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Antimicrobial activity of a probiotic *Lactobacillus Plantarum* against urinary tract infection (UTI) causing pathogens

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ABSTRACT

The aim of this study is to test the probiotic Lactobacillus plantarum against UTI pathogens. The antimicrobial activity of Lactobacillus plantarum and its bacteriocins was examined and it found to be effective against Escherichia coli and the least activity was observed by Streptococcus sp. we also found that bacteriocins can withstand at various temperature and pH without complete lose of its activity. The bacteriocins can stored at -20^{o}C or 4^{o}C for more than 100 days without any considerable changes in their activity Lactobacillus plantarum shows resistant to most of the antibiotic tested.

Keywords: Probiotic *Lactobacillus plantarum*, bacteriocin, antimicrobial activity.

INTRODUCTION

Urinary tract infection is frequently accompanied with urologic abnormalities and can cause end stage renal failure or hypertension if continued. This results in using antibiotics for the purpose of controlling uropathogens produces even superbacteria which are multi-resistant and cause intractable infections that are difficult to treat [7].

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 [17]. Probiotics have been defined as “live microorganisms whichwhen administered in adequate amounts confer a health benefit on the host” [15].

Lactobacilli are an important part of the normal flora commonly found in the mouth , gastrointestinal tract and female genitourinary tract. They also protect the female urogenital tract from pathogen colonization by competitive exclusion of pathogens from the cell surface, co-aggregation with certain pathogenic bacteria , adhere to epithelial cells and bioflim formation based on autoaggregation and surface hydrophobicity [4].

Lactobacilli produce many different bacteriocins of similar activity and are usually predominant species in the vaginas of healthy women. It play an important role in maintaining vaginal health. They produce lactic acid and H_2O_2, which can prevent the overgrowth of other microorganisms in the vagina including *Escherichia coli* and *Gardnerella vaginalis* [5].

The viable lactobacilli can inhibit food-borne and enteric pathogenic micro organisms by producing lactic acid and other antimicrobial substances, yogurt and acidophilus milk have been considered to be healthy probiotic diets [11].

Due to the use of antimicrobial agents is not only select resistance bacteria but it can disturb the balance of body by killing friendly bacteria, when this happen bacteria and yeast can move in and flourish leading to uro-genial tract infection [3].

This need is forced on researchers by the longtime in developing alternative therapy to antimicrobial agents such the application of probiotics to cure urinary tract infection.

MATERIALS AND METHOD

Collection of urine samples

Fifty urine samples were collected from five private hospital, Nagercoil, Kanyakumari in sterile plastic universal containers and transported to laboratory in an ice cold condition.

Isolation and identification of bacteria from urine samples

For isolation of UTI causing organism, loopful of urine samples was streaked on blood agar plate, MacConkey agar plate, EMB agar plate and incubated at 37^{o}C for 24hrs. After incubation the colonies were selected and characterized on the basis of morphological, cultural and biochemical characteristics and were identified with the help of Bergey's Manual of Systematic Bacteriology. These identified organisms were maintained on nutrient agar slants at 4^{o}C and subculture were made for every 30 days.

Collection of *Lactobacillus Plantarum*

Lactobacillus plantarum was obtained as a probiotic material from Bava medical store, Nagercoil, as a live organism.

Isolation and Identification of *Lactobacillus Plantarum*

The probiotic material was inoculated into DeMan Rogosa Sharpe (MRS) broth and incubated at 37^{o}C for 48 hours. After incubation loopful of culture was streaked on the surface of MRS agar plates and incubated for 24 hrs at 37^{o}C . Afterwards, the isolates were selected and characterized on the basis of morphological , cultural and biochemical characteristics and were identified with

the help of Bergey's Manual of Systematic Bacteriology. These identified organisms were maintained on nutrient agar slants at 4^{o}C and subculture were made for every 30 days.

