Experimental Parasitology 146 (2014) 52–63

Contents lists available at ScienceDirect

Experimental Parasitology

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

Full length article

Therapeutic enhancement of newly derived bacteriocins against *Giardia lamblia*

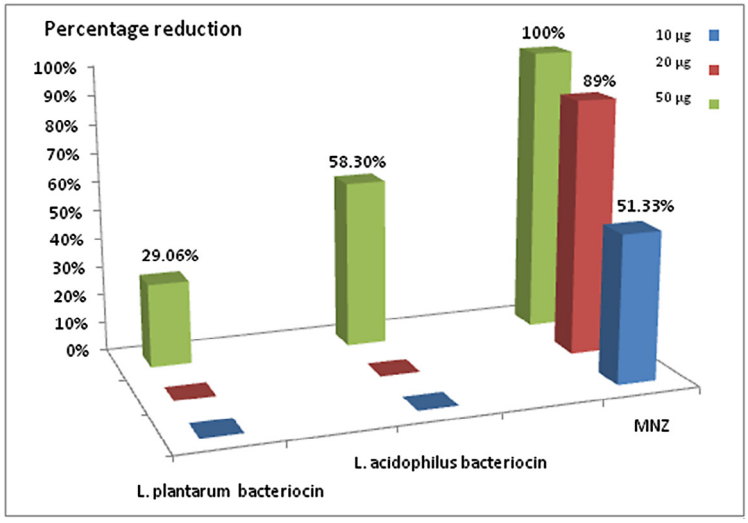
Eglal I. Amer [a,*], Shereen F. Mossallam [a], Hoda Mahrous [b]

[a] Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt
[b] Industrial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt

HIGHLIGHTS

- *L. acidophilus* bacteriocin showed *in vitro* activity against *G. lamblia* trophozoites.
- Bacteriocin induced severe morphological changes using electron microscopy.
- Oral bacteriocin for 5 days was sufficient to induce massive parasite reduction.
- *L. acidophilus* bacteriocin induced amelioration of intestinal pathology.
- Bacteriocin holds great promise as an alternative therapy for giardiasis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 20 September 2014
Accepted 24 September 2014
Available online 6 October 2014

Keywords:
Giardia lamblia
Bacteriocin
Lactobacillus acidophilus
Lactobacillus plantarum
In vivo
In vitro

ABSTRACT

Trials for identifying efficient anti-giardial agents are still ongoing. Nowadays, bacteriocins have attracted the attention as potential antimicrobial compounds. For the first time, the current study evaluated the therapeutic efficacy of bacteriocins derived from newly isolated Egyptian strains of probiotics *Lactobacilli; L. acidophilus* (P106) and *L. plantarum* (P164) against *Giardia lamblia*. Bacteriocins' efficacy was evaluated both *in vitro*; by growth inhibition and adherence assays, and *in vivo*; through estimation of parasite density, intestinal histopathological examination and ultrastructural analysis of *Giardia* trophozoites. *In vivo* bacteriocins' clinical safety was assessed. In vitro results proved that 50 µg of *L. acidophilus* bacteriocin induced reduction of the mean *Giardia lamblia* trophozoites by 58.3 ± 4.04%, while at lower concentrations of 10 and 20 µg of both *L. acidophilus* and *L. plantarum*, non significant reduction of the mean parasite density was achieved. In vitro trophozoites adherence was susceptible to the tested bacteriocins at all studied concentrations with variable degrees, while the highest adherence reduction was demonstrated using 50 µg of *L acidophilus* bacteriocin. In vivo, oral inoculation of 50 µg/mouse *L. acidophilus* bacteriocin for 5 successive days resulted in a noteworthy decline of the intestinal parasite density, along with amelioration of intestinal pathology of infected mice. Ultrastructural examination proved that

* Corresponding author. Fax: +20 3 4831498.
E-mail address: ehabaman2003@yahoo.com (E.I. Amer).

http://dx.doi.org/10.1016/j.exppara.2014.09.005

five doses of *L. acidophilus* bacteriocin showed marked changes in cellular architecture of the trophozoites with evident disorganization of the cell membrane, adhesive disc and cytoplasmic components. This is the first reported study of the safe anti-giardial efficacy of *L. acidophilus* (P106) derived bacteriocin, hence highlighting its great promise as a potential therapeutic safe alternative to existing commercial drugs.

1. Introduction

Giardiasis, the most common intestinal protozoal infection reported in humans, is considered as one of the important causes of diarrheal disease worldwide. Recently, giardiasis was included in the 'Neglected Disease Initiative', estimating that 280 million people are infected each year (Lalle, 2010; Savioli et al., 2006). At present, treatment options include the nitroheterocyclic drugs tinidazole, metronidazole (MNZ) and furazolidone, the substituted acridine, quinacrine, and the benzimidazole, albendazole. Paromomycin is also used in some situations, and nitazoxanide is proving to be useful (Morrone et al., 2004; Sullayman et al., 2002). Among the current recommended treatments, MNZ has been the mainstay of treatment for decades and is still widely used (Escobedo and Cimerman, 2007). However, most of the therapeutically used anti-giardial drugs, including MNZ, cause severe side effects and are not well tolerated by many patients. Furthermore, existing antimicrobial therapies are not always effective, and drug resistance to all available drugs has been demonstrated in the laboratory (Petri, 2003). In addition, clinical resistance has been reported, including cases where patients failed both MNZ and albendazole treatments (Tian et al., 2010). Hence, identifying new anti-giardial agents is an important consideration for the future (Tejman-Yarden and Eckmann, 2011).

