

Evaluation of Antibacterial Activity of Bacteriocin Produced from *Lactobacillus* sp. Isolated from Rhizosphere Soil

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ABSTRACT

Lactic acid bacteria (LAB) can produce antimicrobial substances with capacity to inhibit the growth of pathogenic and spoilage microorganisms in foods. The preservative ability of LAB in foods is attributed to the production of antimicrobial metabolites including organic acids and bacteriocins. The present investigation was planned to isolate LAB from rhizosphere soil samples and to check the antibacterial activity of bacteriocin at different temperature, pH and at different incubation periods against different bacteria. 12 different isolates of Lactobacillus sp. were identified on the basis of various morphological, cultural and biochemical characters and bacteriocins obtained from these isolates were then checked for their antibacterial potential against different bacteria viz., Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Bacteriocin produced from isolate LB 12 showed maximum antibacterial activity against E.coli followed by S. aureus and P. aeruginosa, thus isolate LB 12 was considered for further study. Temperature affected the antibacterial activity of bacteriocin and it was observed that the bacteriocin showed highest zone of inhibition at 60°C against E.coli, S.aureus and P. aeruginosa (26, 24 and 23 mm respectively) and least activity was observed at 121°C. Antibacterial activity of bacteriocin was retained at pH 2-10 but maximum activity was observed at pH 6 against E.coli, S.aureus and P. aeruginosa (24, 22 and 18mm) and incubation period of 24 hrs at 37°C was observed to be the most suitable for bacteriocin activity against test bacteria. Behavior of bacteriocin produced by isolated strains in the present investigation was considered as bacteriocidal.

Keywords: Lactic acid bacteria, Rhizosphere soil, Antibacterial activity, Bacteriocin

INTRODUCTION

Lactic acid bacteria (LAB) are used throughout the world for manufacture of a wide variety of traditional fermented foods. The primary antimicrobial effect exerted by LAB is the production of lactic acid and reduction of pH. LAB are the most important groups for industrial purposes, since their fermentative activity involves a notable preservative capacity as a result of the drop in pH and antimicrobial activity of their metabolites such as lactic and acetic acid, diacetyl or bacteriocins. Many of these lactic acid bacteria produce bacteriocins¹⁻². By definition, bacteriocins are small proteins with bactericidal or bacteriostatic activity against genetically closely related species³. Bacteriocins are ribosomally synthesized antimicrobial peptides that are active against other bacteria, either of the same species (narrow spectrum), or across genera (broad spectrum) and may be produced by both Gram negative and Gram positive bacteria⁴⁻⁶. The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food-borne pathogenic microorganisms⁷.

Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. They are ribosomally synthesized peptides, and this fact creates the possibility of improving their characteristics to enhance their activity and spectra of action⁸. Several types of bacteriocins from food associated lactic acid bacteria have been identified and characterized for example: nisin, diplococcin, acidophilin, bulgarican, helveticins lactacins and plantaricins⁹. At present, nisin is the only bacteriocin commercially available and marketed. Currently, bacteriocins produced from lactic acid bacteria are studied extensively due to their generally recognized as safe (GRAS) status. During the last 20 years, bacteriocins of LAB have been given much attention because some of them exhibit high activity against pathogenic organisms¹⁰. Present investigation reports the isolation and characterization of bacteriocin producing LAB from rhizosphere soil and determination of the antibacterial activity of bacteriocin against different bacteria and to study the effect of temperature, pH and incubation time on bacteriocidal activity of bacteriocin.

MATERIALS AND METHODS

Collection of Soil Samples and Isolation of Bacteria

20 different moist garden soil samples were collected aseptically from fruit trees (mango and guava tree) at 15cm depth from agricultural farm from Faridkot, Ferozepur, Mohali and Bhatinda districts of Punjab, India for the isolation of bacteriocin producing microbial isolates. For the isolation of the LAB, serial dilutions (10^{-1} to 10^{-9}) of the soil sample were prepared using distilled water. Isolation of LAB was done using MRS (de Mann Rogosa Sharpe) agar medium which is the standard culture media to isolate the *Lactobacillus* and incubated at 37°C for 48 hrs¹¹. The strains were sub-cultured onto MRS agar slant incubated at 37°C for 24 hrs and stored at 4°C for further use. A series of morphological, cultural and biochemical tests were performed for characterization of bacterial strains isolated from rhizosphere soil samples and their identification was done on the basis of characteristics described in Bergey's Manual of Determinative Bacteriology¹².