Invitro* Antagonistic Activity of Lactobacillus *Plantarum

The 20 ml of sterilized MRS agar was poured into sterile petriplates, after it get solidified 100 µl of fresh culture of pathogens were swabbed on the respective plates. Then wells were punched over the agar plates using sterile gel puncher. Small drop of MRS agar was put inside the edge of each well before adding *Lactobacillus plantarum* suspension. After surface solidification 100µl of 18 hours culture of *Lactobacillus plantarum* broth was added to each wells. The plates were incubated at 37^{o}C for 18-24 hrs. The inhibitory activity was determined by measuring the inhibition zones around each wells and expressed in millimeter (mm).

Preparation of culture supernatant

The bacteriocin producing *Lactobacillus plantarum* were grown in MRS broth at 37^{o}C for 18-20 hrs. The lactobacilli culture was centrifuged at 10,000 rpm for 5 minutes and then the supernatant was adjusted to pH 6.5-7.0 with 1N NaOH.

Bacteriocin Assay

Bacteriocin activity was detected by agar well diffusion method. Here , 100 µl of each culture supernatants were transferred to the wells in preseeded Muller Hinton agar plates. The plates were then incubated at 37^{o}C overnight and examined for the presence of inhibition zones around the wells and the antimicrobial activity expressed in millimeter (mm).

Effect of temperatur on bacteriocin assay

Temperature stability was investigated by heating 100 µl of each culture supernatant at 40^{o}C, 60^{o}C, 80^{o}C, 100^{o}C and 121^{o}C for 30 minutes. The samples were then assayed for antimicrobial activity using the well diffusion method.

Effect of pH on bacteriocin assay

The pH of culture supernatant was adjusted to 2,4,6,8,10 and then kept at room temperature for 4 hrs. Residual activity was determined by well diffusion method.

Stability of bacteriocin during storage

To determine the stability of bacteriocin, the bacteriocin were stored in an incubator (37^{o}C) , refrigerator (4^{o}C) and freezer(-20^{o}C). At different time interval (every 30 days) the bacteriocin were taken from the stored materials for detection of antimicrobial activity using well diffusion assay.

Antibiotic sensitivity test

Five different commercially available antibiotics (Ampicillin , Chloramphenicol ,Gentamicin, Tetracycline, Tri-methoprime) were tested *in vitro* against *Lactobacillus plantarum* using disc-diffusion method and the diameter of inhibition zones were measured in millimeter (mm).

RESULT AND DISCUSSION

Urinary tract infection remains a major medical problem in terms of number of human afflicted each year. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequently UTI can be treated with broad-spectrum antibiotics. The main problem of antibiotic is the emergence of rapid increase of drug resistance microbes has made therapy of UTI difficult. This resistance problem needs a re-newed effort, to search for new antimicrobial substances from various source like a probiotics.

In this present study, the *in vitro* antagonistic activity of *Lactobacillus plantarum* showed the greater inhibitory effect (20 mm) against *Escherichia coli* while the least activity (9 mm) was found against *Streptococcus sp.* (Fig.1). The antagonism properties of *Lactobacillus sp.* against urinary pathogens were investigated using well diffusion method. *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus casei* produced a greater inhibitory effect towards all five *Escherichia coli* isolates. *Lactobacillus plantarum* showed highest inhibitory activity against *Salmonella typhimurium* (3.75 mm inhibitory zone) while the least activity was demonstrated against *Vibrio cholerae* (0.90 mm inhibitory zone) [10,9].

Result from this study, indicates the antimicrobial activity of bacteriocin produced by *Lactobacillus plantarum* and it had highest inhibitory activity (22mm) against *Escherichia coli* while the least activity (10 mm) was found against *Streptococcus sp.* (Fig.2). The bacteriocin obtained from *Lactobacillus sp.* were tested against *Enterobacter cloacea, Proteus vulgaris, Pseudomonas aeruginosa, Gardnerella vaginalis* and it showed inhibitory activity against *Gardnerella vaginalis* and *Pseudomonas aeruginosa* [14,8]. The antimicrobial agent produced by *Lactobacillus plantarum* AA135 is designated as plantaricin AA135. Plantaricin AA135 were active against *Staphylococcus sp., Bacillus sp., Micrococcus sp., Pseudomonas sp., Salmonella sp.* [12].

