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SCREENING AND MODULATION OF THE BACTERICIDAL POTENTIAL OF PICKLE-BASED LACTIC ACID BACTERIA AGAINST COMMON NOSOCOMIAL PATHOGENS

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ABSTRACT

Fermentation of vegetables is an important process to preserve food. Fermentation is carried out mostly by LAB which are much resistant to salt. Pickles usually contain a variety of LAB which not only improve digestion of fruits and vegetables but also contribute to unique taste by providing components like exopolysaccharides and bacteriocins. The production of bacteriocins by LAB is useful for human body and are beneficial to reduce growth of foodborne pathogens like *S. aureus* and *E. coli* etc. LAB have been used in fermented food industry because they improve the taste of product. From different samples of pickles eighteen Lactic Acid Bacteria (LAB) have been isolated in the current work. The isolates were screened on De Mann Rogosa Sharpe agar (MRS). After preliminary morphological and biochemical examination, twelve out of eighteen strains were identified as *Lactobacillus* species, while four were *Streptococcus* species. Antibacterial activity of eight bacteriocin-producing Lactic acid bacterial (LAB) strains were tested against three pathogenic bacteria *Escherichia coli* (*E.coli*), *Bacillus cereus* (*B.cerus*) and *Staphylococcus aureus* (*S.aureus*) by using disk diffusion method. Maximum antibacterial activity was observed against *B. cereus* and least against *E. coli*. Bradford method was used in order to estimate the protein bacteriocin. The protein content was measured by comparing it with the bovine serum albumin (BSA) as a standard. LAB are beneficial bacteria for human health as these can be used as natural antibacterial tools against common pathogenic bacterial strains involved in different types of deleterious infections.

Keywords: Lactic acid bacteria, pickle, De Mann Rogosa Sharpe agar, *Lactobacillus* sp., *Streptococcus* sp.

INTRODUCTION

Pickling is the oldest method to preserve fruits and veggies though fermentation (Ballester *et al.*, 2022; Akoth *et al.*, 2023). Pickle is a fermented vegetable dish that has unique nutritional value and taste. It is used as traditional in many countries worldwide (Gomathy *et al.*, 2023; Güleç and Yılmaz, 2024). Fermentation of vegetables is an important process to preserve food. It provides a variety of food with enhanced nutrient content (Sionek *et al.*, 2023). These are non-dairy products which are prepared as a result of lactic acid fermentation. These are produced from vegetables and fruits which get fermented in their juices or brine with some salt concentrations and LAB (Sionek *et al.*, 2023). Fermentation is carried out mostly by LAB which are much resistant to salt (Papadopoulou *et al.*, 2023). Pickles usually contain a variety of LAB which not only improve digestion of fruits and vegetables but also contribute to unique taste by providing components like exopolysaccharides and bacteriocins (Banik *et al.*,

2023; Aguirre-Garcia *et al.*, 2024). LAB have been used in fermented food industry because they improve the taste of product (Raj *et al.*, 2022; Sionek *et al.*, 2023). LAB is a homogenous group of bacteria. During carbohydrate fermentation, they produce lactic acid as primary by product. LAB are rod or cocci in shape, Gram-positive bacteria and catalase negative (Harun, *et al.*, 2023). According to reports LAB produce substances which have antimicrobial potential like hydrogen peroxide, acetoin, reuterin, and bacteriocin (Todorov *et al.*, 202; Aleksanyan *et al.*, 2024). The antimicrobial action of LAB is credited to two products, one is organic acid and second is bacteriocin (Chen *et al.*, 2022). LAB have been prime focus of international research due to their antimicrobial chemicals which enhance the probiotic characteristics (Abedin *et al.*, 2023). Bacteriocins are a group of heterogenous bactericidal peptides or proteins mainly produced by bacteria and archaea (Todorov *et al.*, 2022; Shi *et al.*, 2024). These compounds produced by LAB are of special interest.

These compounds are considered as safe by USFDA (U.S. Food and Drug Administration) (Mgomi *et al.*, 2023). These compounds are potentially good substitutes of antibiotics because of no side effects (Todorov *et al.*, 2022).

The production of bacteriocins by LAB are useful for human body and are beneficial to reduce growth of foodborne pathogens like *S. aureus* and *E. coli* etc. They form pores in cell membranes of sensitive bacteria, so they are naturally bactericidal or bacteriostatic (Nisa *et al.*, 2023). The goal of current study is to isolate, detect and characterize the bacterial isolates from various samples of homemade and company made pickles. Additionally, LAB isolates were characterized, and their antimicrobial activity was studied against three pathogenic bacteria. Characterization of bacteriocin was performed by studying enzyme, pH sensitivity and heat stability. The objectives of the study were;

Screening of lactic acid bacteria from pickle

Assessment of antibacterial activity of lactic acid bacteria against common nosocomial pathogens
Characterization of lactic acid bacteria via biochemical tests

Characterization of protein bacteriocin (enzyme and pH sensitivity, Temperature and heat stability

MATERIALS AND METHOD

Sample Collection: The study was organized at Department of Zoology, Government College University Lahore. 12 different samples of pickle were collected. Six samples were commercially prepared pickles including Shezan, Shan, National, Shangrilla, Mitchell's and Mehran. Six samples of homemade pickles from different zones of city Lahore were collected. Storage of samples was done in sterilized plastic bags and properly labelled. All the samples were kept at 4 °C for further processing (Chien *et al.*, 2023).

