Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 8 [9] August 2019 : 117-126

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

In vivo Biopreservation of Fresh Produce Using Lactic Bacteriocins

Seema Garcha
Department of Microbiology, Punjab Agricultural University, Ludhiana 141004, India
*Corresponding author email –sgarcha@pau.edu

ABSTRACT
Production of 'minimally processed food' remains a challenge before food industry. It involves the use of minimum intervention or use of biological means as native microflora to enhance the shelf life without compromising on the nutritional value of the food. Bacteriocins produced by lactics are important agents of food biopreservation. In the present work, bacteriocin preparation from *Lactobacillus plantarum* MTCC 9503 and *Lactobacillus acidophilus*NCDC 291 were applied of the surface of fresh produce to observe their efficacy in preventing mold spoilage. Standard microbiological procedures were used to record mold count. Physico-chemical quality attributes of the fresh produce were also observed and found to be stable throughput time period of incubation. Utility of bacteriocin was compared with that of commercially available bacteriocin, Nisin.
Keywords: *Biopreservation. bacteriocins, fresh produce, physico-chemical quality parameters, mold count*

Received 21.06.2019 Revised 23.07.2019 Accepted 01.08.2019

INTRODUCTION
India, is a leading world food producer but 20-30% of this produce is lost postharvest. Various factors responsible for this loss include poor infrastructure, lack of cold storage facility on farm, etc. Due to tropical and sub-tropical location, India has high average temperature which accelerates microbial growth. Micro-organisms are the major cause of food spoilage. Postharvest manhandling results in internal bruises, splitting, skin breaks and water losses [1]. Postharvest loss is compounded by the fact that India processes a dismal 2% of the total produce. This percentage is significantly higher in other countries.

Food preservation endeavors to enhance shelf life of food and also maintain its nutritional value. Use of heat, low temperature, modified atmosphere, chemical additives, radiations, etc. are principle conventional preservation techniques. Though efficient, they have certain inherent disadvantages. Loss of nutrients like vitamins, incidence of food allergies being notable among them. Newer methods of food preservation on the anvil include use of high hydrostatic pressure, high pulsed electric field, ozonization and biopreservation. Biopreservation involves the use of biological means or native microflora or their products to extend shelf life of the food[2, 3]. It is used to prepare "minimally processed foods" with diminutive compromise on nutritional value of the food.

Lactic acid bacteria have major potential for use as biopreservatives. They naturally dominate native microflora. Most of the lactic are known to produce bacteriocins at late log or early stationary phase. Bacteriocins are ribosomally synthesized, extra-cellularly released low molecular weight proteins or conjugate proteins. They have antimicrobial activity against food borne pathogens and also food spoilage agents. Nisin, bacteriocin produced by *Lactococcus lactis* subsp *lactis* was approved for use in food by US-FDA[4].

Bacteriocins have broad spectrum of activity[5,6]. They are effective against bacteria. *Lactobacillus plantarum* is known to inhibit the growth of Gram positives and Gram negatives and also molds. Plantaricin 35d produced by *L plantarum*is effective against *Aeromonas hydrophila*[7]. *L plantarum* isolated from molasses produced bacteriocins effective against *E coli* and *Acinetobacter baumanii*and also some Gram positive bacteria [8,9].*L plantarum* BFE 905 has been reported to inhibit the growth of *Listeria monocytegenes*[10]*Bacillus cereus*, *Cperfringenes*, *C sporogenes*[11]. Lactic bacteriocins are also known to be antimycotic. Consortium of *Lactobacillus* species reduced growth and inhibited aflatoxin production by *Aspergillus* sp. They inhibited spore germination and also mold growth.

Fresh produce as tomato, kinnow, pears and peaches are acidic in pH. It makes them more susceptible to mold spoilage. The present study was planned with the objective of determining the efficacy of *Lactobacillus* bacteriocins in preventing mold spoilage*in vivo*.

MATERIAL AND METHODS

Lactobacillus plantarum MTCC 9503 and *Lactobacillus acidophilus* NCDC 291 were procured from standard culture collections, Microbial Types culture Collection (MTCC), Institute of Microbial Technology, Chandigarh and National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal. Growth phase at which maximum bacteriocin production takes place was identified after plotting the growth kinetics of both the cultures. Bacteriocin preparation was partially purified by the previously reported method [12].

