CHARACTERIZATION OF BACTERIOCIN PRODUCE BY LACTIC ACID BACTERIA ISOLATED FROM SAUERKRAUT

Shaikh Rajesh Ali*
Assistant Professor, Post Graduate Dept. of Microbiology,
Acharya Prafulla Chandra College, New Barrackpore, West Bengal-700131, India.
Email: rajumicro1979@gmail.com

ABSTRACT
Food loss due to spoilage is a significant problem worldwide. About 30-40 % foods are lost due to improper handling, lack of preservation and by other means. Food processing industries use different type of chemicals to minimized the economic loss but some time these chemicals proven negative consequence for human health. The green technology with using natural preservative can use consider as alterative. Lactic acid bacteria are probiotic microorganism used for fermentation have the ability to control food spillage and food born pathogen. Some of LAB produce bacteriocin like molecule that provide added advantages. Bacteriocins are proteinaceous extracellular metabolite secreted by some microorganisms have known for its ability to destroy other microorganisms.

In this research we try to find out some fermentative microorganism belong to lactic acid bacteria family having bacteriocin production ability. In this study we screened lactic acid bacteria from sauerkraut (fermented cabbage) using MRS agar media and find out bacteriocin producer belong to Lactobacillus sp. confirmed by different morphological and biochemical test. The partially purified bacteriocin`s activity was assayed using four different indicator strains belong to food spoilage and pathogen groups to prove its bio-preservative ability and prevention of food born disease.

Our isolated Lactobacillus sp. have the ability to produce bacteriocin that are heat stable and active under wide range of pH.

INDEX TERMS: Lactic acid bacteria, Bacteriocin, Indicator strain, Proteinase K.

INTRODUCTION

The presence of spoilage bacteria in food represents a serious problem to mankind with respect to health and economics. With industrialization in food sector many chemical preservatives are added in food product for long time storage (Bali et al., 2011). Many such chemical food additives create undesirable effect in human life. With modernization of community demand of minimally processed foods that are chemical free is increasing. Given these demand, scientist, researcher and industrial microbiologist try to find out new alternatives for natural food preservation is of priority. From literature it is found that probiotic microorganisms to inhibit pathogens and other spoilage microorganisms through different mechanisms such the production of antimicrobial agents (e.g., bacteriocin) or organic acids (e.g., lactic acid, hydrogen peroxidase) (Danielsen and Wind, 2003; Collado et al., 2007; Longdet et al., 2011; O'Shea et al., 2011; Zendo, 2013; da Silva Sabo et al., 2014). Now a day different types of probiotic microorganisms from fermented food product used as preservative to develop functional food (De Jong, A et al., 2006).

Sauerkraut is a fermented food product produce by lactic fermentation of cabbage (Holzapfed et al., 2003; Clarke T.C., 2015). It is considered to be a functional food that are full of probiotic microorganism especially lactic acid bacteria (LAB). The lactic acid bacteria can ferment different types of sugar to produce organic acid and other antimicrobial metabolites such as bacteriocin that prevent food spoilage and different pathogenic microorganisms (Thokchom et al., 2012; Buckenhüskes et al., 1993; Mokoena et al. 2016). Bacteriocins first time invented by Gratia in 1925; are proteinaceous antimicrobial substance extracellularly released by bacteria to inhibit the growth of similar or closely related bacteria (Deshmukh PV.et al., 2013; Delves-Broughton et al., 1996; Holzapfel et al., 1995; Tagg. J.R.et al., 1976). These bacteriocins are produced by Gram-positive (Chavan & Riley, 2007), Gram-negative (Lee & Kim, 2011) and also by Archae. Although spectrum of activity considerable varies but it mostly effective against gram positive bacteria (Yang, E. et al.

2012; Cotter et al., 2005). Most of bacteriocin are effectively bind to the membrane of the cell and impair its function. Bacteriocin of lactic acid bacteria are consider effective food preservative due its thermostability, diverse pH activity, do not change organoleptic properties and more easily degraded by proteolytic degradation by gastrointestinal enzymes (Gautam N et al., 2009; Cleveland J et al., 2001).

