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Research Article

IN VITRO ANTAGONISTIC ACTIVITY OF A PROBIOTIC *LACTOBACILLUS PLANTARUM* AGAINST WATER BORNE PATHOGENS

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ABSTRACT

Objective: Lactic acid bacteria (LAB) produce various substances that exhibit antagonistic activity against other bacteria. Detection of antimicrobial substance produced by lactic acid bacteria against the water borne pathogens is the objective of this work. Materials: The bacteriocin producing probiotic strain *Lactobacillus plantarum* was isolated from a grass silage samples and identified based on the characteristics of *Lactobacillus plantarum* as present in Bergey's manual of Systemic Bacteriology. The metabolite bacteriocin was extracted from the isolated strain and the antagonistic activity was evaluated against water borne pathogens. Results: The bacteriocin producing strain, *L.plantarum* exhibited the inhibitory zone ranged from 16 mm to 24 mm against water borne pathogens. Conclusion: Results from this study concluded that the compound bacteriocin produced by *L. plantarum* have the potential to inhibit water borne pathogens.

Keywords: Bacteriocin, *L.plantarum*, Water borne pathogens, Antagonistic activity.

INTRODUCTION

Probiotics are living microorganisms that are similar to symbiotic microorganisms found in the human gut. They are also called "Friendly bacteria" or "Good bacteria" [1,2]. Probiotics are the microorganisms and, when administered in adequate amounts, confer a health benefits on the host [3]. Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as Complementary and Alternative Medicine (CAM) [4]. Lactic acid bacteria are regarded as a major group of probiotic bacteria; they are commercially used as started cultures for the manufacture of probiotic foods [5].

Beneficial effects conferred by Lactobacilli, including inhibition of gram negative and gram positive pathogenic bacteria, were described by Maragkoudakis *et al*. [6] and Charlier *et al*. [7]. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public [8]. Amin *et al*. [9] reported the isolation of sixty lactobacilli strains from fresh vegetables which were grown in Man- Rogosa- Sharpe medium (MRS) broth, exhibited remarkable antimicrobial activity against a panel of pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Shigella dysenteriae, Bacillus anthracis* and *Staphylococcus aureus.* The aim of this study was to investigate the antagonistic activity of bacteriocin producing *Lactobacillus plantarum* isolated from grass silage against water borne pathogens.

MATERIALS AND METHODS

Isolation and identification of bacteriocin producing *L. plantarum*

The probiotic *L. plantarum* strain was isolated from the grass silage following the method described by Stroem *et al.* [10]. The isolated strain was identified based on the biochemical profile and fermentation pattern studies. The biochemical profiles were carried out according to Bergey's manual of Systemic Bacteriology [11]. The API 50CHL test (BioMerieux, France) was applied for identification by fermentation patterns. The *L. plantarum* strain was grown on MRS agar (Himedia, Mumbai) and stored at 4°C. Liquid culture was grown in MRS broth (Himedia) and stored at 4°C.

Test organisms

The test organisms used in the attempt such as *Escherichia coli, Salmonella typhi, Vibrio cholerae* and *Shigella dysenteriae* were procured from Microbial Type Culture Collection, Chandigarh, India. All cultures were maintained in the refrigerator at 4°C.

Optimization of culture conditions

To determine the optimum temperature for bacteriocin production, the *L.plantarum* strain was grown in MRS broth at different incubation temperatures such as 25, 30, 35, 37 and 40°C for 12 hrs. The MRS broth was prepared and adjusted at different pH values such as 5.5, 6.0, 6.5, 7.0, and 7.5 using 0.1N NaOH solution. To determine the efficient incubation time for bacteriocin, the bacterial strain was grown in MRS broth at different incubation times such as 12, 24, 36, 48, 60 and 72 hrs at 37°C. The culture supernatant from the tested production medium was assayed for bacteriocin production (AU/ml) and the results were recorded.

Extraction of bacteriocin

The *L.plantarum* strain was propagated in 250 ml of MRS broth (pH=6.8) for extraction of bacteriocin, a culture supernatant were obtained by centrifugation (10,000 rpm for 20 min at 4°C). The cell free supernatant was precipitated with ammonium sulphate (40% saturation). The mixture was rotated for 2 hrs at 4°C and later centrifuged at 10,000 rpm for 20 min. The precipitates were dialysed and resuspended in 10 ml of 0.05M potassium phosphate buffer (pH=7.0).