In vivo efficacy of bacteriocins against four fungal agents of food spoilage, *Aspergillus*, *Penicillium, Fusarium* and *Alternaria* were tested [13]. Tomato,Pears, Peaches, Kinnowof nearly uniform size and shape were procured from local market. They were cleaned using 70% (v/v) ethanol and then rinsed thoroughly with water. Each fruit was divided into at least three groups of approximately same weight and each group placed in a separate plastic bag. These plastic bags were treated as a sample. Each group was inoculated with mold suspension to a final concentration of 10^3 spores of each of the fourmoldmentioned earlier. After inoculation the fruits were allowed to dry at room temperature for one hour. Thereafter, it was treated for five minutes by immersion in partially purified bacteriocin of various concentrations, 20,000 to 30,000 AU of acidophilus preparation and 15,000 to 25,000 AU of plantarum preparation. Concentration of partially purified bacteriocins was selected on basis of the results of a study on *in vitro* antimycotic efficacy of these partially purified bacteriocins (data not shown). Samples were dried again and stored in plastic bags at two temperatures- 22°C and refrigeration temperature. Kinnow was however incubated at ambient temperature only because it is a winter fruit and possesses a protective rind. Control and test were run simultaneously. Each bag was removed periodically and treated as a sample for recording of microbial and physico-chemical quality observations. Fruits were macerated in saline under aseptic conditions before plating to record mold count. Standard microbiological procedures were followed for plating of appropriate dilutions of fruits on Potato Dextrose Agar. TSS, % acidity, weight change and color were also recorded. Firmness was tested using handheld firmness tester. Water activity was also estimated using HC2-AW-USB water activity meter. CPCS1 statistical software was applied to the observations to determine significance.

RESULTS

The objective of this investigation was to ascertain ability of partially purified bacteriocin to prevent mold growth on fruits of considerable commercial value (Pears, Peaches, Tomato and Kinnow). Partially purified bacteriocin preparation of *Lactobacillus plantarum* MTCC 9503 and *Lactobacillus acidophilus* NCDC 291 and mold spore suspension were applied onto the surface of these fruits and later incubated. Thereafter, quality parameters and mold count were recorded periodically. Results of physico-chemical quality parameters` recordings are presented in Tables 1-4. Since the effect of bacteriocin application was minimal, the cumulative results of all observations are presented collectively, there being no significant difference among sample and control groups. Application of partially purified bacteriocin of both lactics, mentioned earlier, did not affect the physico-chemical quality parameters, namely, TSS, % acidity, water activity, adversely.All fruits were acidic in nature and % acidity did not change after application of bacteriocin preparation. TSS and water activity also remain unchanged. Non-significant decrease in % firmness, % weight loss and color were recorded. After eight days of incubation, maximum weight lossof 9.3% was recorded in Peaches and minimum in Kinnow (4%). Application of a solution as partially purified bacteriocin, is expected to affect the firmness. It decreased by 8.9% in Pears on sixth day, increasing markedly from 4.5% on second day of incubation. Loss of firmness was more pronounced in Peaches, 18.1% and 10.4% in Tomato, on sixth day of incubation. Decrease in firmness was expectantly marginal, 5.2% in Kinnow due to the presence of rind which protects against dessication. Color was recorded subjectively. Deteriorationof natural color seemly of the same extent was observed in Pears, Peaches and Tomato was recorded. It did not affect the acceptability of the fruits However, color of Kinnow remained unchanged.

Total mold count of fruits in each group was plated periodically and recorded for a time period of upto six days (Pears, Peaches and Tomato) and eight days (Kinnow). Additionally, Nisin, a commercial preparation was also run to compare its efficacy with that of partially purified bacteriocin. Results are presented in

Figs 1-8. Partially purified bacteriocin of both lactics, *Lactobacillus plantarum* MTCC 9503 and *Lactobacillus acidophilus* NCDC 291 was effective in delaying the onset of mold spoilage in all the fruits which formed subject of this study. Plantarum preparation at 20,000 AU concentration was as effective as acidophilus preparation at 30,000 AU. At refrigeration temperature, plantarum at 25,000 AU was as effective as acidophilus at 30,000 AU. Mold growth between second and fourth day was slow. It amassed between fourth and sixth day. Fruit group treated with Nisin (25,000 AU) recorded the least mold growth. Mold count was mathematically less in Pears compared to its count with other fruits.