Some bacteria, fungi, plants, insects and vertebrates excrete antimicrobial peptides, which are known to have inhibitory effects directed against enveloped viruses, bacteria, fungi, parasites as well as cancer cells (Pálffy et al., 2009). Bacteriocins are a diverse family of ribosomally synthesized proteins produced by probiotic bacteria. According to the generally accepted definition, probiotics are live microorganisms beneficially affecting the health of a host animal (Fuller, 1989). To be effective, probiotic bacteria must exhibit a number of functional characteristics, including the resistance to technologic processes, gastric acidity, bile toxicity and the ability to adhere to the intestinal epithelium. Several reports have indicated that the survival and viability of bacteria from most probiotic products in gastric juice is rather poor, and only few of them exhibit stability and resistance to adverse environmental conditions (Dunne et al., 2001; Nighswogner et al., 1996). Therefore, bacteriocins derived from probiotic bacteria could provide the beneficial role of bacteria away from these conditions. Although research in this area is still in its infancy, there is intriguing evidence to suggest that bacteriocins are characterized by their antimicrobial activity, most frequently against bacteria which are phylogenetically closely related (narrow spectrum of activity). However, a surprisingly high level of inter-specific killing was observed; with almost half of the bacteriocins affect more than one taxon. Furthermore, the relationship between killing ability and phylogenetic distance is nonlinear. This nonlinear relationship indicates killing outside of the producer strain's own species. The observation of a broad killing range for numerous enteric bacteriocins requires that the ecological role proposed for bacteriocins should be reconsidered (Dawson and Scott, 2012; Dobson et al., 2012). Nowadays, bacteriocins have attracted the attention as potential substitutes for, or as combined therapy to currently used antimicrobial compounds due to their powerful killing activity, stability and low toxicity to humans (Hassan et al., 2012; Joerger, 2003). Therefore, this study was designed to evaluate, for the first time, the possible in vitro and in vivo therapeutic efficacy of bacteriocins derived from newly isolated Egyptian strains of probiotics *L. acidophilus* (P106) and *L. plantarum* (P164) against *Giardia lamblia* (*G. lamblia*).

2. Material and methods

2.1. Bacteriocins

2.1.1. Probiotic strains isolation and maintenance

Two probiotic strains; *L. acidophilus* and *L. plantarum* have been isolated from healthy breast-fed 15 day old Egyptian infants, and were identified as P106 and P164; respectively by Mahrous (2006). They were used after the selection had been done according to Bergey's Manual of Determinative Bacteriology, 9th edition with confirmation of the identification by SDS-PAGE technique and API System (Holt et al., 1994). The strains were tested for their probiotic characteristic i.e. gastric acid resistance, bile salt tolerance, antibacterial activity, adhesion to human mucus. Their antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells of Gram negative *E. coli* ATCC25922 and Gram positive *Bacillus subtilis* NIB3610. *Lactobacillus* strains were cultivated in MRS (de Man Rogosa Sharpe) broth (Lab M, IDG, UK) and incubated at 37 °C in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) provided with disposable BBL gas generating pack (CO2 system envelopes, Oxoid, Ltd., West Heidelberg, VIC, Canada). Isolates were stored at –20 °C in MRS broth supplemented with 25% (v/v) glycerol. For routine analysis, the strains were subcultured twice in MRS broth at 37 °C for 24 h.

2.1.2. Bacteriocin extraction, preparation and purification

Isolated *L. acidophilus* and *L. plantarum* strains were grown in MRS broth at 37 °C for 24 h. After incubation, the bacterial cells were removed by centrifugation and 200 mL of the crude bacteriocins supernatant was treated with 1.4 g streptomycin sulfate for nucleic acid precipitation. The supernatant was fractionated by the addition of 525 g solid ammonium sulfate with continuous stirring to get 80% saturation. The precipitated fraction was collected by centrifugation at 10,000 rpm for 10 min at 4 °C and dialyzed against 10 volumes of phosphate buffer pH 7.5. The buffer was changed several times, until equilibration occurred after overnight dialysis. The dialyzed solution was used to find the concentration of protein. Further purification was carried out using column chromatography with a Superdex-G 200 packed column (1.5 × 3 cm), using 0.1 M phosphate buffer (pH = 7) as the eluant. The purified sample was dissolved in the eluting buffer at 10 mL and applied to the gel permeation column at room temperature. An elution flow rate of 1.5 mL/15 min was employed and fractions were collected sequentially, and monitored by UV absorption at 280 nm. The bacteriocin-containing samples (fractions 15–25) were pooled and the resultant solutions were dialyzed against distilled water (molecular weight cut-off 10 kDa) overnight (Van Reenen et al., 1998).

2.1.3. Bacteriocin activity units

Twofold serial dilutions of cell free neutralized supernatant of the producer strain and of partially purified (80% ammonium sulfate precipitated) bacteriocins were prepared and were followed for agar well diffusion assay. Plates were incubated overnight at 30 °C and zones of inhibition around each well were measured in mm. The bacteriocin titer was expressed as arbitrary or activity unit/mL. One arbitrary unit (AU) of bacteriocin is defined as the reciprocal of the