Antagonistic assay

The antagonistic activity of *Lactobacillus plantarum* against water borne pathogens was carried out by overlay method [12]. The *Lactobcillus plantarum* strain was inoculated as 2 cm line on MRS agar plates and incubated at 37°C for 24 hrs. The plate was overlaid with MRS soft agar containing 1 ml of indicator organism and incubated at 37°C for 24 to 48 hrs. Clear zone of inhibition around the colony was observed. The inhibition zones were measured and compared with standard antibiotics [13]. The activity index is calculated by using the following formula,

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Quantification of bacteriocin

Quantification of bacteriocin was done by agar well diffusion assay method [14]. Overnight MRS broth containing *L.plantarum* was centrifuged at 10,000 rpm for 10 min and then the resulting supernatant was purified by membrane filtration (0.45 mm pore size). This supernatant was serially diluted up to 1: 200. Then 50 µl of two fold diluted sample was transferred to the wells of 5 mm diameter in the MRS agar plate which were already inoculated with indicator strains. The plates were incubated at 37°C for 24 hrs, the inhibition zone was observed. The antibacterial activity (Arbitrary Unit; AU/ml) was calculated with the inhibitory effect of bacteriocin

against indicator strains using one arbitrary unit was (AU) defined as reciprocal of the highest dilution showing an inhibition zone.

RESULTS AND DISCUSSION

The isolated colony was confirmed with the information of Bergey's manual of Systemic Bacteriology and the information supplied by commercial suppliers (API 50 CHL test; BioMerieux, France). The biochemical profile and fermentation pattern of the isolated *L. plantarum* strain is presented in Table 1.

The optimized culture conditions for the production of bacteriocin were 37°C temperature, pH 6.5 and 48 hrs of incubation. The highest quantity of bacteriocin produced was ranged from 3200 AU/ml to 3800 AU/ml (Figs 1-3). One-way ANOVA test revealed that each parameters showed significant difference (P<0.05) between them.

Table 1: Biochemical profile of *L.plantarum* strain and results of API test

Biochemical test	*L.plantarum* strain
Gram staining	+
Catalase	+
Oxidase	+
Citrate	+
Fermentation patterns (API test results)	
Arabinose	+
Cellobiose	+
Fructose	+
Galactose	+
Glucose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melibiose	+
Raffinose	+
Rhamose	-
Salicin	+
Sorbitol	+
Sucrose	+
Trehalose	+
Xylose	+
Adonitol	+
Inulin	+
Inositol	+

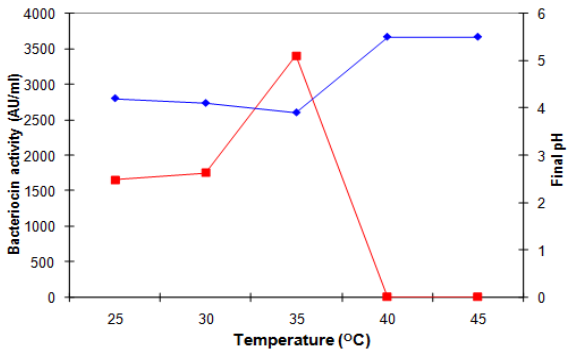

Fig. 1: Effect of temperature(°C) on bacteriocin production by *Lactobacillus plantarum* (Each data represents an average of five individual replicates).

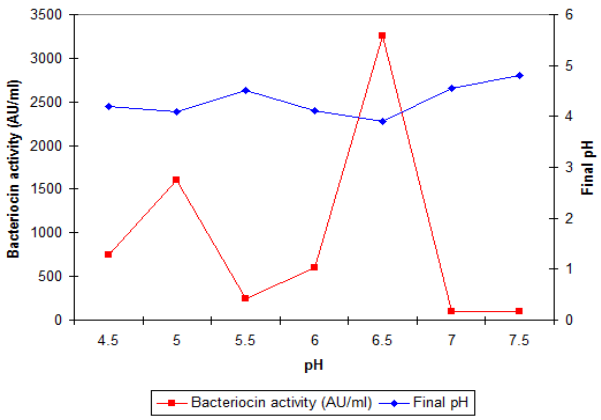

Fig. 2: Effect of pH on bacteriocin production by Lactobacillus plantarum (Each data represents an average of five individual replicates).