In Pears, plantarum preparation at 25,000 AU was effective (22°C) as against 25,000 AU (acidophilus preparation) and 15,000 AU (plantarum preparation) at refrigerated temperature. In Peaches, significant reduction on mold count was observed using Nisin 20,000; 25,000 AU acidophilus and 15,000 AU of plantarum preparation (22°C). At refrigerated incubation, 25,000 AU (acidophilus preparation) and 15,000 AU (plantarum preparation) were effective. Kinnow was incubated at ambient temperature only. 50 % reduction in mold count was recorded using 25,000 AU of Nisin. 20,000 AU and 30,000 AU of plantarum and acidophilus preparation extended the similar benefit. At refrigerated incubation of Tomato, Nisin 20,000 AU efficacy in reducing mold count was effective as 30,000 AU (acidophilus preparation) and 20,000 AU (plantarum preparation).

Table 1- Physico-Chemical Quality Parameters of Pears

Parameters→	TSS	% acidity	% weight loss	% change in firmness	Water activity	Color
Days						
0	9.37±0.002	0.39±0.001	0.0	0.0	0.9476±0.002	+++
2	9.41±0.000	0.38±0.002	4.1	4.5	0.9474±0.001	+++
4	9.61±0.001	0.39±0.000	5.7	7.9	0.9473±0.001	+++
6	9.60±0.001	0.39±0.001	6.8	8.9	0.9473±0.001	++

Color- +++Shiny Green; ++Green; +Dull Green

Table 2- Physico-Chemical Quality Parameters of Peaches

Parameters→	TSS	% acidity	% weight loss	% change in firmness	Water activity	Color
Days						
0	9.97±0.03	0.53±0.01	0.0	0.0	0.9499±0.010	+++
2	9.94±0.02	0.51±0.01	5.4	11.5	0.9497±0.002	+++
4	9.81±0.01	0.51±0.03	8.9	16.5	0.9498±0.002	+++
6	9.80±0.01	0.51±0.01	9.3	18.1	0.9496±0.001	++

Color- +++Highly Yellow Red
++Yellow Red
+Dull Yellow Red

Table 3- Physico-Chemical Quality Parameters of Kinnow

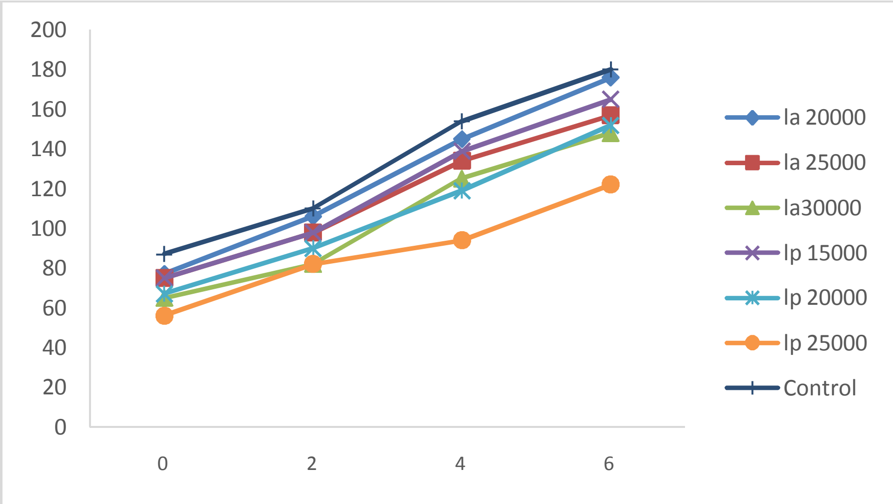
Parameters→	TSS	% acidity	% weight loss	% change in firmness	Water activity	Color
Days						
0	11.93±0.01	0.41	0.0	0.0	0.9479±0.000	+++
2	12.01±0.02	0.39	1.4	2.3	0.9476±0.001	+++
4	12.13±0.01	0.39	2.5	4.3	0.9477±0.001	+++
6	12.11±0.01	0.38	3.1	4.8	0.9476±0.001	+++
8	12.11±0.02	0.38	4.0	5.2	0.9476±0.001	+++

Color- +++Orange; ++Moderately Orange; +Light Orange

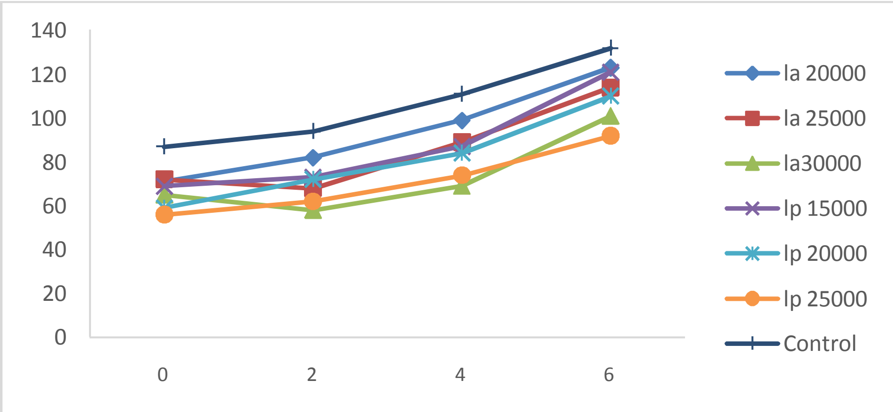
Table 4-Physico-Chemical Quality Parameters of Tomato