The aim of this study to isolate lactic acid bacteria; Lactobacillus sp. from sauerkraut and analyse the production of antimicrobial substance other than organic acids such as bacteriocin and characterization of it to fulfil the need of natural preservation and to encourage green technologies in food industry.

MATERIAL AND METHOD

A. MICROORGANISM STRAINS

The bacterial strain Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus was used as test organism. All culture was obtained from P.G. department of Microbiology, Acharya Prafulla Chandra College, Kolkata. They were subculture and maintained on nutrient agar media and used for further experimental work. The isolated strain from sauerkraut maintained in MRS media and made glycerol stock for future work.

B. ISOLATION OF LACTIC ACID BACTERIA FROM SAUERKRAUT

Lactic acid bacteria isolation was carried out based on Abbasiliasi et al. with minor modification. To isolate LAB, 5gm fermented cabbage are mixed with 95 ml phosphate buffer saline (PBS) and mixed well. The mixture was crushed by motor pastel and sup are collected. The crushed product was filtered through Whatman filter paper 20. The filtrate was used for microbial analysis.

The filtrate was diluted up to 10^{-6} and 0.1 ml of it plated on Plate Count Agar (PCA) media (Enzymatic Digest of Casein/tryptone 5 g/l, Yeast Extract 2.5 g/l, Glucose 1.0 g/l, Agar 18.0 g/l, pH 6.5) and incubated at 37^0C for 48 hours.

Fifty colonies were randomly selected and used for Lactobacillus sp. screening. In this process colonies from PCA plates were subculture in MRS (deMan Rogosa Sharpe) agar media (Protease Peptone 10g/l, Beef Extract 10 g/l, Yeast Extract 5 g/l, Dextrose 20 g/l, Polysorbate 80 1 g/l, Ammonium Citrate 2 g/l, Sodium Acetate 5 g/l, Magnesium Sulphate 0.1 g/l, Manganese Sulphate 0.05 g/l, Disodium Phosphate 2.0 g/l, Agar 12.0 g/l, pH 6.5) agar supplemented with 0.01% (w/v) sodium azide (as an inhibitor for growth of Gram-negative bacteria).

The colonies grow in MRS media further subculture on MRS agar contained 1 % (w/ v) $CaCO_3$ and incubated at 37^0C for 48 hours. Twelve different colonies that produce zone of clearance are selected for further study.

C. SCREENING FOR BACTERIOCIN PRODUCERS

To isolate bacteriocin producing lactic acid bacteria, primary screening is performed by inhibitory effect on test organisms (Tagg et al., 1976). The test organisms (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus) ware lawn culture on nutrient agar media and the selected microorganism from MRS media were spotted over it and incubate for 48 hours at 37^0C.

Six isolates that produce good clear zone on test organisms were selected and grown in MRS broth culture to evaluated bacteriocin production. The MRS broth after incubation 48 hours was centrifuge at 8000rpm for 10 minutes at room temperature and supernatants were collected. The pH of supernatants was adjusted to 6.0 with 2(N) NaOH. The cell free extract was further purify using 0.22µm membrane filter.

The antimicrobial activity of filtrates was carried out by agar well diffusion method. In this method a 6mm diameter wells were made on pre-inoculated nutrient agar plate and 100 µl of culture supernatant were added in each well. The inhibitory activity was measured and record by zone of clearance after 24 hours of incubation at 37^0C. The inhibitory activity was performed against four indicator microorganisms viz. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus.

Isolate`s cell free extract which showed the antimicrobial activity on all indicator organisms was selected for sensitivity test against proteolytic enzyme and producer were lactobacillus confirmed by gram staining and biochemical test according to Bergey's Manual.

D. PROTEOLYTIC SENSITIVITY OF BACTERIOCIN

Cell free extract of crude bacteriocins was assayed against proteinase K enzyme in presence of 50mM Tris-HCl buffer (pH 8.0). Crude extract of bacteriocin without addition of proteinase K enzyme was used as a control. All tubes were assayed for antimicrobial activity against test organisms using agar well diffusion method (Ogunbanwo et al., 2003; Udhayashree et al., 2012). The cell free supernatants that was sensitive to proteolytic enzyme (proteinase K), no transparent halo zone surrounded the wells, were considered to be presence of bacteriocin.