Bacterial Isolation from Samples: 45gm of normal sterilized saline was taken, in which 1gm of pickle was added. Pickle from different samples were taken and inoculated in different saline tubes. It was shaken well and was led with normal saline to gradient dilution 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} times. It was poured on plates containing MRS media. Plates were upturned and incubated for the growth of bacterial strains at 37°C for 48 to 72 hours (Nemska *et al.*, 2022; Yaekob *et al.*, 2024).

Preparation of Pure Culture: Various isolated colonies of different morphological characteristics were randomly selected and isolated to get pure culture. Then different isolated colonies of bacteria were picked. By using sterilized loop colonies were streaked on prepared MRS agar plates from the culture plate and again incubation of plates were done at 37°C for 72 hours as lactic acid bacteria are slow growing bacteria and they require more for their

growth. Glycerol 40% was prepared to preserve colonies at -80 °C (Shafique *et al.*, 2024).

Characterization of Bacterial Isolates: For the morphological and biochemical characterization of obtained isolates, tests were performed. Microscopic observation of bacterial colonies of isolates was done for morphology, texture and color. Gram's staining, motility test and endospore staining were performed. Different biochemical tests such as Citrate utilization test, Methyl red test, Indole test, Triple Sugar Iron Agar (TSI) test and Vogues Proskauer test were done to determine and characterize lactic acid bacteria (Shafique *et al.*, 2024).

Screening for pathogenic bacterial strains: In order to check the pathogenicity of isolated bacterial strains blood agar media test was done.

Blood agar test: A blood agar test was conducted for the isolation of indicator pathogenic bacterial strains used for assaying antibacterial activity. Medium for blood agar test was arranged, 14 g of nutrient agar was dissolved in 300ml of distilled water and mix it well and make the volume up to 500ml. Then blood agar medium was sterilized and the medium was allowed to cool till the temperature has fallen down to 50°C. When medium was cooled, then 5 ml of non-coagulated mammalian blood was added in medium. The flask was thoroughly shaken for mixing and then plates were poured with 20 ml of medium in each plate. Let the blood agar media in plates to get solidify and after solidification streak the isolated strains on blood agar media and after streaking incubate the inverted plates at 37 °C for 24 hours (Al-Ajeeli, 2024).

Determination of antibacterial Activity of Lactobacilli

Preparation of Sample Filtrate: In MRS broth, inoculation of isolated lactobacilli strains was done and then the broth was incubated for 48-72 hours at 37°C to obtain broth culture of each strain. Culture broth was centrifuged for 20 minutes at $10,000 \times g$ after completion of incubation period. After centrifugation, supernatant was collected by using a 0.2 µm sterile syringe filter. To assure the production of bacteriocin, supernatant broth (cell-free) check the antibacterial activity against different food borne microbes (Divyashree *et al.*, 2024).

Media for growth of food pathogens was collected

Pure cultures of food borne pathogens included *E. coli*, *S. aureus* and *Bacillus cereus* were inoculated into brain heart infusion broth (BHIB) from slants and incubation of broth was done for 24 hours at 37°C. Centrifugation of broth culture was done after overnight incubation and pellets collected were suspended in 9 ml saline. This suspension was then spread over the nutrient agar media plates to check the antibacterial activity of the sample filtrate. Before the antibacterial activity pathogenicity test was done to check the hemolysis by pathogenic strains.

Disc Diffusion Method: Disc diffusion method was performed to assess the antibacterial activity of isolates of LAB.

A lawn of different three bacterial isolated strains with the help of spreader was done on nutrient agar media. The plates were allowed to dry and then small filter paper discs of about 5mm diameter were dipped in 100 µl culture free filtrate obtained from each LAB isolate. After dipping, disks were placed on agar media plates with the help of sterilized forceps. Incubation of plates was done for 48 hours at 37°C. After 48 hours, the zones of inhibition were measured. Bacteriocin activity was depicted in diameter (mm) of inhibition zones. If the diameter (mm) of ZOI (Zone of inhibition) was greater than 1mm results were considered positive. The experiment was performed in triplicate (Ngamsurach and Praipipat, 2022; Jeannot *et al.*, 2023).

Protein Estimation: For determination of protein concentrations Bradford method was applied by using a protein assay kit (Filgueiras and Borges, 2022).

As standard, bovine serum albumin (BSA) was used in this study.