Parameters→	TSS	% acidity	% weight loss	% change in firmness	Water activity	Color
Days						
0	3.97±0.00	0.44±0.01	0.0	0.0	0.9555±0.000	+++
2	3.97±0.01	0.44±0.01	3.7	6.8	0.9554±0.001	+++
4	3.99±0.01	0.46±0.01	5.6	9.1	0.9555±0.001	+++
6	3.98±0.02	0.45±0.02	6.8	10.4	0.9554±0.001	++

Color- +++Bright Red; ++Red; +Light Red

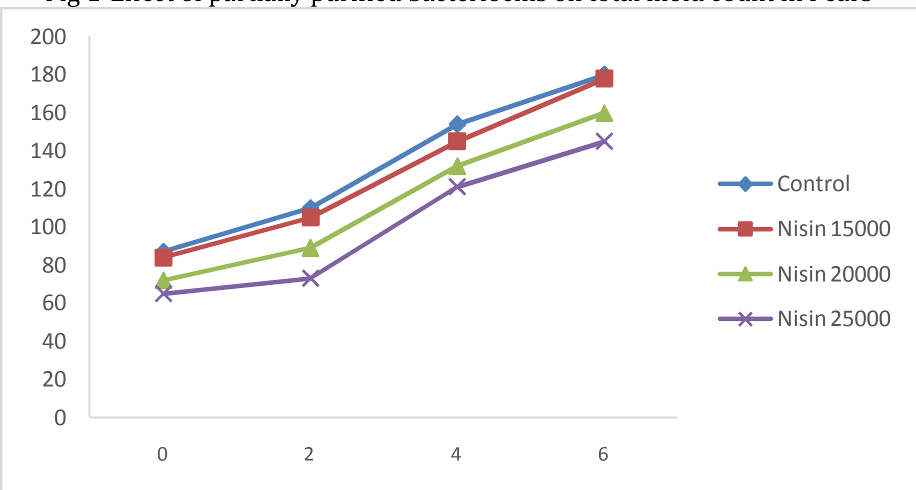


A-22°C incubation

