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Antagonistic Activity of *Lactobacillus* Against Uti Pathogens

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ABSTRACT

Urinary tract infections (UTIs) are the most frequent community-acquired infections in world wide. The antimicrobial activity *lactobacillus* and its bacteriocins production were studied. A total 25 isolates were obtained from 35 urine samples. The most recurrently encountered pathogens were *E. coli* (9) 25.71%, *Klebsiella pneumonia* (7) 20%, *Proteus mirabilis* (4) 11.42%, *Pseudomonas aeruginosa*(4) 11.42%, and *Candida albicans* 1(2.8%) respectively. It was observed that the *lactobacillus sp* effectively inhibit the UTI pathogen such as *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans.* Based on antimicrobial activity, the potential strains were selected for optimization of bacteriocin activity at different temperature and pH value. The effect of NaCl on bacteriocin activity was also studied. It was clearly noted that the *Lactobacillus* showed very strong inhibition against *Pseudomonas aeruginosa* (15.5mm) and *Klebsiella pneumonia* (13.2mm), strong inhibition against *Proteus mirabilis* (12.1mm) and moderate inhibition against *Escherishia coli* (10mm) and *Candida albicans* (10.0mm). The activity of bacteriocin was stable between the pH 4.0 to 5.0 and it didn't show any antimicrobial activity above pH 5.0. The activity of bacteriocin was stable at temperature less than 80^0C and above 1% NaCl concentration increased the production of bacteriocin from 25-75%. Thus the above study indicates that *Lactobacillus* can be used as a alternative therapy to treat the UTI infection and to overcome the emergency of rapid increase of drug resistance UTI pathogens.

Key words: Urinary tract infection, Lactobacillus, Antimicrobial activity, bacteriocin.

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INTRODUCTION

Urinary tract infections (UTIs) are the most frequent community-acquired infections in world wide. In each year, about 5% of women can be affected by UTI infection. Urinary tract infections are treated by the use of broad spectrum of antibiotics [Hooton, 2003]. This antibiotics treatment leads to increase the drug resistance microbes that has been made therapy of UTI is difficult. Infectious diseases caused by resistant microorganisms are accountable for increased health costs as well as high morbidity and mortality, especially in developing countries [Pfaller *et al.*, 1997]. In recent years, there has been increased focus on the use of probiotic such as *Lactobacillus sp.* for prophylaxis and treatment of urinary tract infection [Uehara *et al.*, 2006]. In humans, *Lactobacilli* are an essential part of the normal flora usually found in the mouth, gastrointestinal tract and female genitourinary tract. They also protect the female urogenital tract from pathogen colonization by the production of lactic acid which turns the environment acidic, that inhibit the growth of some harmful bacteria. *Lactobacilli* are commonly found in vaginas of healthy women where it maintain vaginal health due to production of lactic acid, bacteriocins and H2O2 , that suppress the growth of pathogens such as *Escherichia coli* and *Gardnerella vaginalis* [Eschenbach *et al.*, 2009]. The lactic acid bacteria have several scientifically established and clinically proved health effects such as reduction and prevention of diarrhea of different origin, alleviation of lactose intolerance symptoms, improvement of the intestinal microbial balance by antimicrobial activity, prevention of food allergy, enhancement of immune potency, antitumerogenic activities (Anterson *et al.*, 2001). Therefore, the aim of this study was to determine the antagonistic activity of *Lactobacillus* against UTI pathogens.

METERIALS AND METHOD

Collection of Urine Samples

Twenty five urine samples were collected from Excellent Hospital in Velachery, Chennai, Tamil Nadu India. The mid stream urine samples were taken after cleaning the genital area with soap and water. The samples were collected by using in sterile plastic universal containers. Then the samples were transported to the laboratory in an ice cold condition. The samples were divided into two parts; one was used for microscopic examination, and the other for culturing.

Processing of the Specimens

- Macroscopic examination was done.
- Microscopic examination was done.

Culture method

Samples were plated on nutrient agar, EMB agar, MacConkey agar and Sabarouds dextrose agar. All the bacterial isolates are identified by their characteristic colony morphology, gram staining, motility, catalase test, oxidase test, coagulase test and biochemical reactions based on the guidelines described in the Koneman's Colour Atlas and Textbook of Diagnostic Microbiology.

ISOLATION AND IDENTIFICATION OF *LACTOBACILLUS*

Collection of Milk Samples

Containers were washed thoroughly and were sterilized at 121^0C for 15 minutes in autoclave. Aseptically cow milk samples were collected in sterile containers.

Cultivation of *Lactobacillus*

The collected fresh milk samples were inoculated in (Deman, Rogosa and Sharp) MRS broth and agar medium by spread plate method then incubated at 37^0C for 48 hours.