By using micropipette protein sample of quantity 100µl in the form of cell free supernatant was added into Bradford reagent of quantity 0.9 ml and incubated for 15 mins at room temperature. After incubation, optical density (OD) was taken at 595nm on Agilent spectrophotometer.

Characterization of Bacteriocin

Sensitivity to enzymes: Crude bacteriocin was treated with proteolytic and non-proteolytic enzyme to check its sensitivity. By using crude bacteriocin in 0.01 M phosphate with adjusted pH 7 different Proteolytic enzymes including Proteinase K, pepsin, trypsin with a concentration of 2mg/ml were treated. In a test tube 0.15 ml of bacteriocin was taken, 0.15 ml of phosphate buffer and 0.15 ml of proteolytic enzyme was added to the test tube. Samples were incubated at 37°C for 2 hours and then boiled at 100°C for 3 to 5 mins. Same procedure was performed with non-proteolytic enzyme which was taken to be catalase. Then bacteriocin activity was assayed.

Thermal stability assay: A thermostatically controlled water bath was used to evaluate the stability of bacteriocin activity and heat resistance at 60°C to 100°C at increments of 10°C (60, 70, 80, 90, 100°C) and in an autoclave at 121°C for 10 minutes. To 5.0 ml sterile MRS broth, 1.0 ml bacteriocin was added in test tubes and heated to these temperatures mentioned above. Then all the samples were immediately cooled to room temperature in ice slurry water. Then each test tube was checked for antibacterial activity (Wang *et al.*, 2022).

Sensitivity to pH: Bacteriocin pH stability was evaluated by pouring 5.0 ml sterile MRS broth in different test tubes and adjusted the pH ranges from 3.0 to 10.0 range with the use of NaOH and HCl. 0.5 ml of bacteriocin was put in to each test tube. At room temperature, samples were incubated for about 30

minutes. After incubation samples were tested for bacteriocin activity (Qadi *et al.*, 2023).

Preparation of glycerol stocks: Glycerol stocks were prepared by adding 200µl of 50% autoclaved glycerol to 800µl of freshly grown bacterial broth culture in different labeled eppendorf. Then bacterial culture was mixed by inverting tube slightly several time until thorough mixing or vortex and then were stored at -20°C.

Determination of optimal growth conditions

Growth curves: Preparation of growth curves of pure bacterial isolates were done. For this purpose, in 100 ml conical flasks, as a starter culture 20 ml of MRS broth was poured and labeled properly.

Effect of growth conditions on bacteriocin production:

Determination of Optimum pH: To find out optimum pH for each bacterial isolate, 5ml MRS broth was prepared as a different test tube was used for different strain and pH was set up to 5, 6, 7, 8, and 9 using sterile 1N NaOH and HCl.

Determination of Optimum Temperature: To Figure out the optimum temperature for each strain, a media of 5 ml MRS broth was prepared in test tubes in four sets, each test tube was properly labeled and used for each strain.

Effect of incubation time: To find out the effect of incubation time on bacteriocin production for each LAB isolate, a medium containing 5 ml of MRS broth was prepared in test tubes. Each test tube was properly labeled and used for each strain. Sterilization of broth was done in autoclaved at 15 lb pressure for 15 minutes. The inoculation of 100 µl of fresh isolated strains of LAB was done in broth media and incubated at 37°C for different hours such as 24, 48, 72 and 96 hours. Optical density was taken by spectrophotometer after 24 hours, then 48 hours, 72 hours and finally after 96 hours under sterilized conditions at 590nm. Lastly, a graph was plotted between optical density along Y-axis and each incubation time on X-axis, the optimum temperature of each strain was checked. The graph indicated the optimum incubation time for each bacterial strain.

Effect of different carbohydrate sources: To figure out the effect of different carbohydrate sources on bacteriocin production for each LAB isolate, MRS broth of 5ml was prepared in test tubes in four sets, in each set 4 different carbohydrate sources were added during media preparation including glucose, sucrose, lactose and galactose respectively. Each test tube was properly labeled and used for each strain. The broth was sterilized by using autoclave for 15 minutes at 15 lb pressure.

The inoculation of each isolated strain of LAB was done in media and incubation was done for 72 hours at 37°C. After 72 hours, by using spectrophotometer at 590nm, optical density was observed. Lastly, a graph was plotted between optical density along Y-axis and each carbohydrate source on X-axis. the

optimum temperature of each isolated strain was determined.

Effect of NaCl concentration: Effect of NaCl on bacteriocin production was checked adding 2, 4 and 6% NaCl concentration (w/v) in MRS broth during media preparation. The medium was sterilized for 15 minutes at 15 lb. 100 µl of fresh LAB culture was used to inoculate the medium for each isolated strain. Incubated was done for 72 hours at 37°C. After 72 hours at 590nm by using spectrophotometer optical density was. To find out optimum temperature, a graph was plotted taking NaCl concentrations on X-axis and optical density along Y-axis.