E. CHARACTERIZATION OF CRUDE BACTERIOCIN

IIE1. EFFECT OF PH ON THE ANTIBACTERIAL ACTIVITY OF BACTERIOCIN

About 2 ml of each crude bacteriocin extracts (L_{B1}, L_{B2}) were distributed in different test tube and the pH was adjusted to 2, 4, 6, 8 and 10 by using 1 (N) HCl and 1 (N) NaOH solutions and for 4 hours at room temperature. Thereafter, the antimicrobial activities were assayed using agar well diffusion method by taking 100 µl samples from each tube against test organisms. The antimicrobial activity was expressed as arbitrary unit (AU) per ml which is defined as the area of inhibition zone per volume of bacteriocin sample (mm^2/ml) (Usmiati & Marwati, 2007). The activity of bacteriocin can be calculated using formula:

$$\text{Bacteriocin activity } (mm^2/ml) = (Lz - Ls) / V$$

Where: Lz = The area of transparent zone (mm^2)
Ls = The area of well (mm^2)
V = volume of sample (ml

IIE2. EFFECT OF TEMPERATURE ON THE ANTIBACTERIAL ACTIVITY OF BACTERIOCIN

The effect of temperature on antimicrobial activity of bacteriocin was assayed at pH 4.0 by taking 2ml of each crude extracts in test tube and incubating in water bath at different temperature, viz. 50^0C, 60^0C, 80^0C, 100^0C and 120^0C for 30 minutes. The stability of heat treated bacteriocins were determined by antimicrobial activity using agar diffusion method against test organisms as describe above. Crude extract produce halo zone after heat treatment considered stable bacteriocins and activity was determined quantitively (AU/ml) using above formula.

IIE3. EFFECT OF DETERGENTS ON THE ANTIBACTERIAL ACTIVITY OF BACTERIOCIN

The detergent's effect on bacteriocin activity was assayed using EDTA (Merck, US), SDS (Sigma Aldrich Corporation, US), Triton X-100 (Merck, US) and Tween 20 (Sigma-Aldrich Corporation, US) at the final concentration 10 mg/ml. 2ml of each cell free extracts were incubated with detergents for 1 hours at room temperature. 100 µl of each treated sample were evaluated for antimicrobial activity by the procedure as described above (agar well diffusion assay) and the activity units for each treatment were determine and recoded.

RESULT AND DISCUSSION

IIIA. ISOLATION OF LACTIC ACID BACTERIA FROM SAUERKRAUT

Sauerkraut also called acidic cabbage is a fermented food product rich in lactic acid bacteria content. The lactic acid bacteria are bio-preservative due to its antimicrobial activity against food spoilage and pathogenic microorganisms by different means. Lactic acid bacteria lower the pH of food sample also have the ability to produce bacteriocin like molecule that are inhibitory to other microbial entity. In our experiment we randomly select fifty colonies from PCA plate and streak on MRS media for searching Lactobacillus sp. Lactobacillus is a gram-positive bacterium so Sodium azide is added in MRS media to avoid any unwanted growth of gram-negative bacteria and to increase selective nature of MRS media. After incubation in MRS media, we found 22 colonies produce growth. When further screening was done in CaCo3 containing MRS agar only 12 colony produce clear zone due to acid production provide the hints for lactic acid bacteria.

IIIB. SCREENING FOR BACTERIOCIN PRODUCERS

Twelve colonies from $CaCO_3$ supplemented MRS plate are further screened for antimicrobial activity using four test organisms (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus) and found only six organisms produce clear zone after incubation of plates. This zone of inhibition may be due to lactic and/or bacteriocin. To further screen for bacteriocin producing strain we collect cell free extract after growing them in MRS broth and neutralizing the lactic acid by 2(N) NaOH. The filtrate cell free extract assayed for antimicrobial activity and found one isolate`s extracts produce zone of inhibition on strain Escherichia coli and Pseudomonas aeruginosa; three isolate's extract do not produce clear zone and remaining

two isolate's cell free extract produce clear zone on all four-test organism. The isolates that produce zone of inhibition in all four-indicator strain are due to bacteriocin production and designated as L_{B1} and L_{B2}.

