Original Article

Antagonistic effect of bacteriocin against urinary catheter associated *Pseudomonas aeruginosa* biofilm

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Abstract

**Context:** *Pseudomonas aeruginosa* is a gram negative opportunistic bacteria causes several infections commonly colonize these devices and developing biofilms. Bacteria in biofilm can be up to 1,000 times more resistant to antibiotics than the same bacteria circulating in a planktonic state. **Case Report:** A total of 10 isolates of *Pseudomonas aeruginosa* were isolated from catheter associated urinary tract infections. While carbenicillin was the most effective antibiotic, all isolates developed multidrug resistant. Both crude and purified bacteriocin showed marked inhibition activity against planktonic and biofilm of the highly resistant isolate *P. aeruginosa* P7. **Conclusion:** Bacteriocin extracted from a locally isolated *L. acidophilus* has an anti *P. aeruginosa* biofilm activity also it can be used as a therapeutic agent after adequate in vivo experimentation.

**Keywords:** Antagonistic, catheter, *Pseudomonas aeruginosa*, bacteriocin, biofilm.

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Introduction

A biofilm is a thin layer of micro-organisms that adhere to the surface of an organic or inorganic structure, together with their secreted polymers. Biofilms are the predominant phenotype of nearly all bacteria in their natural habitat, whether pathogenic or environmental [1]. Indeed, bacteria in a biofilm environment can be up to 1,000 times more resistant to antibiotics than the same bacteria circulating in a planktonic state [2].

Urinary catheters are tubular latex or silicone devices, which when inserted may readily acquire biofilms on the inner or outer surfaces. The longer the urinary catheter remains in place, the greater the tendency of these organisms to develop biofilms and result in urinary tract infections [3]. *Pseudomonas aeruginosa* is a gram negative opportunistic bacteria causes several infections commonly colonize these devices and developing biofilms [4]. This study attempts to evaluate the impact influence of bacteriocin extracted from *Lactobacillus acidophilus* on biofilm of *P. aeruginosa* formed on Foley catheter.

Materials and Methods

**Isolation and Identification**

Ten isolates of *P. aeruginosa* were isolated from catheterized patients presented with urinary tract infections. After withdrawal of catheter, it was cut into pieces of 1 cm in length, rinsed in phosphate buffered saline then cultivated in 5 ml of Brain Heart Infusion broth (BHIB) at 37°C for 24 hr. Identification was done according to protocol illustrated by Holt et al [5].

In order to isolate *L. acidophilus*, one ml sample was taken from yoghurt and cultured by spreading on MRS agar (Hi-Media, India, pH 5.5). The plates were incubated at 37°C in anaerobic jar for 24-48 hour. Thereafter, smooth convex whitish to creamy colonies were isolated, sub-cultured on MRS agar medium and incubated for 24-48 hour [6]. The lactobacilli were initially identified by their ability to grow on the selective MRSA, gram-positive staining, rod shape, and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth [Hi-Media, India], were conducted as described in the second edition of Bergey's manual [7].
**Antibiotic susceptibility**

Antibiotic susceptibility test towards piperacillin, imipenem, Cefotaxime, Carbenicillin, Amikacin, Ceftriaxon, Ciprofloxacine, Gentamicin, Tobramycin and Trimoxazole was done according to the method of Bauer et al [8]. The isolate which resist the highest number of antibiotics was elected for further experiments.

**Preparation of crude bacteriocin**

An overnight culture of *L. acidophilus* was adjusted with MRS broth in accordance to McFarland turbidity standard tube no. 0.5 as measured by absorbance (0.08-0.1 at 625nm) using Cary 100 spectrophotometer (Varian PTY ltd., Australia) corresponding to approximately 1.5-2 × 10^8 cfu/ml. Afterward, the culture was propagated in the same broth at 37°C for 24 hr under anaerobic conditions. Cells were separated by centrifugation at 6000 rpm/min for 10 min at 4°C. The resulting supernatants were filtered through a 0.2-μm membrane filter. All supernatants were cultured on MRS agar in order to confirm the absence of lactobacilli cells. Thereafter, they were stored at 4°C until the assay. In parallel, Aliquots of supernatants neutralized with 1N NaOH were prepared as well [9].

**Purification of bacteriocin**

The bacteriocin present in the supernatant fraction was concentrated by ammonium sulphate precipitation (700 g/l). After the mixture had been stirred overnight at 4°C, the precipitate was pelleted by centrifugation at 10,000 g. The collected precipitate was then dissolved in 0.05M sodium acetate buffer pH 5.0 and dialyzed using a 1 kDa cut-off membrane against the same buffer at 4°C overnight [10]. Inhibitory activity was assayed before and after purification. Protein concentration after each purification step was determined [11].

**Inhibitory activity of crude and purified bacteriocin on planktonic P. aeruginosa P7**

Well diffusion method described by Ikeagwu et al [12] was followed to detect inhibitory effect of *L. acidophilus* supernatants as well as purified bacteriocin on planktonic *P. aeruginosa* P7.

