosaceus* SB83 to be used in the prevention of neonatal listeriosis

S. Borges, J. Barbosa, J. Silva, P. Teixeira

CBQF – Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Centro Regional do Porto da Universidade Católica Portuguesa, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

Corresponding author: pcteixeira@porto.ucp.pt

Introduction

The normal vaginal microbiota is dominated by lactobacilli, at 10^7 to 10^8 CFU/mL of vaginal fluid in healthy premenopausal women (Bolton *et al.* 2008). Lactobacilli contribute to the prevention of genital infections and play a role in the maintenance of a healthy state. The capacity that lactobacilli have to adhere and compete for adhesion sites in the vaginal epithelium and the capacity to produce antimicrobial compounds (hydrogen peroxide , lactic acid , bacteriocin-like substances and biosurfactants) are important in the impairment of colonization by pathogens. Furthermore, the production of lactic acid may help to maintain a low vaginal pH, approximately 4-4.5, that makes the vaginal environment more conducive to lactobacilli growth (Bolton *et al.* 2008). There is often a loss of colonization by lactobacilli caused by antibiotic therapy, douching, sexual activity, hormone deficiency, and contraceptive measures. The selection and use of "vaginal probiotics" can be important to restore a healthy vaginal microbiota (Pascual *et al.* 2008). Probiotic have been defined as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". Lactic acid bacteria (LAB) may play a major role in preventing illness of the host, including bacterial vaginosis, yeast vaginitis, urinary tract infection and sexually transmitted diseases. The administration of probiotics by mouth and intravaginally has been shown to be safe, and for pregnant women this restoration could be important to lower the risk of preterm labor (Reid and Bocking 2003). *Listeria monocytogenes* is a bacterium that, during pregnancy, can cause many serious complications including preterm labor, chorioamnionitis, spontaneous abortion, stillbirth and neonatal infection. Vaginal colonization by pathogens can result in transmission to the fetus/neonate by vertical transmission. Therefore, the vaginal application of LAB could be a preventive strategy to reduce the global burden of neonatal infections.

The aim of this study was to select, identify and characterize an isolate of LAB to be considered as vaginal probiotic candidate.

Methods

Screening for antimicrobial activity of 56 isolates of LAB against 29 clinical isolates of *L. monocytogenes* (serotypes 1/2a, 1/2b and 4b)

Quantification of antibacterial activity (Van Reenen *et al.* 1998)

Identification of bacteriocin-producing lactic acid bacteria by ribosomal 16S rDNA sequencing

Survival of LAB in simulated vaginal fluid at pH of 4.2, at different time intervals (4, 8, 24 and 48 h) (Borges *et al.* 2011)

Based on the bacteriogenic activity and survival in simulated vaginal fluid, one isolate of LAB (*Pediococcus pentosaceus* SB83) was selected.

Determination of virulence factors (production of gelatinase, lipase and Dnase, hemolytic activity and presence of virulence genes *esp*, *agg*, *gelE*, *efaAfm*, *efaAfs*, *cylA*, *cylB* and c*ylM*)

Biofilm assay in Man Rogosa and Sharpe medium (MRS) and simulated vaginal fluid (SVF) at pH 4.2, 5.5 and 6.5 (Borges *et al.* 2011)

Antibiotic susceptibility testing (determination of minimum inhibitory concentration (MIC; µg/mL) of seventeen antibiotics

Results and Discussion

62.5% (35/56) of LAB demonstrated anti-listerial activity by production of a bacteriocin

Bacteriocin activity of LAB isolates against *L. monocytogenes* (serotype 1/2a, 1/2b and 4b) varied between 400 to 6400 AU/ml, although values of 1600 and 3200 AU/ml were more common.

All strains with anti-listerial activity were identified as *Pediococcus* spp. (3 isolates were *P. acidilactici* and 32 isolates were *P. pentosaceus*)

In general, the behavior of *Pediococcus* spp. in vaginal fluid was similar ($P = 0.30833$).

- During 24 h there were no great reductions in viable cells. log (N/N_0) varied between 0.03 and -0.4
- After 48 h a greater reduction was observed - six isolates showed a decrease of more than 1.0 log. At this time, the most sensitive isolate had decreased by 1.6 log and the most resistant had reduced by only 0.2 log viable cells. Thus, after 48 h, survival was demonstrated to be strain dependent ($P < 0.0001$).

Characterization of *P. pentosaceus* SB83

The presence of virulence factors were not shown by *P. pentosaceus* SB83

P. pentosaceus SB83 produced biofilm in MRS and SVF at different pH values.

- In SVF, *P. pentosaceus* SB83 produced more biofilm at pH 4.2 (normal vaginal pH) than at higher pH (pH 5.5 and 6.5)
- In MRS, biofilm increase with increasing pH

P. pentosaceus SB83 was considered sensitive to cloramphenicol, gentamicin, streptomycin, kanamycin, erythromycin, and ampicillin. This strain was considered resistant to tetracycline and intrinsically resistant to vancomycin (EFSA, 2008) (Table 1).

Table 1 - MIC (µg/mL) of seventeen antibiotics for *P. pentosaceus* SB83

Antibiotic	Vancomycin	Chloramphenicol	Nitrofurantoin	Erythromycin	Tetracycline	Ciprofloxacin	Rifampicin	Gentamicin	Streptomycin	Oxacillin	Kanamycin	Ceftazidime	Penicillin G	Ampicillin	SXT	Meropenem	Imipenem
µg/mL	512	4	128	0.125	16	16	8	0.25	8	8	4	32	0.5	2	>32	1.5	0.125

These *in vitro* results provide a basis for the use of *P. pentosaceus* SB83 as a vaginal probiotic, to prevent colonization of *L. monocytogenes* in pregnant women

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