In the present study, the activity of bacteriocin was found in different temperature and it was seen that the bacteriocin from *Lactobacillus plantarum* shown the highest zone of inhibition at temperature of 60^0C (22 mm) (Fig.3). The bacteriocin were subjected to the temperature of 68^0C,100^0C,121^0C for 10min, 15min, and 20min, it was found that the bacteriocin was stable at 68^0C for 20minutes [18]. The bacteriocins produced from *Lactobacillus gasseri* TL093c and TL143a strains were inactivated by heating at 60^oC for 10 minutes [15].

In the present study, the activity of bacteriocin was found in different pH ranges. The maximum activity was found at pH 6 (25 mm) against *Escherichia coli.* At different pH ranges the diameter of inhibitory zone doesn't show any considerable changes (Fig.4). The most bacteriocins are resistant to acidic pH more than basic pH. The inhibitory activity of bacteriocin isolated from *Lactobacillus acidophilus* strain occurred between pH 3.0 and 5.0 and the inhibitory activity was lost when the pH was raised to 5.3 [16,2,3].

In the present study, the stability of bacteriocin were determined at various storage condition and at the storage temperature of -20^0C and 4^0C it doesn't show any considerable changes in their activity after 120 days but the bacteriocin stored at 37^0C showed considerable loss of its activity

after 90-120 days of interval. The maximum zone of inhibition (24 mm) was seen in 30 days of interval at 4^0C. From this study, the bacteriocin can be effectively stored at -20^0C and 4^0C for more than 100 days without any changes in their antimicrobial activity (Fig.5). A crude extract of plantaricin AA135 could be stored at -20^0C or 4^0C for atleast 100 days without substantial loss of its activity. However, storage at 37^0C caused some loss of activity, possibly due to the action of proteolytic enzymes which might be found in culture supernatant [1].

In the present study, sensitivity of *Lactobacillus plantarum* to five different antibiotics was determined. The antibiotic Tri-methoprime showed maximum zone of inhibition (15 mm) whereas the Ampicillin, Chloramphenicol and Gentamicin doesn't shown any activity against *Lactobacillus plantarum.* (Fig.6). The *Lactobacillus sp.* are resistant to some antibiotics. This resistance may be attributed by many factors including enzymatic inactivation, decrease intracellular drug accumulation or presence of gene that confer antibiotic resistance. *Lactobacillus plantarum* was more resistant to all tested antibiotics such as Ampicillin, Amoxicillin, Cephalexin, Tetracycline, Vancomycin than other *Lactobacillus sp.* [19,6].

Fig.1: Antimicrobial activity of Lactobacillus plantarum against UTI causing pathogens