Production of Crude Bacteriocin

The isolated strains were grown in MRS broth seeded with 5% inoculums of overnight culture incubated at 30°C for 48 hrs. After incubation, cells were removed from the growth medium by centrifugation at 10,000 rpm for 15 mins at 4°C. The cell free supernatant was adjusted to pH 6.0 using 1N NaOH and was used as crude bacteriocin¹³.

Bacteriocin Assay

The bacteriocins obtained from selected *Lactobacillus* strain were checked for their antibacterial potential against the selected bacterial cultures by using agar well diffusion method. The antibacterial activity of LAB isolates were checked against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The supernatant from a 48 hrs culture of *Lactobacillus* was filtered and sterile supernatant were placed in 4mm diameter wells that had been cut in Mueller Hinton agar plates previously seeded with bacteria in the diffusion agar¹⁴.

Sensitivity of Bacteriocin to Temperature, pH and Incubation Time

Heat stability

Temperature stability was investigated by heating 5 ml of bacteriocin at 60°C, 80°C, 100°C and 121°C for 15 min. The heat-treated bacteriocin samples were then assayed for antibacterial activity by well diffusion method¹⁵.

Effect of pH

Aliquot (5 ml) of bacteriocin was taken in test tubes and the pH values of the contents were adjusted from 2 to 10 individually, using either dilute NaOH or HCl (1 M NaOH or 1 M HCl) solution. The samples were allowed to stand at room temperature for 2 hrs and the antibacterial activity was assayed by well diffusion method¹⁵.

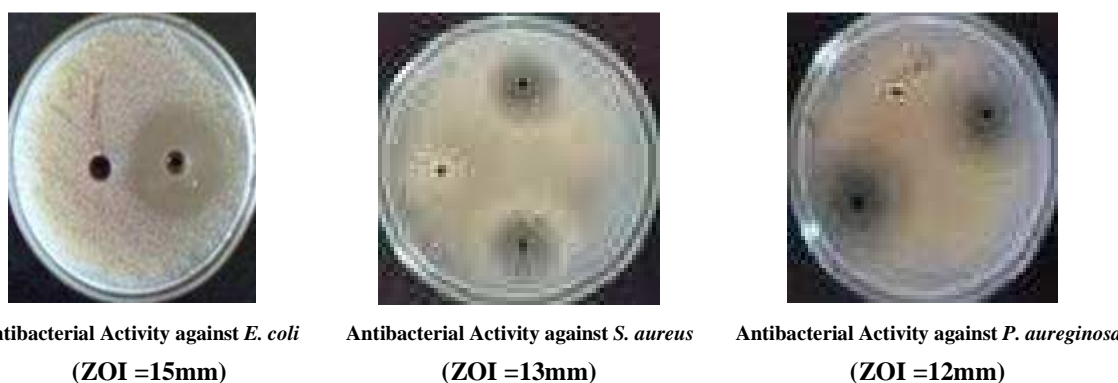
Effect of Incubation Period

Active cultures of producer organisms (1% v/v) were inoculated in 100 ml aliquots of sterile composed media. Inoculated flasks were incubated at 37° C for 12, 24, 48 and 72 hrs and at the end of each incubation period, bacteriocin activity was observed against different bacteria by inoculating culture supernatant against indicator organism¹⁶.