Lactobacillus Characterization

Grams staining, catalase test and vancomycin sensitivity test were done and the isolates were designated as Lb series and numbered randomly (Lb-1 to Lb-4) (Barbara *et al.*, 2010).

Screen for Antimicrobial Activity By Kirby Bauer Method

The four isolates of *Lactobacillus* namely Lb-1 to Lb-4 are screened for antimicrobial activity against UTI pathogens such as *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans.* The antimicrobial activity was detected by using agar well diffusion method. The test pathogens were inoculated on MHA plates after comparing with 0.5 Macfarland standards. 0.5mm diameter wells were made on preinoculated Muiller Hinton agar plates and supernatant was transferred at the rate of 50µl into each wells. The plates were kept in a 3 hours allowing the diffusion of samples into each wells followed by incubation at 37^oC for 48 hours without inverting. Zone of inhibition formed around the wells were measured.

Preparation of Culture Supernatant

From the MRS plates the potential strains were selected for optimization of bacteriocin activity. The potential isolates (Lb-2) were grown in (Deman, Rogasa and Sharp) MRS broth at 37^oC for 18-20 hours. The cultures were centrifuged at 10,000 rpm for 5 minutes and then the supernatant were adjusted to pH 6.5 to 7.0 with 1N NaOH (Sengul *et al.*, 2003).

Optimization of Bacteriocin Activity

Sentivity to Heat

100ml of culture supernatant were heated for 10 minutes at different temperatures such as 60^{o}C, 70^{o}C, 80^{0}C, 90^{o}C and 100^{o}C and then antimicrobial sensitivity testing was performed by agar well diffusion method.

Sensitivity to Different pH Values

The pH of culture supernatants was adjusted to different pH values such as 3.0, 4.0, 5.0, 6.0, 9.0 and then kept at room temperature for 4 hours. Then antimicrobial activity of bacteriocin in different pH was measured by agar well diffusion method.

Effect of NaCl on Bacteriocin Activity

After sterilization by autoclaving, MRS broth with 1%, 2%, 3% NaCl were aseptically transferred to the growing cultures and grown overnight. Bacteriocin production and antimicrobial activity of Bacteriocin was measured by agar well diffusion method.

RESULT AND DISCUSSION

Urogenital infections are worldwide common problem that affects the bladder, kidneys, urethra, periurethra and cervix which are leading to cause of hospitalization for patients with UTI infection. This present study investigates an antimicrobial activity of *lactobacillus* against UTI causing pathogens.

Prevalence of UTI Infection

The UTI infections were significantly high in female (68.57%). Trivedi *et al.*, 2014 reported 40 to 50% of female population were frequently encounter with UTI infection. Spahiu and Hasbahta, (2010) reported in his study, the males 67. 86% are predominantly affected with the age group of 1-28 days of infants. They also proposed female (56.07%) were mainly affected in the age group of 7-16 years than male.

Microbiological Profile of Urinary Tract Infection

In the present study, a total numbers of 12 isolates are detected from 10 UTI infected patients. *E.coli* (41.6%) was found to be largest isolate followed by *Klebsiella pneumonia* (25%), *Proteus mirabilis* (16.6%), *Pseudomonas aeruginosa* (8.3 %) and *Candida albicans* (8.3%) respectively. Bashir et *al.*, (2008) reported, *E. coli* was predominant isolate (66%) followed by *Candida sp.*(13.89%) *and Pseodomonas spp.* (13.8%), *Klebsiella spp.* (11.11%) and *Enterobacter spp.* (2.7%). *Proteus* and *Morgenella species* were found to be <1%.

Isolation of Lactobacillus from Cow Milk Samples

Lactobacillus was isolated from cow milk. *Lactobacillus* was identified as Gram- positive bacilli, non- motile, catalase negative, oxidase negative, facultative anerobic or microaerophilic bacteria

and vancomycin resistant. This study correlates with the study of Bushra *et al.*, 2009 reported the *Lactobacillus* species was isolated from different sources such as yoghurt, cow milk and vagina.

Antimicrobial Activity of *Lactobacillus*

Among the four isolates tested, the isolates Lb-2 exhibited highest antimicrobial activity followed by Lb-1, Lb-3 and Lb-4 (Table 1). Hence the isolate Lb-2 was considered as potential. The potential *Lactobacillus* strain (Lb-2) was exhibited highest inhibitory activity against UTI pathogens such as *Escherishia coli* (10.0mm), *Klebsiella pneumonia* (13.2mm), *Pseudomonas aeruginosa* (15.5mm), *Proteus mirabilis* (12.1mm) and *Candida albicans* (10.0mm). Among the test micro organisms, the maximum zone was observed. Datta *et al.*, 2013 reported the lactobacillus species exhibited antagonistic activity against various pathogenic microorganism such as *E.coli*, *Klebsiella*, *S. aureus*, *Proteus*, *Streptococcus*, and *Pseudomonas*. The highest zone of inhibition was observed in *Pseudomonas* (25mm). Least results were found in *Proteus* (5mm) and *Streptococcus* (5mm).