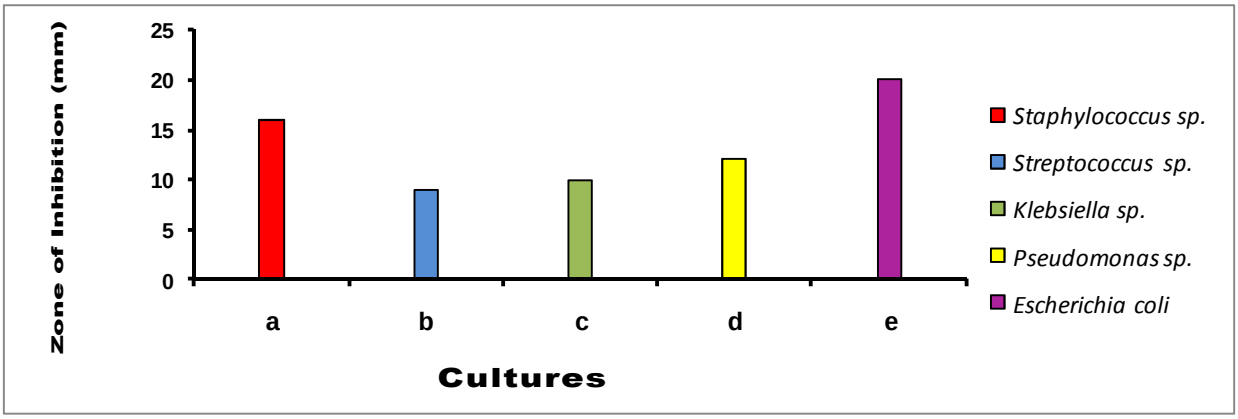


Fig.2: Antimicrobial activity of bacteriocin isolated from Lactobacillus plantarum against UTI causing pathogens

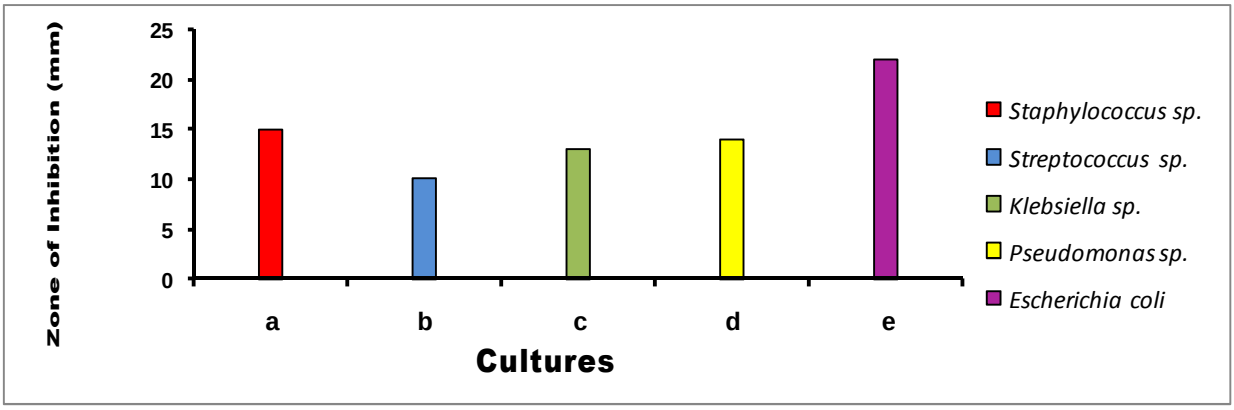


Fig.3: Effect of temperature on antimicrobial activity of bacteriocin from Lactobacillus plantarum