The L_{B1} and L_{B2} are belonging to gram-positive rod-shaped bacteria and belong to different group Lactobacillus sp. confirmed by biochemical test. Both bacteria are common in most of the biochemical test (H_2S production, Nitrate Reduction, Urease, Indole, Methyl Red, VP and Oxidase) except catalase and citrate initialization test. Although both isolates are gram positive but L_{B2} is slightly longer then L_{B1} and produce yellowish pigment in nutrient agar medium. The sugar fermentation test revels that the L_{B1} distinct from L_{B2} (Table 1). Both isolated strains belong to Lactobacillus family confirmed by comparing with Bergey's Manual of Determinative Bacteriology (Holt et al.,1994) with the help of software ABIS online.

Table 1: Different biochemical test for L_{B1} and L_{B2}.

Morphological/Biochemical test		**Properties of L_{B1}**	**Properties of L_{B2}**
Morphological test	Colony morphology	Circular, glossy, smooth	Circular, glossy, smooth
	Pigmentation	Non- pigmented	Yellow-pigmented
	Bacteria Shape	Rod Shaped	Rod Shaped
	Gram character	Gran positive	Gram positive
	Size	Small	Slightly longer
Biochemical test	H_2S production	-	-
	Nitrate Reduction	-	-
	Catalase	-	+
	Urease	-	-
	Indole	-	-
	Methyl Red	-	-
	VP	-	-
	Citrate	+	-
	Oxidase	-	-
Carbohydrate fermentation test	Glucose	+; gas production	+; gas production
	Sucrose	+; gas production	+; gas production
	Maltose	+; gas production	+; gas production
	Lactose	+; no gas production	+; gas production
	Mannitol	+; gas production	-
	Inositol	+; no gas production	-

IIIC. PROTEOLYTIC SENSITIVITY OF BACTERIOCIN

Bacteriocins are protein in nature and have antimicrobial activity. The activities are sensitive toward proteolytic enzyme. The table 2 shows that after treatment the crude extract with proteinase K the inhibitory activity was lost and confirming the bacteriocin production activity of L_{B1} and L_{B2} (Fig 1).

Table 2: Effect of proteolytic enzyme on bacteriocin activity

Treatment	**Zone of inhibition (bacteriocin activity)**			
	Escherichia coli	**Pseudomonas aeruginosa**	**Staphylococcus aureus**	**Micrococcus luteus**
L_{B1} Cell free extract	++	++	+++	+++
L_{B1} Cell free extract + Proteinase K	-	-	-	-
L_{B2} Cell free extract	++	+++	+++	++++