RESULTS AND DISCUSSION

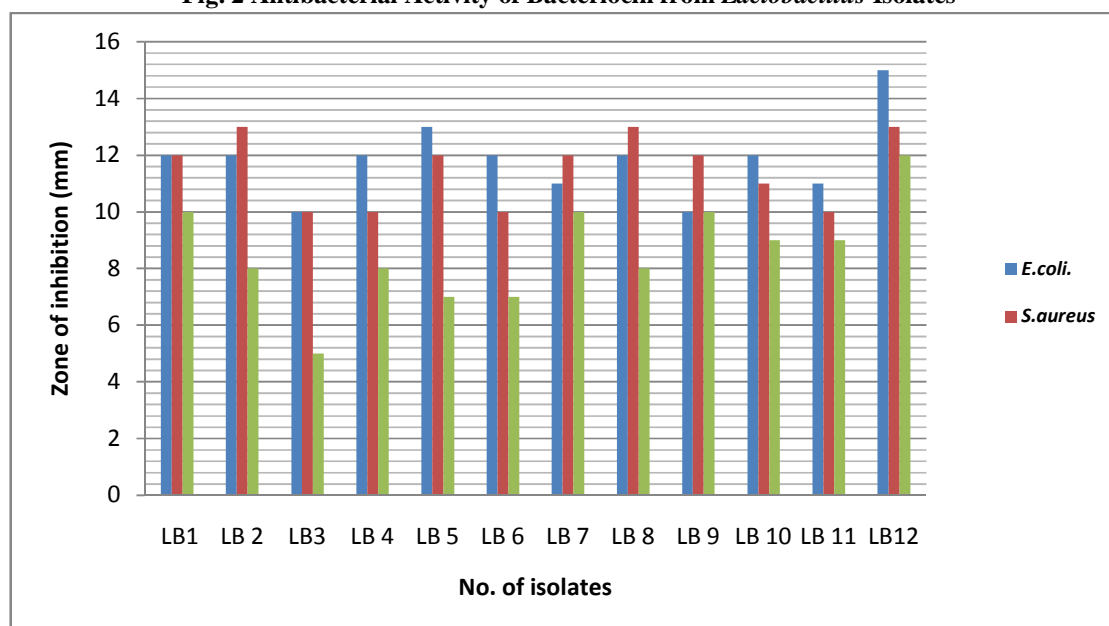
Bacteriocins produced by LAB have received considerable attention during recent years for their possible application as a biopreservatives in food. 12 presumptive strains were isolated from 20 different rhizosphere soils and based on morphological, cultural and biochemical characterization they were identified as *Lactobacillus* sp. Bacteriocins produced by *Lactobacillus* strains was used as an antimicrobial agent against various pathogens. The susceptibilities of various bacteria viz., *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by the bacteriocin of *Lactobacillus* strains are presented (Fig. 1) which showed inhibitory activity against *E. coli*, *P. aeruginosa* and *S. aureus* and maximum zone of inhibition was observed against *E. coli* (15mm) and minimum against *P. aeruginosa* (12mm).

Fig. 1 Antibacterial Activity of Bacteriocin by Isolate LB 12



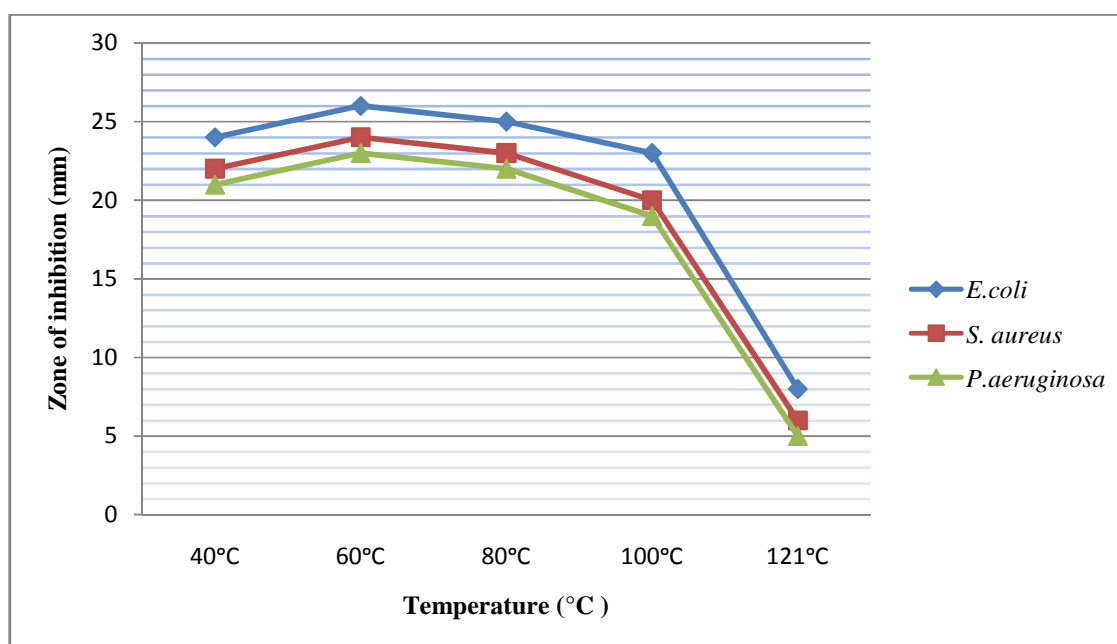
The antibacterial activities exhibited by 12 different isolates of *Lactobacillus* sp. are presented in Fig. 2. On the basis of maximum zone of inhibition against all tested bacteria, isolate LB-12 was selected for evaluation of effect of temperature, pH and incubation time on antibacterial activity of bacteriocin.