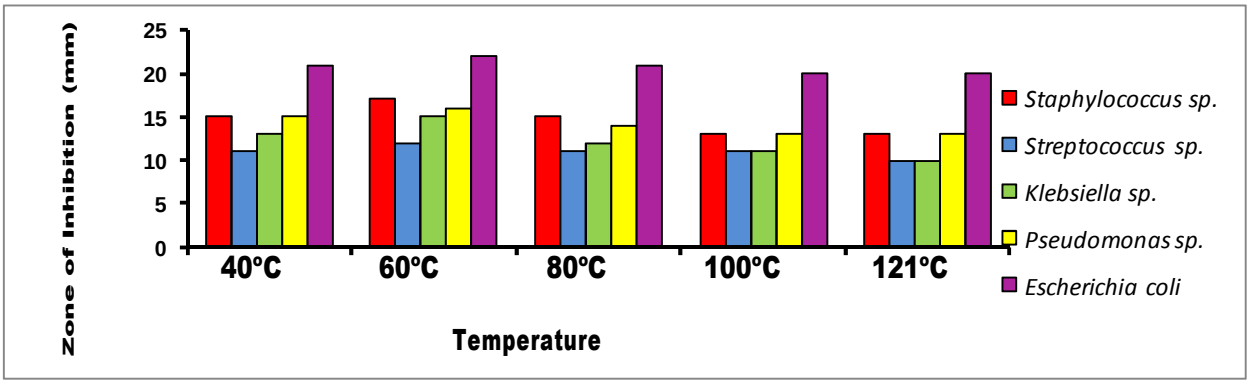


Fig.4: Effect of pH on antimicrobial activity of bacteriocin from Lactobacillus plantarum

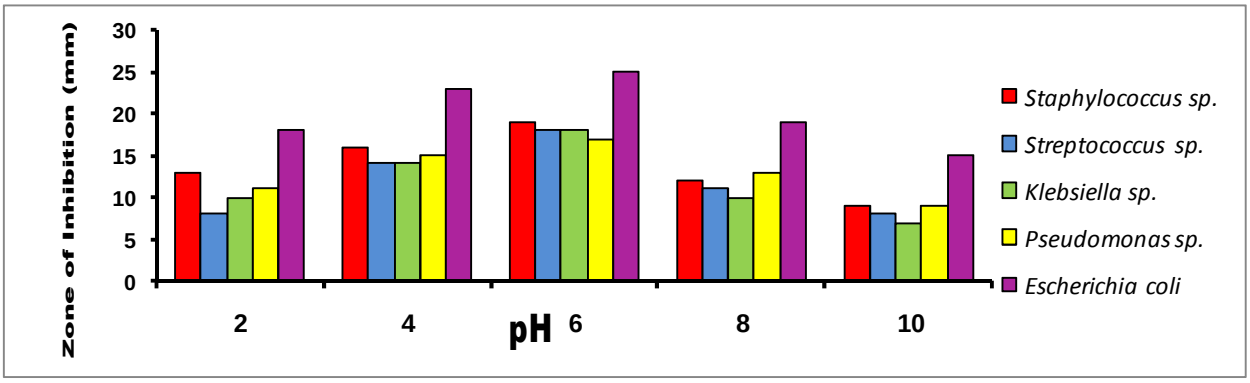


Fig.5: Effect of time on bacteriocin activity during different storage condition against Staphylococcus sp.

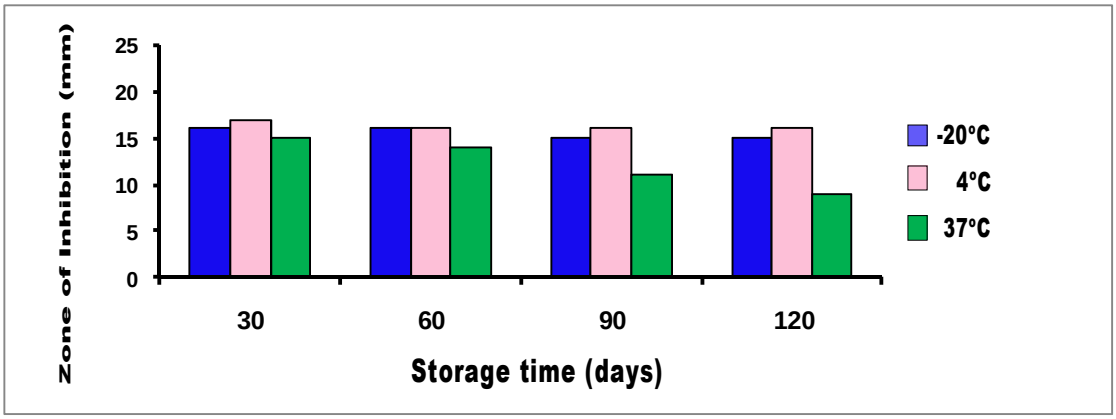


Fig.6: Effect of time on bacteriocin activity during different storage condition against Streptococcus sp.