RESULTS

Isolation of bacterial strains: Out of 12 pickle samples 18 bacterial strains were isolated and purified

on MRS agar plates. Bacterial strains were isolated as a consequence of morphology of the colony. All these experiments were conducted under sterilized laboratory conditions (Liu et al., 2022).

Morphological characterization: Through morphological characterization, 13 bacterial strains were selected out of which 11 were off white and 2 of them were yellow in color. Optical characterization showed that majority of them were opaque. Surface texture of most of the strains were shiny or glittery. Most of the colonies showed raised elevations. Most of them were circular and a few had irregular margins (Table 1). All of the strains were gram-positive, non-motile and non-endospore formers (Table 2).

Table 1: Colony morphology of LAB isolates.

Strains	Form	Colour	Texture	Margin	Elevation	Optical Character	Surface
SH1	Circular	White	Buttery	Entire	Raised	Opaque	Shiny
SH2	Circular	White	Buttery	Entire	Raised	Opaque	Shiny
SH3	Circular	White	Buttery	Entire	Raised	Opaque	Smooth
SH4	Circular	White	Moist	Entire	Raised	Opaque	Shiny
SH5	Circular	White	Buttery	Entire	Raised	Opaque	Smooth
SH6	Circular	White	Buttery	Entire	Umbonate	Opaque	Smooth
SH7	Circular	White	Buttery	Entire	Umbonate	Opaque	Shiny
SH8	Circular	Light Orange	Buttery	Entire	Umbonate	Opaque	Shiny

Table 2: Colony morphology (II) of LAB isolates.

Strains	Gram Staining	Endospore Staining	Motility Test
SH1	+ve	-ve	Non-Motile
SH2	+ve	-ve	Non-Motile
SH3	+ve	-ve	Non-Motile
SH4	+ve	-ve	Non-Motile
SH5	+ve	-ve	Non-Motile
SH6	+ve	-ve	Non-Motile
SH7	+ve	-ve	Non-Motile
SH8	+ve	-ve	Non-Motile

Biochemical characterization: Catalase test was performed for selected 8 strains of lactic acid bacteria and all strains showed negative results for catalase test as these bacteria do not have capacity to degrade hydrogen peroxide. 7 strains of lactic acid bacteria gave negative result for gelatin hydrolysis while 1 of them gave positive result. All of the 8 strains showed positive result for carbohydrate fermentation test as all the lactic acid bacteria are acid producers and majority of them gave yellow color after performing the test. None of them showed gas production so the selected LAB isolates were non gas formers. When urease test was performed, 7 of the LAB isolates gave negative result while one of them gave purple color after performing the test. All of the strains gave negative result for citrate utilization. When nitration reduction test was performed, majority of the strains gave negative result and most of them appeared yellow while one of them appeared purplish red. Litmus milk broth test was performed and all the LAB strains gave positive result (Table 3).

Table 3: Bactericidal activity (measurement of zone of inhibition in mm) of LAB isolates against bacterial pathogens.

Strains	<i>E.coli</i>	<i>S. aureus</i>	<i>B. cereus</i>
	Zone of inhibition (mm) M ± SD	Zone of inhibition (mm) M ± SD	Zone of inhibition (mm) M ± SD
SH1	2.5 ± 0.30	4.0 ± 0.20	8.0 ± 0.20
SH2	2.0 ± 0.25	4.0 ± 0.02	6.0 ± 0.70
SH3	2.0 ± 0.02	3.0 ± 0.25	3.0 ± 0.03
SH4	2.5 ± 0.03	2.5 ± 0.30	4.0 ± 0.25
SH5	3.0 ± 0.25	NA	3.5 ± 0.25
SH6	NA	3.0 ± 0.20	NA
SH7	1.0 ± 0.20	NA	2.5 ± 0.03
SH8	1.5 ± 0.03	3.5 ± 0.25	NA

KEY: mm (millimeter), M= Mean, SD= Standard deviation, NA=No activity

Blood agar test: Blood agar test was conducted for isolation of indicator pathogenic bacterial strains used for assaying antibacterial activity. Blood agar test was performed on 3 of the pathogenic strains (*E. coli*, *S. aureus* and *B. cereus*) that were taken and checked for hemolysis and these pathogenic strains showed beta-hemolysis so the 3 pathogenic strains were considered as indicator strains to check antibacterial activity of isolates of lactobacilli.

Antibacterial activity of LAB isolates: Antibacterial activity of LAB isolates was checked by performing disk diffusion method. Out of 18 LAB strains, 8 of them showed antibacterial activity by giving medium

zone of inhibition against indicator bacterial pathogens (*E. coli*, *S. aureus* and *B. cereus*). Two of the isolates showed small zones of inhibition. 8 of the isolates revealed no zone of inhibition against indicators strains which means they did not show any antibacterial activity. Hence, 8 (SH1, SH2, SH3, SH4, SH5, SH6, SH7, SH8) of the LAB strains were considered to be bacteriocidal against pathogenic strains. Bacteriocin activity was represented in diameter (mm) of inhibition zones. If the diameter (mm) of ZOI was greater than 1mm, the results were considered positive (Table 4).