The titers of crude and purified bacteriocin were quantified by two fold serial dilutions of bacteriocin in saline solution and aliquots of 50 μl from each dilution were placed in wells in plates previously seeded with the *P. aeruginosa* P7. These plates were incubated aerobically at 37°C for 24 h and examined for the presence of 2 mm or larger clear zones of inhibition around the wells. The antimicrobial activity of the bacteriocin was defined as the reciprocal of the highest dilution showing inhibition of *P. aeruginosa* P7 and was expressed in activity units per ml (AU/ml) [13].

**Biofilm assay**

The method of Jones and Versalovic [14] was followed with some modifications in order to produce biofilm on urinary catheter. Briefly: Foley catheter was cut into 1 cm pieces posted in 20 ml of BHIB containing *P. aeruginosa* P7 cultured in a final concentration of 1.5 × 10^8 cfu/ml. Containers were incubated aerobically at 37°C for 24 hrs. Media and planktonic cells were removed by decantation and two washes with distilled water (DW) were done. Two hundred microliters of BHIB supplemented with bacteriocin (1/32 v/v) were added and incubated for another 24 hrs. All pieces were washed twice with DW while the adherent cells were stained with crystal violet (1% w/v) for 10 minutes. Thereafter, the catheter pieces were washed with DW. Finally, the crystal violet was redissolved with ethanol and the OD at 625nm was determined spectrophotometrically. Hence absorbency will represent the biofilm thickness. Foley catheter pieces placed in sterile BHIB were designated as a blank. Control was prepared by cultivating Foley catheter pieces with bacterial culture free of bacteriocin. Simultaneously, viable count following the procedure described by Harley and Prescott [15] was carried out to determine the viability of bacterial cells within the biofilm. All assays were done in triplicates.

**Statistical analysis**

Data is presented as mean ± standard deviation. ANOVA and LSD_0.05 were employed for data analysis using Microsoft office Excel 2007 application.

**Discussion**

A total of 10 isolates of *Pseudomonas aeruginosa* were isolated from catheter associated urinary tract infections. Morphologically all of these isolates were gram negative, non-sporing, capsulated, and motile bacilli, produced typical grapes like odor. They were also positive for oxidase, pyocyanin production as well as growth test at 4 and 42°C. All isolates were non glucose fermenter and succeeded in grown on cetrimide agar.

Data presented in Figure 1 showed that most isolates (9 isolates) were resistant to ceftriaxon; while carbenicillin was the most effective antibiotic. All isolates developed multidrug resistant. However, isolate P7 developed resistance against the highest number of antibiotics under investigation; therefore, it was elected for further experiments.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Antibiotic resistance of *Pseudomonas aeruginosa* isolated from urinary catheter associated urinary tract infections.

Purification steps of bacteriocin are depicted in Table 1.
The bacteriocin of *L. acidophilus* was recovered with an increase in specific activity from 10 to 26.6 AU/mg after precipitation by 70% saturation of the culture broth with ammonium sulphate. These results agreed with findings of Mojgani et al [13], Invanova et al [16] and Ogunbanwo et al [17]. Mojgani et al [13] reported that the increase in activity could be due to release of active monomers from bacteriocin complexes. During salt precipitation various amount of the protein was fractionated as a surface pellicle, this might be due to the association of bacteriocin molecules with the hydrophobic globular micelle like structure in the supernatant fluid.

Purified bacteriocin showed significant activity against the multi drug resistant *P. aeruginosa* isolate over the crude bacteriocin (*P* < 0.05) as it shown in Table 2.

Treating the biofilm formed on the Foley catheter by *P. aeruginosa* P7 with crude and purified bacteriocin caused a significant reduction (*P* < 0.05) in absorbancy (i.e. biofilm thickness) reached 0.536 ± 0.04 and 0.299 ± 0.07, respectively, in comparison to control (0.733 ± 0.13) as it diagrammed in Figure 2.

![Fig. 2](image)

| Titer | Inhibition zone diameter [mm] |
|-------|------------------------------|
| neate | 12.3 ± 1.5                   |
| 1/2   | 10 ± 1.7                     |
| 1/4   | 7.6 ± 1.1                    |
| 1/8   | 5.3 ± 0.57                   |
| 1/16  | 4.3 ± 0.57                   |
| 1/32  | 1.6 ± 0.57                   |
| Purified bacteriocin ± SD | 15 ± 1.7 |
|       | 13.6 ± 1.1                   |
|       | 12.3 ± 0.57                  |
|       | 11.6 ± 0.57                  |
|       | 10.6 ± 1.1                   |
|       | 7 ± 1                        |

SD: standard deviation. *P* = 9.34 × 10⁻¹⁵. LSD: 1.8. Each datum is the mean of triplicate. Means with similar letters have insignificant differences.

Table 2 Inhibition of multi drug resistant *Pseudomonas aeruginosa* P7 by *Lactobacillus acidophilus* bacteriocin.

The present work can conclude that although *P. aeruginosa* biolim is hardly to be eradicated by pseudomandal antibiotics, the bacteriocin extracted from a locally isolated *L. acidophilus* has an anti *P. aeruginosa* biolim activity also it can be used as a therapeutic agent after adequate in vivo experimentation.

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