Fig. 2 Antibacterial Activity of Bacteriocin from *Lactobacillus* Isolates



Temperature affected the antibacterial activity of bacteriocins produced from *Lactobacillus* sp. Activity of bacteriocin was recorded at different temperature and it was seen that the bacteriocin produced from isolate LB-12 showed highest zone of inhibition at 60°C against *E. coli*, *S. aureus* and *P. aeruginosa* (26, 24 and 23 mm respectively) and minimum at 121°C (8, 6, 5 mm respectively) (Fig.3). Present study revealed that temperature beyond 60°C leads to decreased antimicrobial activity of bacteriocin and results of current investigation are similar to previous studies in which 68°C temperature was found to be suitable for bacteriocin activity¹⁵. Bacteriocin was heat reliable as it is inactivated partially at 80°C for 10 min and completely at 100°C for 10 min¹⁷. There is no change in antimicrobial activity when bacteriocin was heated at 60, 70, 80, 90 and 100°C and antimicrobial activity was considered to be heat resistant¹⁸⁻¹⁹. Partially purified bacteriocin was effective upto 100°C temperature²⁰.

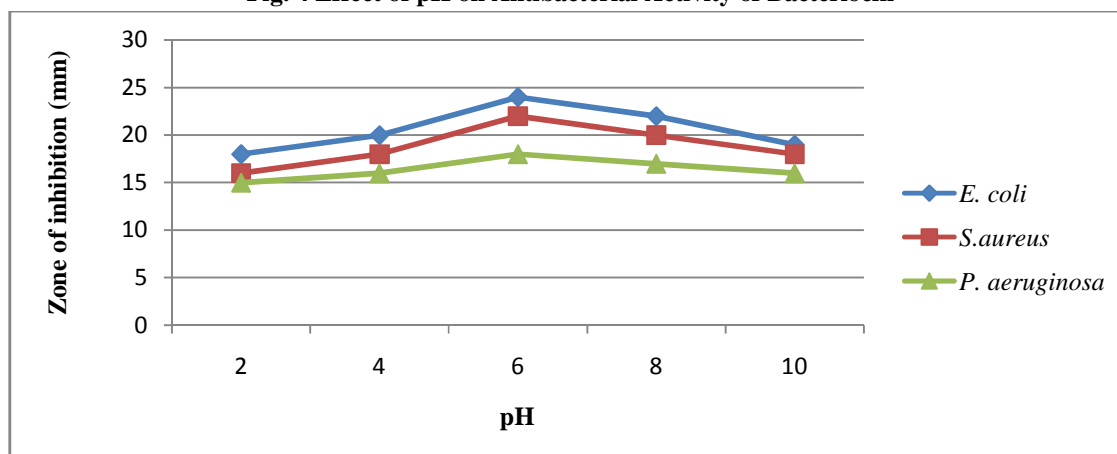
Fig. 3 Effect of Temperature on Antibacterial Activity of Bacteriocin