Table 4: Protein content of bacteriocin by LAB isolates.

Strains	Absorbance (595nm)	Protein Content (mg)
SH1	0.618	9.686
SH2	0.480	7.523
SH3	0.437	6.849
SH4	0.461	7.225
SH5	0.506	7.931
SH6	0.296	4.639
SH7	0.495	7.758
SH8	0.381	5.971

Protein (bacteriocin) estimation by Bradford method: Absorbance was taken at 595 nm plotted on y-axis and the concentration of protein plotted at x-axis. Protein content was calculated by putting the value of absorbance (y) in the equation displayed by setting linear trend line.

Characterization of bacteriocin

Sensitivity to enzymes: Bacteriocin was treated with proteolytic and non-proteolytic enzymes to check out its sensitivity. While treating with proteolytic enzymes like proteinase-K, pepsin and trypsin, bacteriocin showed no antibacterial activity as these enzymes degrade proteins and this treatment confirmed that bacteriocin is protein in nature. On the other hand, when bacteriocin was treated with catalase which is

non proteolytic enzyme, it showed significant antibacterial activity as it was not degraded.

Heat stability: The stability of bacteriocin activity and heat resistance was examined at 4°C and then in a thermostatically controlled water bath at 60°C to 100°C at increments of 10°C (60, 70, 80, 90, 100°C) and then in an autoclave at 121°C for 15 minutes. Bacteriocin showed its significant antibacterial activity at 60, 70, 80, 90 and 100°C but there was no activity shown at 4°C and 121°C, as at this temperature no bacterial growth occurs.

Sensitivity to pH: pH stability of bacteriocin was examined by adjusting to different pH ranges from 3.0 to 10.0. Bacteriocin showed its significant activity at pH 3.0 to 9.0 but there was no antibacterial activity seen at pH 10.0

Table 5: Effect of temperature, effect of enzymes and effect of pH on bacteriocin activity.

Effect of Temperature		Effect of Enzyme		Effect of pH	
Treatment	bacteriocin activity	Treatment	bacteriocin activity	Treatment	bacteriocin activity
4°C	-ve	Proteinase	-ve	3.0	-ve
60°C	+ve	Trypsin	-ve	4.0	-ve
70°C	+ve	Pepsin	-ve	5.0	+ve
80°C	+ve	Catalase	+ve	6.0	+ve
90°C	+ve			7.0	+ve
100°C	+ve			8.0	+ve
121°C	-ve			9.0	+ve
				10.0	-ve

Determination of optimal growth conditions

Growth curves: For obtaining growth curves of the strains, O.D. was determined for 14 hours and 15minutes. With reference to growth curve, the LAB strain SH1 exhibited a lag phase after 1 hour time interval, log phase was observed till 9 hours and 15 minutes, after that stationary phase started. Strain SH4

exhibited a lag phase after 1 hour time interval, log phase was observed till 7 hours and 15 minutes, after that stationary phase was observed which persisted for 7 hours. SH5 strain showed lag phase of one hour, log phase was observed for 7 hours and 15 minutes after that stationary phase was observed, which persisted for 7 hours.

Determination of Optimum pH: In general, LAB isolates were able to grow over a wide range of pH (5, 6, 7, 8 and 9). The optimum pH of MRS medium was seen to be 6.0 and growth of SH1 was maximum at pH 8. SH2 had maximum growth at pH 9. Least growth was observed to be at pH 5 whereas LAB strains showed significant growth at pH 6, 7, 8, and 9.

Determination of optimum temperature: Optimum temperature for growth was 37°C and strains exhibited best growth at 30°C and 37°C and bacteriocin activity was maximum at 37 °C.

Effect of incubation time: It was observed that growth of all strains was maximum at 72 and 96 hrs. Maximum growth was seen to be at 96 hours by SH3 and least growth was observed at 24 hours by SH7.

Effect of different carbohydrate sources: Effect of 4 carbohydrate sources (Glucose, sucrose, lactose and galactose) was observed on the growth of LAB isolates and it was seen that growth was achieved with all sources. Growth was maximum with galactose and achieved by SH1 and least was seen by SH5 with glucose.

Effect of NaCl concentration: It was observed that growth and bacteriocin activity was seen at all concentrations. Although it was noted that growth was not affected by addition or absence of NaCl.

DISCUSSION

In the present research, a diverse bacterial microflora was obtained from pickle samples which included 18 bacterial strains. Among these 18 isolates, 8 LAB strains showed antibacterial activity.