L_{B2} Cell free extract + Proteinase K	-	-	-	-

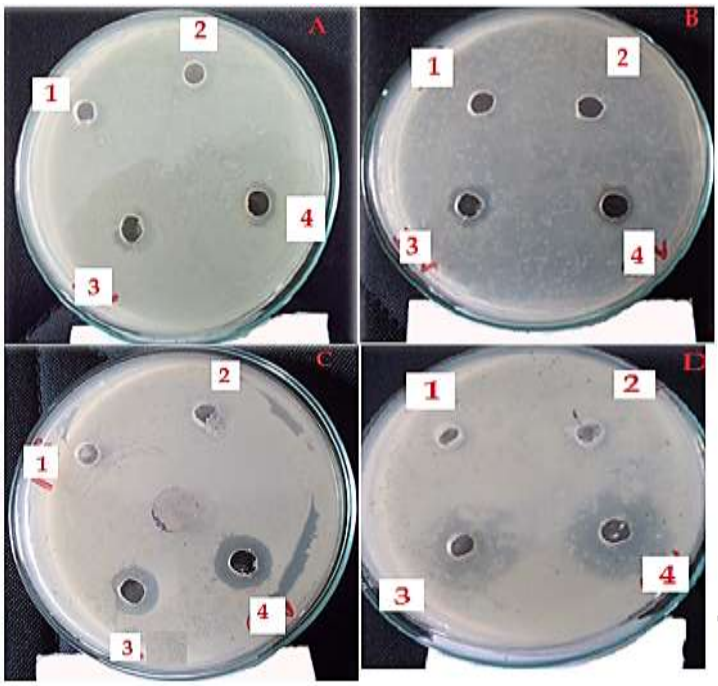

Fig. 1: Proteolytic sensitivity of bacteriocin. Analysis of zone of inhibition with proteinase k treatment (1, L_{B1}; 2, L_{B2}) and without proteinase k treatment (3, L_{B1}; 4, L_{B2}) against indicator strain E. coli (A); P. aeruginosa (B); S. aureus (C) and M. luteus (D).

IIID. Characterization of Crude Bacteriocin

Bacteriocin producing strain especially Lactobacillus sp. can be used as good preservative in food processing industry especially if bacteriocin was heat stable. In our study after heat treatment of cell free extract of L_{B1} and L_{B2} for 30 min at different temperature, we found the L_{B2} produce heat stable bacteriocin which can show the maximum activity at 80^0C and after 100^0C its activity was rapidly decreed. Whereas, the L_{B1} show the activity zone in between 50 to 80^0C with maximum at 60^0C (Fig. 2). The maximum activity of bacteriocin for L_{B1} was 8.254 AU/ml and for L_{B2} was 7.779 AU/ml in their respective temperature against the test organism Pseudomonas aeruginosa.

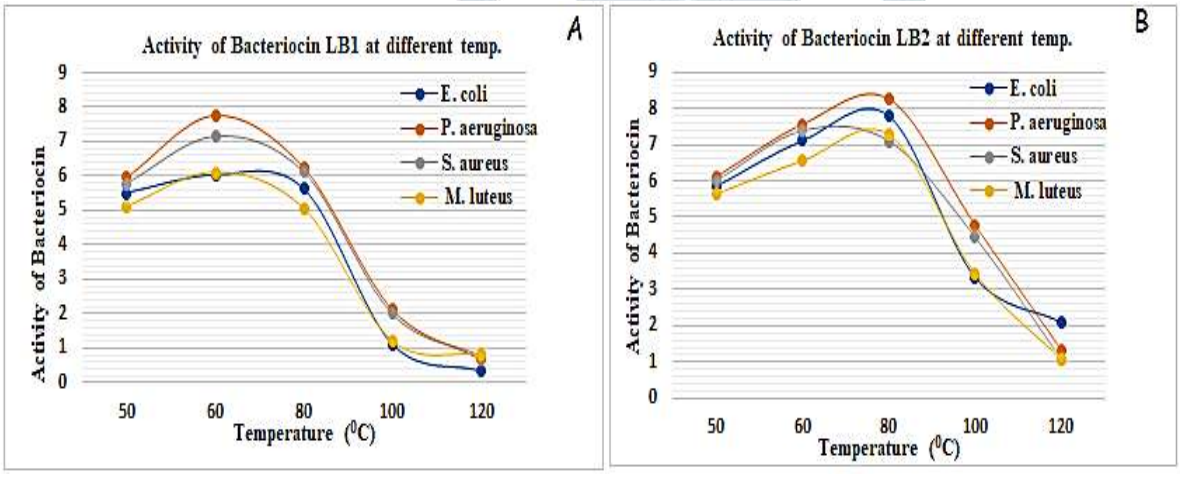

Fig 2: Bacteriocin activity of cell free extracts (A, L_{B1}; B, L_{B2}) assayed at different temperature against different indicator strains (E. coli, P. aeruginosa, S. aureus and M. luteus)

The effect of pH on activity of bacteriocin was assayed in terms of zone of inhibition on test organisms. Both bacteriocin (L_{B1} and L_{B2}) shows activity at a range pH 4 to 8. The L_{B1} activity was rapidly decrease then L_{B2} at pH 10 indicate some extent of pH stability of L_{B2}. The L_{B1} shows maximum activity (6.652 AU/ml) at pH 6.0 against Pseudomonas aeruginosa and the L_{B2} shows maximum activity (7.123 AU/ml) at same pH against Staphylococcus aureus (Fig. 3).