Effect of pH on Antibacterial Activity of Bacteriocin

Activity of bacteriocin against different bacteria at different pH ranges was determined and maximum antibacterial activity was found at pH 6 against *E. coli*, *S. aureus* and *P. aeruginosa* (24mm, 22mm and 18mm respectively) (Fig. 4). Bacteriocin when subjected at different pH the diameter of inhibitory zone does not show any considerable change and previous studies also showed no change under wide pH range from 3 to 9 and all samples retained antimicrobial activity against *L. innocua* 4030 in the pH range 2-10, increased pH after 9 decreases the activity of bacteriocin²¹⁻²². Antimicrobial activity was retained at pH range 5-8 and activity was more stable in acidic than basic conditions¹⁹. Bacteriocin produced by *L. plantarum* and *L. brevis* OG1 retained their antimicrobial activity in an acidic range (pH 2.0-6.0) only²³. The inhibitory activity of bacteriocin isolated from *Lactobacillus acidophilus* strain occurred between pH 3.0 and 5.0 and inhibitory activity was lost when the pH was raised to 5.3^{15,24}. Bacteriocin of *B. mycoides* when treated at pH 3.0-11.0, the maximum activity was observed at neutral pH²⁰. The effect of pH on inhibitory effects by the bacteriocin producing *Lactobacillus* strains using modified-MRS broth indicated that the highest antimicrobial productions were recorded mostly between pH 5.5 and 7.5 followed by gradual decrease between pH 8.0 and 9.0, although there was no growth/survival of the *Lactobacillus* strains at pH 3 after 24 h incubation²⁵.

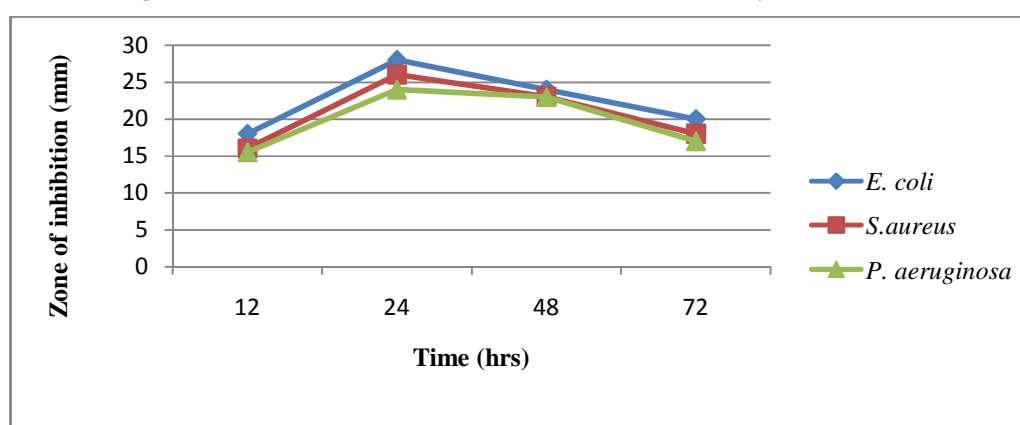
Fig. 4 Effect of pH on Antibacterial Activity of Bacteriocin



Effect of Incubation Period on Bacteriocin Activity

The effect of incubation period at 37°C on bacteriocidal activity of bacteriocin was studied and it was observed that at the end of 24 hrs of incubation bacteriocin showed maximum antibacterial activity against test microorganisms, while at 48 and 72 hrs the inhibitory action was found to be comparatively less (Fig. 5). Previous studies also indicated that 24 hrs of incubation period is most suitable for bacteriocin activity²⁶.

Fig. 5 Effect of Incubation Period on Antibacterial Activity of Bacteriocin



CONCLUSION

LAB exerts strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. There have been very limited studies comparing the activity of bacteriocins from LAB against pathogens under well controlled experimental conditions. *Lactbacillus* sp. produce antimicrobial substances like bacteriocin that ensure other organisms are obstructed in their growth patterns. The bacteriocins produced by the LB 12 strains showed a strong inhibitory activity with highest zone of inhibition at temperature 60°C, pH 6 and incubation period of 24 hrs at 37°C against *E. coli*, *S. aureus* and *P. aeruginosa*. Present study highlight the possibility that bacteriocin and LAB will be notified of great interest in terms of security especially in combination with other antimicrobial compounds in further research. Antimicrobial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganism.

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