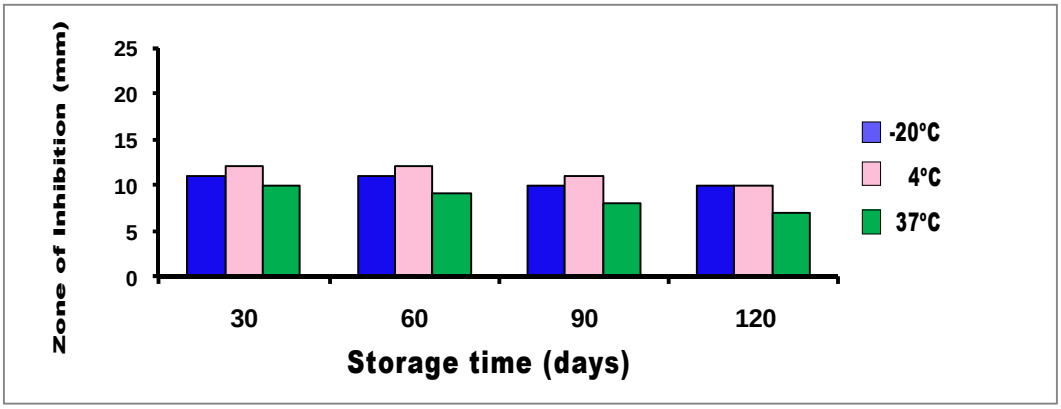


Fig.7: Effect of time on bacteriocin activity during different storage condition against Klebsiella sp.

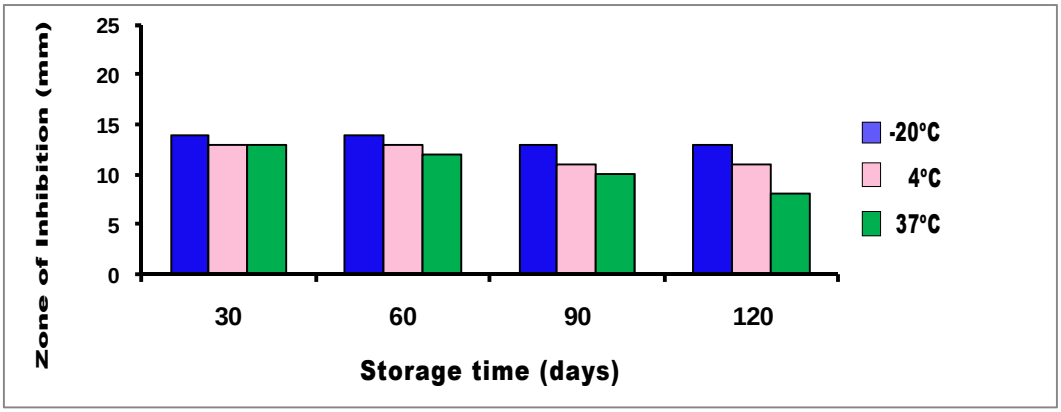


Fig.8: Effect of time on bacteriocin activity during different storage condition against Pseudomonas sp.