Bacteriocin activity showed positivity against both gram-positive and gram-negative bacterial strains, *S. aureus*, *B. cereus* and *E. coli*, respectively. This result is conforming to the previous work where crude bacteriocin was not showing positive activity against not active against gram-negative bacteria it might be due to the other membrane of gram-negative bacteria which affected the site of bacteriocin action (Natthida and Piyawan, 2011). It has been reported that the growth of LAB isolates at 6% and 8% NaCl concentration was slight but in present research, LAB isolates had significant growth at these concentrations. A study revealed that bacteriocin showed its maximum antibacterial activity towards *E. coli* which is gram-negative than the gram-positive strain (Vinod et al., 2006). In the same study, the maximum antibacterial activity was obtained at pH 4 and 5 whereas in present study, maximum activity was observed to be at pH 6, 7 and 8.

A previous study reveals that bacteriocin activity was unaffected or increased during treatment with trypsin and protease although they are proteolytic enzymes (Aslam and Qazi, 2010). In present research, antibacterial activity was totally inhibited by trypsin. Bacteriocin activity seen when treated with catalase but it is minimum when treated with proteinase-K, pepsin and trypsin.

Earlier it was observed that *Lactobacillus* sp. attained maximum inhibitory activity at incubation time of 24-48 hours and *Pediococcus* sp. attained its significant antibacterial activity at 48 hours and the partial purification was done by washing method (Tejpal, 2012). We have observed that *Lactobacillus* sp. and *Streptococcus* sp. had their maximum activity at 72 hours of incubation time but washing was done with the same method during purification.

The total protein content of bacteriocin of 8 LAB strains was employed using Bradford method. Protein content was measured by comparing it with BSA standard curve. By setting linear and trend line, protein content was calculated by putting the values of absorbance at y-axis (Figure 1). The higher protein content was observed to be 9.686 for strain SH1 (*Lactobacillus* sp.) and lower was observed to be of SH6 (*Streptococcus* sp.) which was calculated as 4.639.

In a previous study, *Lactobacillus brevis* gave lower protein content than *Streptococcus pyogenes* but another specie of *Lactobacillus* gave a higher protein content than *Streptococcus pyogenes* (Tolinacki et al., 2010). In present study, protein content both higher and lower were observed *Lactobacillus* but in both cases species were different. *Streptococcus* sp. lied at the middle of the two protein contents.

Graphs has been plotted for optimum temperature in which temperature at x-axis and absorbance at y-axis has been observed. Absorbance of all the LAB isolates have shown a slight variation at different points. Absorbance has observed to be maximum at room temperature for SH1 which is 0.48 and least for SH6 at 45°C which is 0.001. In other study bacteriocin activity has been maximum at 40 °C whereas in present study it was observed to be maximum at 37 °C (Figure 2 a and b).

Maximum growth and bacteriocin activity have been observed at 96 hours in SH3 which is 0.96 and least activity has been seen at 24 hours whose optical density is 0.002 for SH7. Variation in antibacterial activity has been observed at 48 and 72 hours. Some studies have revealed maximum bacteriocin activity at 48 hours.

Graphs were plotted for pH at x-axis and optical density at 595 nm for all the LAB strains. Optical density for all LAB strains is 1.387 maximum at pH 9 for strain SH2 and least was seen at pH 5 which was 0.005 (Figure 3). It was somewhat increased at pH 6 then a light decreases at 6.5 and attained maximum activity at Ph 6 and 7. But in the study Nithhida and Piyawan reported in their research that increase in bacteriocin activity was observed at 6.5 and a light decrease occurred at 7.5 and 8.

Effect of different carbohydrate sources has been plotted against a graph in figure 4.13 in which different carbohydrates has been taken at x-axis and optical density at 590 nm. Variation in bacteriocidal activity of LAB isolates has been observed. There is a slight decrease in activity observed at glucose

concentration and then an increase at sucrose. Maximum activity observed at lactose and galactose which is 0.92 for SH1 and least at glucose while Con and Karasu revealed in their study that minimum bacteriocin activity has been observed at sucrose.

A comparison graph has been plotted against NaCl concentrations where concentrations taken at x-axis and optical density at 590 nm taken at y-axis (Figure 4). The graph is a comparison among different NaCl concentrations. Bacteriocin activity was

significant at all concentration but maximum at 4% NaCl whereas in a previous study, bacteriocin did not show any activity at 6% and 8% NaCl concentrations (Aslam and Qazi,2010). Variations were seen from 2% to 4% NaCl concentrations when antibacterial activity was gradually increased to maximum and then a slight decrease occur when activity was seen at 6% NaCl concentration but it did not decrease too much. Still was significant enough to be inhibitory against the pathogenic strains