Table 1: Antimicrobial Activity of Bacteriocin

Test organisms	Lb-1	Lb-2	Lb-3	Lb-4
	Zone of inhibition mm in diameter			
E.coli	7.0	10.0	5.0	5.0
Klebsiella pnumoniae	10.0	13.2	7.0	10.9
Proteus mirabilis	9.3	12.1	8.8	9.6
Pseudomonas aeroginosa	12.3	15.5	11.8	12.4
Candida albicans	6.8	10.0	9.1	6.6

Optimisation of Bacteriocin Activity

The activity of bacteriocin was stable between the pH 4.0 to 5.0 and it didn't show any antimicrobial activity above pH 5.0 (Figure 1). The activity of bacteriocin was stable at temperature less than 80^0C (Figure 2) and above 1% NaCl concentration increased the production of bacteriocin from 25-75% (Figure 3). Selvamohan and Sujitha, 2010 reported the activity of bacteriocin from *Lactobacillus plantarum* showed highest zone of inhibition at temperature of 68^0C for 20 minutes. The bacteriocin activity was stable at pH 3.0 to 5.0 and the inhibitory activity was lost when the pH was raised above 5.0. The highest bacteriocin activity was observed with Lf3 against *V. cholera* subsp *Inaba* at 30^0C, pH 6.0, and 1.5 to 2.0% of NaCl concentration (Asha and Gayathri, (2012).

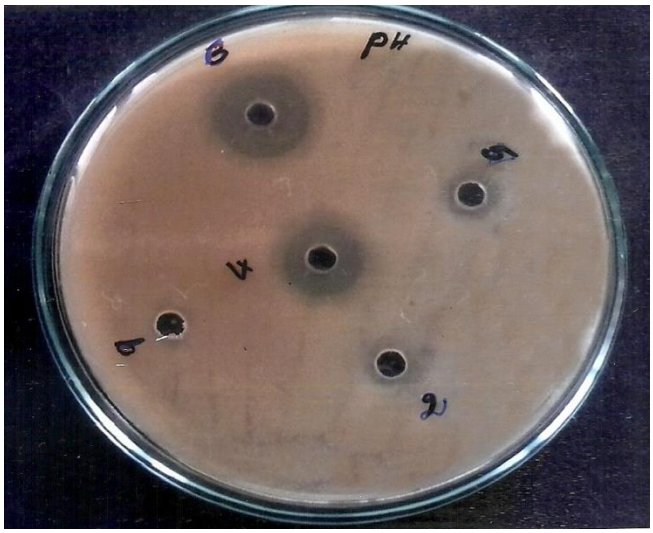

Figure -1 Effect of pH on the Bacteriocin activity of *E.Coli*

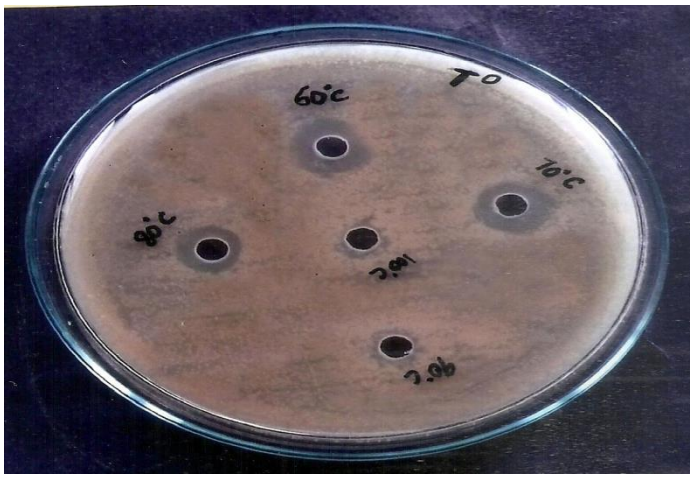

Figure - 2 Effective of Temperature on the bacteriocin activity on *E.Coli*

Figure -3 Effect of NaCl in the Bacteriocin Activity

CONCLUSION

The conclusion of our study is *Lactobacillus* exhibits very effective antimicrobial activity against UTI pathogens such as *E.coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Candida albicans*. Hence the *Lactobacillus* used as alternative therapy to treat the UTI infection and to overcome the emergency of rapid increase of drug resistance UTI pathogens.

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