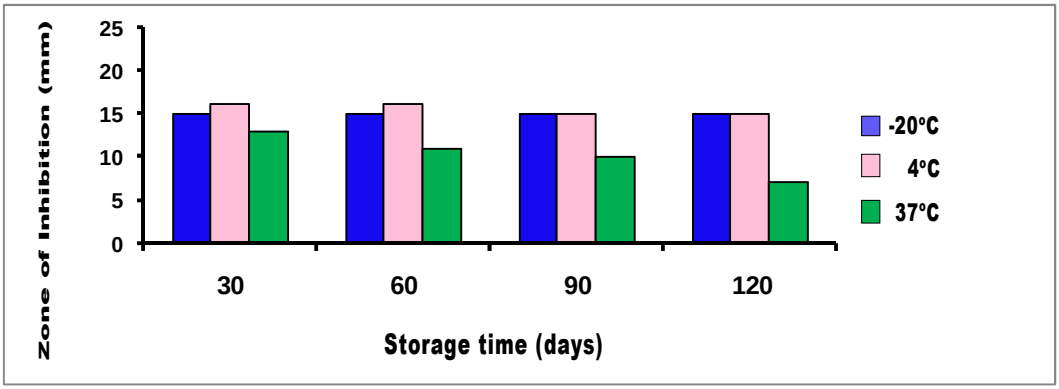


Fig.43: Effect of time on bacteriocin activity during different storage condition against Escherichia coli

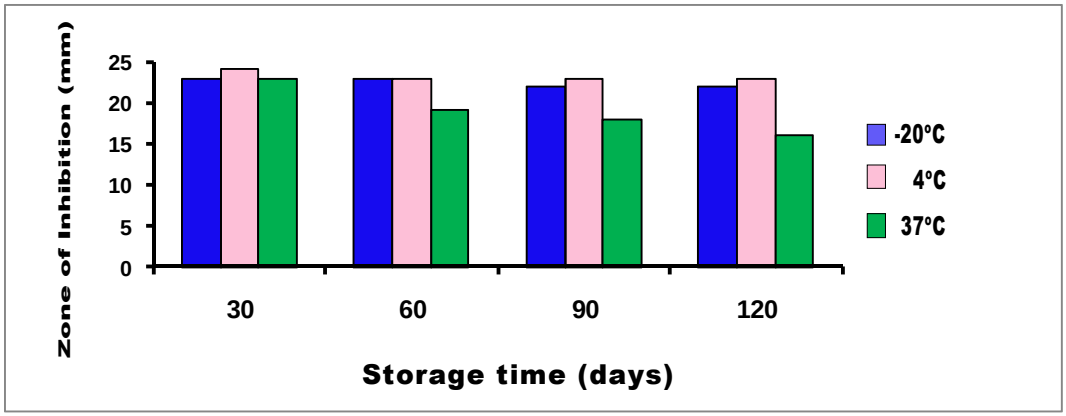
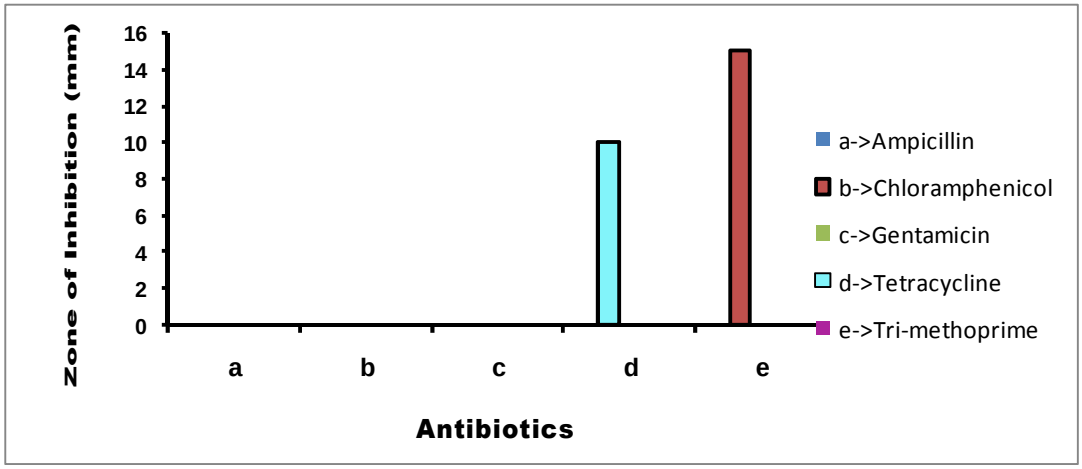


Fig.44: Sensitivity of Lactobacillus plantarum to different antimicrobial agents



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