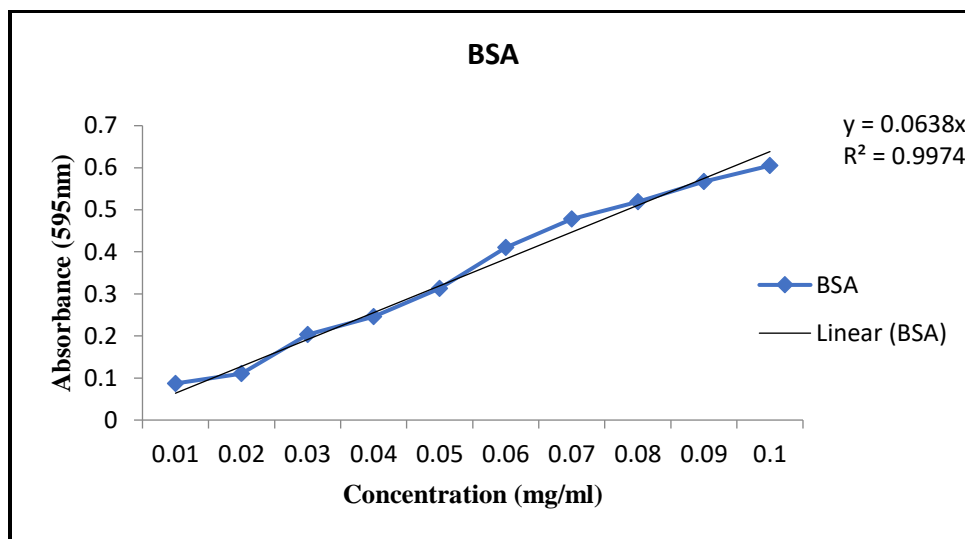


Figure 1: Standard curve of BSA for protein (bacteriocin) estimation.

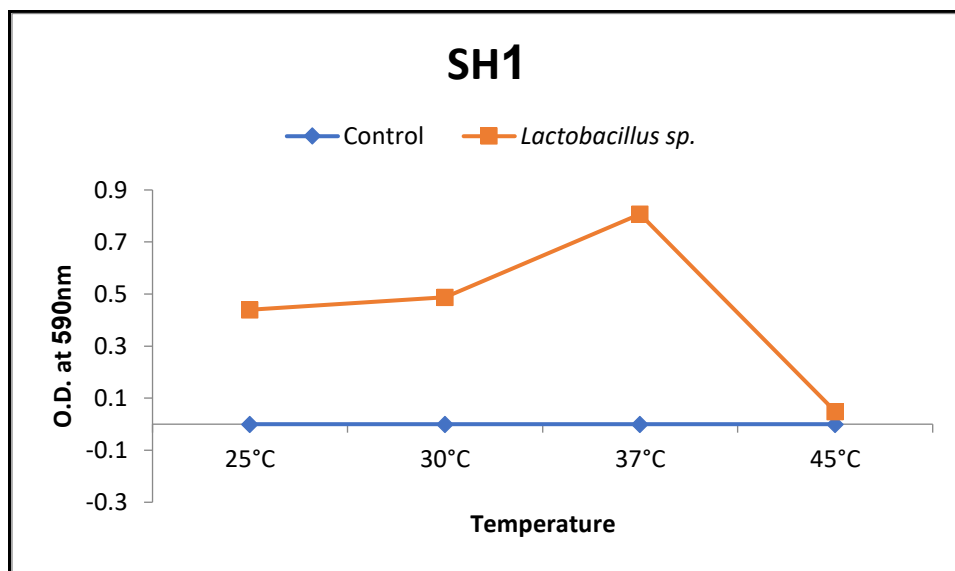


Figure 2(a): Effect of temperature on LAB isolate SH1 (*Lactobacillus* sp.).

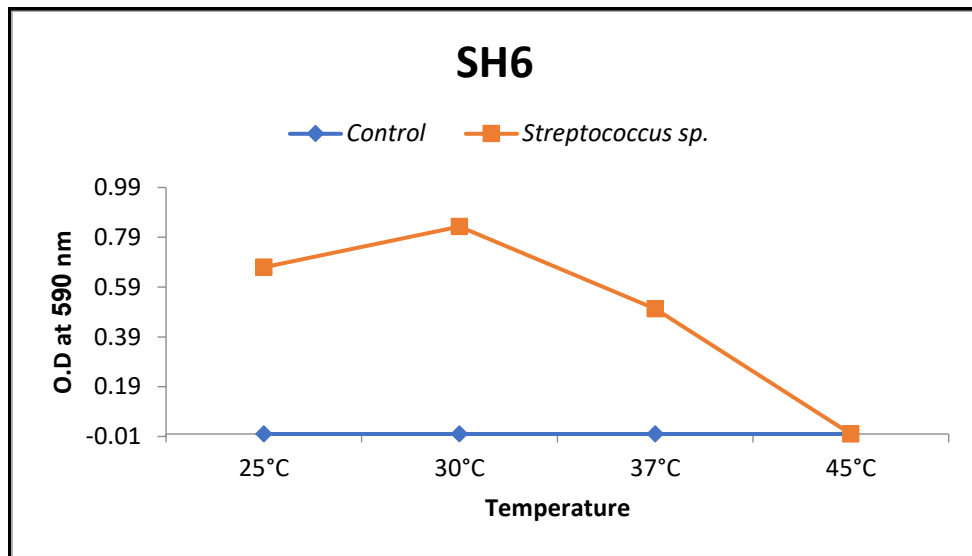


Figure 2(b): Effect of temperature on LAB isolate SH6 (*Streptococcus sp.*).