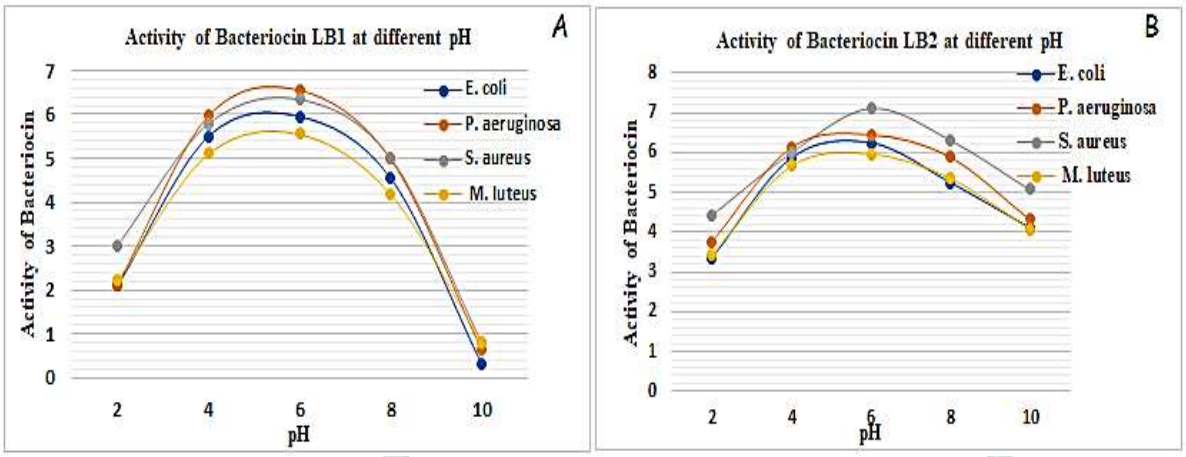

Fig 3: Bacteriocin activity of cell free extracts (A, L_{B1}; B, L_{B2}) assayed at different pH against different indicator strains (E. coli, P. aeruginosa, S. aureus and M. luteus)

The treatment of detergent showed variable activity in bacteriocin activity of isolated strains. We found when cell free extract was treated with SDS and EDTA it stimulates bacteriocin activity, Triton X100 has no effect but Tween 20 decease the activity in both strains. For L_{B1} maximum activity found against Pseudomonas aeruginosa when treated with EDTA (12.213 AU/ml) and SDS (11.132 AU/ml); for L_{B2} maximum activity found against Staphylococcus aureus when treated with EDTA (11.011 AU/ml) and SDS (11.431 AU/ml). The Tween 20 decrease about 50% activity both the cases in respect to control (Fig. 4).