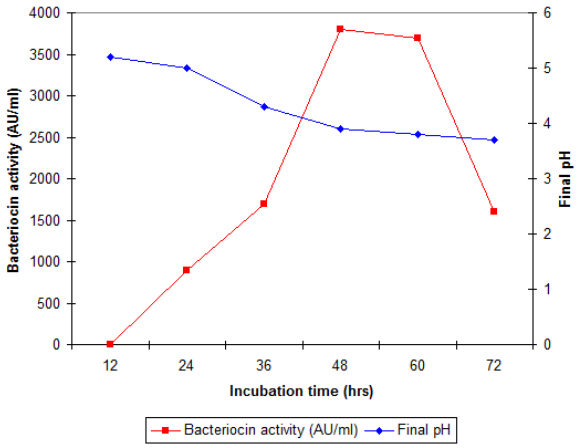

Fig. 3: Effect of incubation time(hrs) on bacteriocin production by Lactobacillus plantarum (Each data represents an average of five individual replicates).

The antagonistic activity of *Lactobacillus plantarum* against water borne pathogens showed clear zone around the colony (Fig 4).

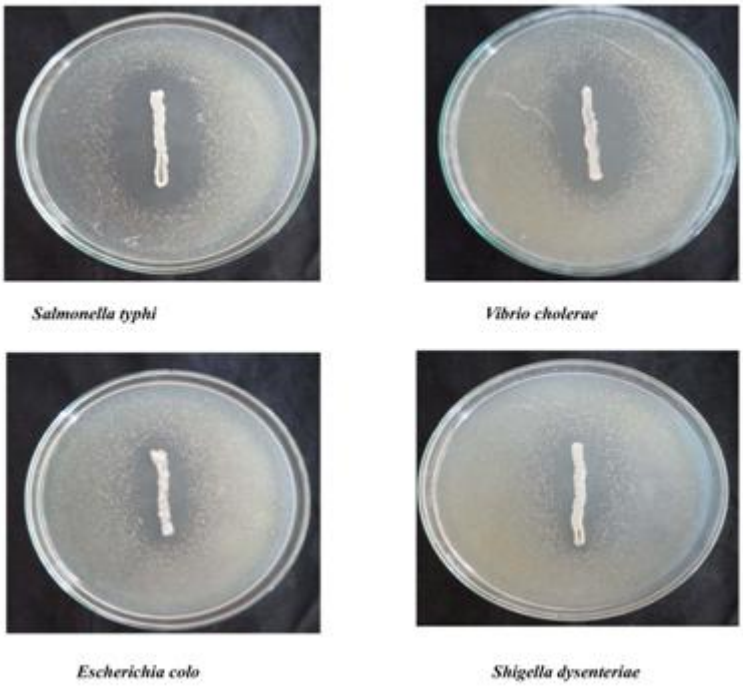

Fig. 4: Antagonistics activity of Lactobacillus plantarum against water borne pathogens

It indicated that the strain *Lactobacillus plantarum* produced bacteriocin which was active against the indicator strains used in the present study. The maximum zone of inhibition (24 mm) was recorded against *Salmonella typhi* as indicator strain. Followed by this, 21 mm, 20 mm, and 16 mm, was recorded against *Vibrio cholerae, E.coli*, and *Shigella dysenteriae* respectively (Table 2).

Table 2: Antagonistic activity of *Lactobacillus plantarum* (IZ, AI and FI) against water borne pathogens

Water borne pathogens	Inhibitory zone in mm (IZ) of sample	Inhibitory zone in mm (IZ) of Standard	Fold Increase in Percentage (FI)	Activity Index (AI)
Salmonella typhi	24	17	29.2	1.412
Vibrio cholerae	21	16	23.8	1.313
Escherichia coli	20	17	15	1.176
Shigella dysenteriae	16	15	12.5	1.067

Lima *et al*. [15] reported that *A. actinomycetemcomitans* showed antagonistic activity against a wide range of gram positive and Gram negative bacteria. Hammond *et al*. [16] reported that *A. actinomycetemcomitans* FDC Y4 produces a bacteriocin active against *S. sauguis*, *S. uberis*, *A. viscosus,* and *A. actinomycetemcomitans* strains, but not against other bacteria.

Similarly in the present report *L.plantarum* strain produced bacteriocin which was active against water borne pathogens such as *Salmonella typhi*, *Vibrio cholerae*, *E.coli* and *Shigella dysenteriae*. Fazeli *et al.* [17] reported that the *L.plantarum* strains have differ in their antagonistic activities against *Salmonella* which could be due to the metabolite secreted by the *L.plantarum* strains specially type of bacteriocins. In accordance to this study, similar findings are observed in the present attempt.

CONCLUSIONS

As per the observed data, it can be concluded that the bacteriocin producing *Lactobacillus plantarum* has wide range of antibacterial spectrum against water borne pathogens. The bacteriocidal activity exerted by this strain makes it usable as a topical therapeutic agent to combat many infections and could provide medicinally value added advantages to the human beings.

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