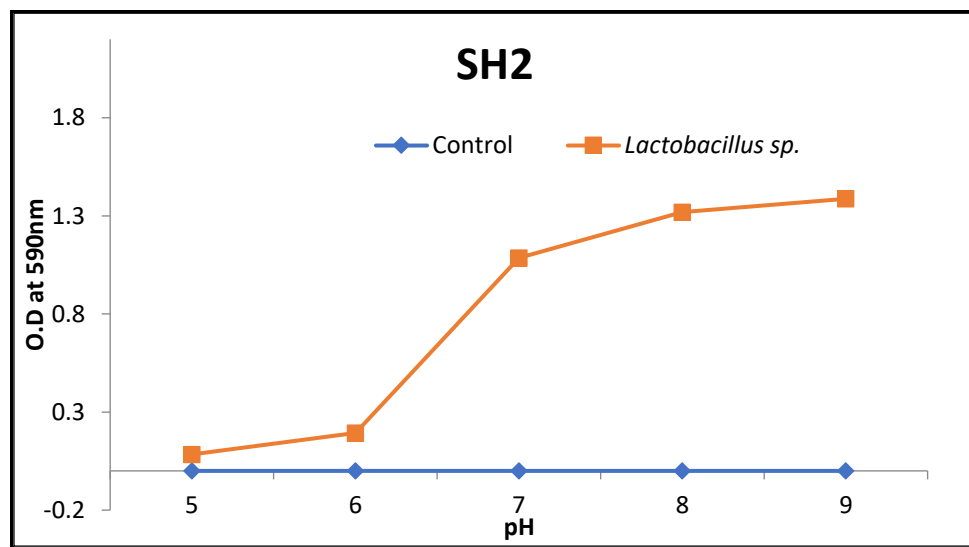


Figure 3: Effect of pH on LAB isolate SH2 (*Lactobacillus sp.*).

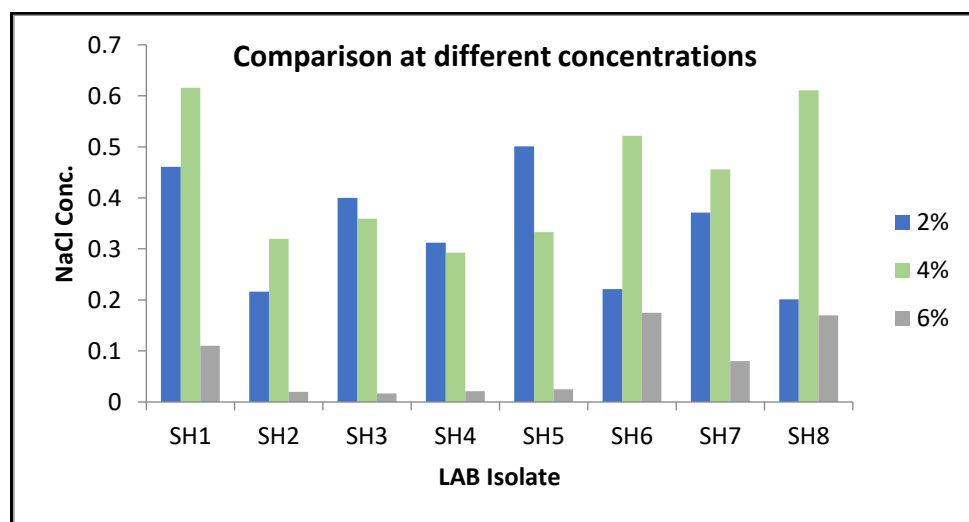


Figure 4: Comparison of effects of different NaCl concentrations on LAB isolates (2, 4, 6%).

CONCLUSIONS

Lactic acid bacteria are very closely linked with the human environment. They are typically associated with a number of spontaneous food fermentations such as pickles, which are a good example of fermented foods. Before commercialization, there is need to work further to identify and study LAB at species level and their bioactive compounds. The present study revealed that LAB are human friendly and are beneficial for health as they are inhibitory against food borne bacterial pathogens which can cause variety of food borne diseases. Hence, the study revealed that *Lactobacillus* sp. and *Streptococcus* sp. isolated from pickles can inhibit the growth of food pathogens entailing *E. coli*, *S. aureus*, and *B. cereus*.

CONFLICT OF INTEREST

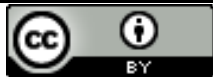
There is no conflict of interest.

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