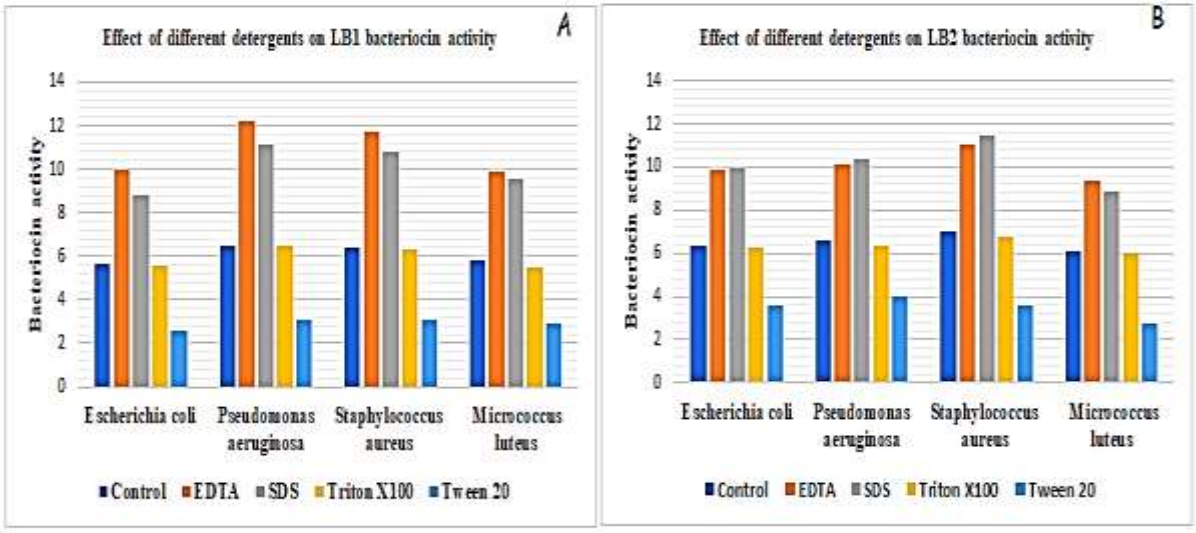

Fig 4: Effect of different detergents (EDTA, SDS, Triton X100 and Tween 20) on bacteriocin activity of cell free extracts (A, L_{B1}; B, L_{B2}) assayed against different indicator strains (E. coli, P. aeruginosa, S. aureus and M. luteus)

CONCLUSION

Prevention of food loss due to microbial spoilage is a challenging field in industrial sector. Although different types of chemicals are used to minimized the economic loss but some of them have negative aspect on human life. The antimicrobial products from microorganisms are natural preservative can be use as alternative. In our study we successfully isolate and characterized two such Lactobacillus sp. L_{B1} and L_{B2} from sauerkraut, that have potential to produce antimicrobial substance bacteriocin (tested against four indicator microorganisms) confirmed by proteolytic cleavage. Bacteriocin of both strains are effective against wide range of temperature and pH and the activity was enhance by some detergent (EDTA and SDS). In this study we proved L_{B1} and L_{B2} produce high quality of bacteriocin, that can be optimized for quantity and extraction in future and can be

used as preservative to minimized food loss, improve immunity (probiotic) as well as to prevent food born illness.

ACKNOWLEDGEMENTS

The author is highly thanks to Dr. Saktibrata Bhowmik (Principal of Acharya Prafulla Chandra College), Dr. Prajna Mondal (Head, Dept. of Microbiology, APC college) for continuous support, encouragement and guidance for completing this work. I thanks to all my students for their help and special thanks to my wife Beauty Parvin Sultana who always encourage and support to conduct the research work.

REFERENCES

1. Abbasiliasi S, Tan JS, Ibrahim TAT, Ramanan RN, Vakhshiteh F, Mustafa S, Ling TC, Abdul Rahim R and Ariff A 2012 Isolation of Pediococcus acidilactici Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry BMC Microbiology 12 260-271
2. Bali V, Panesar PS, Bera MB. Isolation, screening and evaluation of antimicrobial activity of potential bacteriocin producing lactic acid bacteria isolate. Microbiol J 2011:1(3);113-9.
3. Buckenhüskes, H. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiol. Rev. 1993, 12, 253–271.
4. Chavan, M.A., & Riley, M.A., "Molecular Evolution of Bacteriocins in Gram-Negative Bacteria", Bacteriocins: Ecology and Evolution, pp. 19-43, 2007.
5. Clarke T.C., Black L.I., Stussman B.J., Barnes P.M., Nahin R.L. Trends in the use of complementary health approaches among adults: United States, 2002–2012. Natl. Health Stat. Rep. 2015; 79:1–16.
6. Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: Safe, natural antimicrobials for food preservation. Int J Food Microbiol 2001;71(1):1-20
7. Collado M., J. Meriluoto, and S. Salminen. 2007 Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Lett. Appl. Microbiol. 45: 454–460
8. Cotter, P.D.; Hill, C.; Ross, R.P. Bacteriocins: Developing innate immunity for food. Nat. Rev. Genet. 2005, 3, 777–788.
9. da Silva Sabo S., M. Vitolo, J.M.D. Gonzalez and R.P. de Souza Oliveira. 2014. Overview of the Lactobacillus plantarum as a promising bacteriocin producer among lactic acid bacteria. Food Res. Int. 64: 527–536
10. Danielsen, M. and A. Wind A. 2003. Susceptibility of Lactobacillus spp. to antimicrobial agents. Int. J. Food Microbiol. 82: 1–11.
11. De Jong, A.; van Hijum, S.A.; Bijlsma, J.J.; Kok, J.; Kuipers, O.P. Bagel: A web-based bacteriocin genome mining tool. Nucleic Acids Res. 2006, 34, W273–W279.
12. Delves-Broughton. J., P. Blackburn, R.J. Evans and J. Hugenholtz, (1996). Applications of the bacteriocin, nisin. Antonie Van Leewenhoek. 69: 193-202.
13. Deshmukh PV, Thorat PR. Bacteriocins: A new trend in antimicrobial food packaging. Int J Adv Res Eng Appl Sci 2013;2(1):1-2.
14. Gautam N, Sharma N. Bacteriocin: Safest approach to preserve food products. Indian J Microbiol 2009;49(3):204-11.
15. Holt, J.G., Krieg, N.R., Sheath, P.H.A., Staley, J.T. and Williams, S.T. (1994) Bergey's Manual of Determinative Bacteriology (9th Edition). Baltimore, Maryland, USA: Williams & Wilkins.
16. Holzapfel, W., Schillinger, U., and Buckenhuskes, H. J. 2003. Sauerkraut. In. Handbook of Fermented Functional Foods, (E.R. Farnworth Ed.) pp. 343- 59. Raton, Fl: CRC Press.
17. Holzapfel. W.H., R. Geizen, U. Schillinger, (1995). Int. J. Food microbial., 24, 343-362
18. Lee, H., and Kim, H. Y. (2011) Lantibiotics, class I bacteriocins from the genus Bacillus. J. Microbiol. Biotechnol., 21, 229-235.
19. Longdet I.Y., R.J. Kutdhik and I.G. Nwoyeocha. 2011. The probiotic efficacy of Lactobacillus casei from human breast milk against shigellosis in albino rats. Advances Biotech. Chem. Proc. 1: 12–16.
20. Mokoena, M.P.; Mutanda, T.; Olaniran, A.O. Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. Food Nutr. Res. 2016, 60, 1–12.
21. O'Shea, E., P. Cotter, C. Stanton, R. Ross and C. Hill. 2011. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanism: bacteriocins and conjugated linoleic acid. Int. J. Food Microbiol. 152: 189–205
22. Ogunbanwo ST, Sanni AI, and Onilude AA. Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactobacillus brevis OG1. Afr J Biotechnol 2003;2(8):219-27.

23. Tagg. J.R., A.S. Dajani and L.W. Wannamaker, (1976). Bacteriocins of Gram-positive bacteria. Bacteriol. Rev. 40: 756-772.
24. Thokchom, S.; Joshi, S.R. Probiotic and bacteriocin efficacy of lactic acid bacteria from traditionally fermented foods: A review. Assam Univ. J. Sci. Technol. 2012, 10, 142–155
25. Udhayashree, N., Senbagam, D and Senthilkumar, B. 2012. Production of bacteriocin and their application in food products. Asian Pacific Journal of Tropical Biomedicine: S406-S410.
26. Usmiati, S and Marwati, T. 2007. Selection and optimation of process of bacteriocin production from Lactobacillus sp. J. Post-Harvest. 4 (1): 27-37.
27. Yang, E.; Fan, L.; Jiang, Y.; Doucette, C.; Fillmore, S. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express 2012, 2, 48.
28. Zendo T., N. Eungruttanagorn, S. Fujioka, Y. Tashiro, K. Nomura, Y. Sera, G. Kobayashi, J. Nakayama, A. Ishizaki, and K. Sonomoto. 2005. Identification and production of a bacteriocin from Enterococcus mundtii QU 2 isolated from soybean. J. Appl. Microbiol. 99: 1181–1190