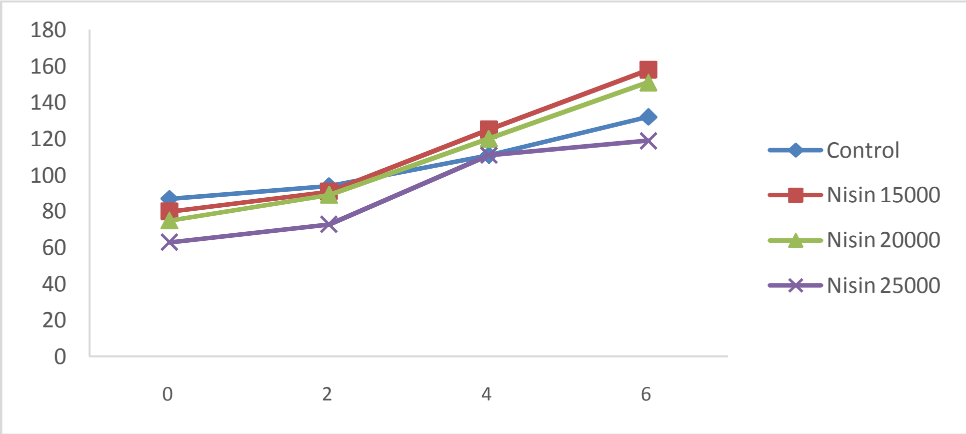


B- Refrigeration temperature incubation

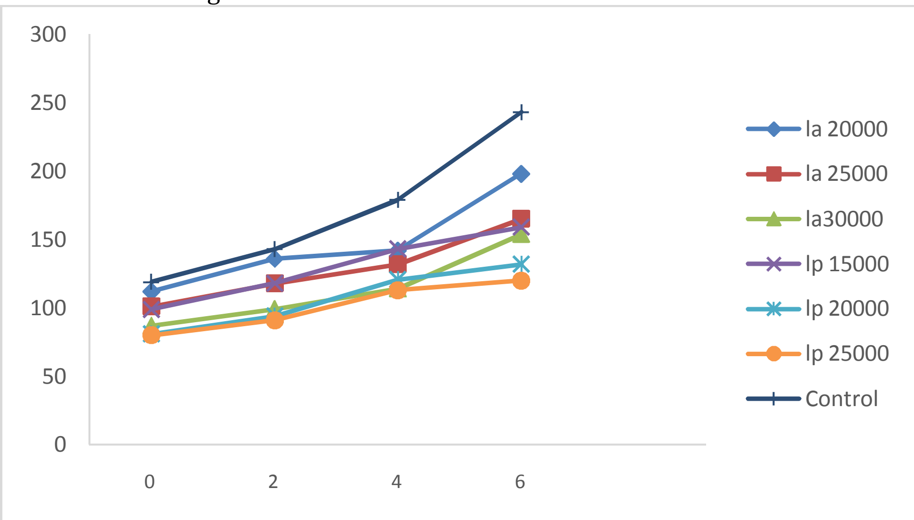
Fig 1-Effect of partially purified bacteriocins on total mold count in Pears



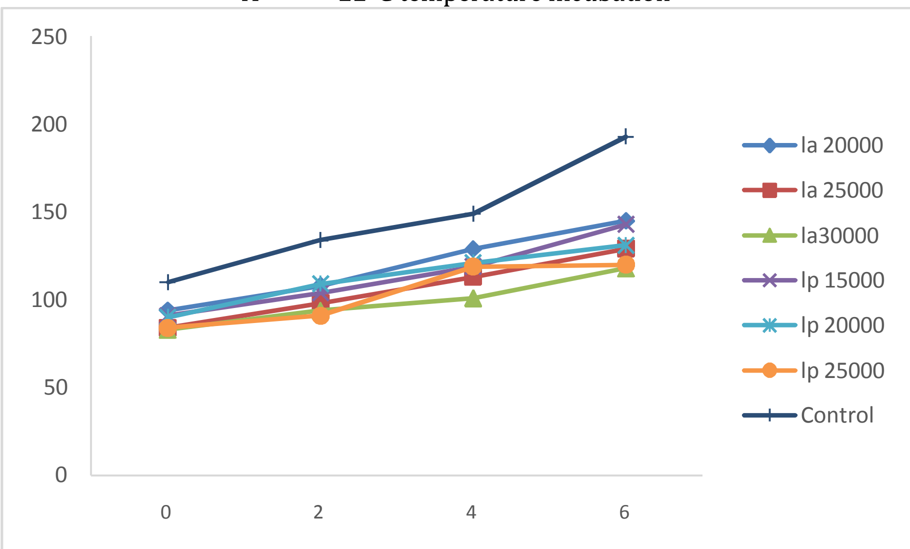
A- 22°C incubation



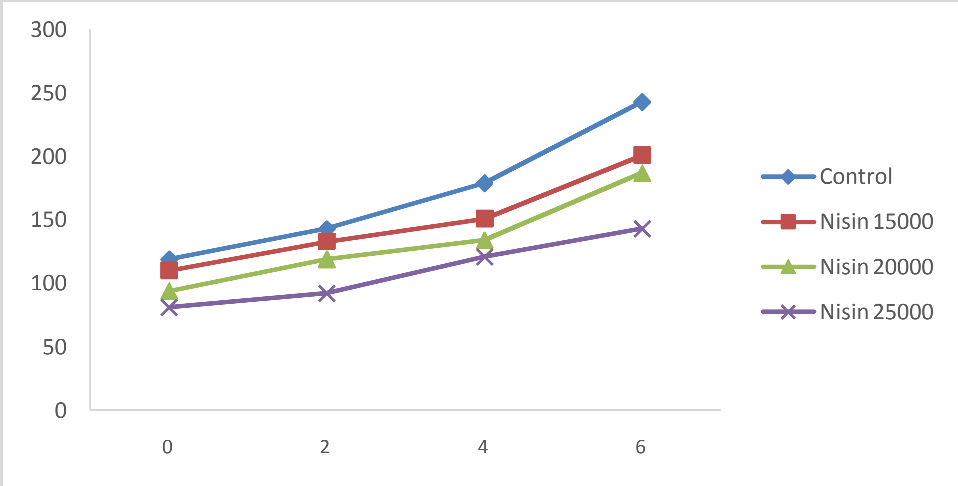
B-Refrigeration temperature incubation
Fig 2-Effect of Nisin on total mold count in Pears



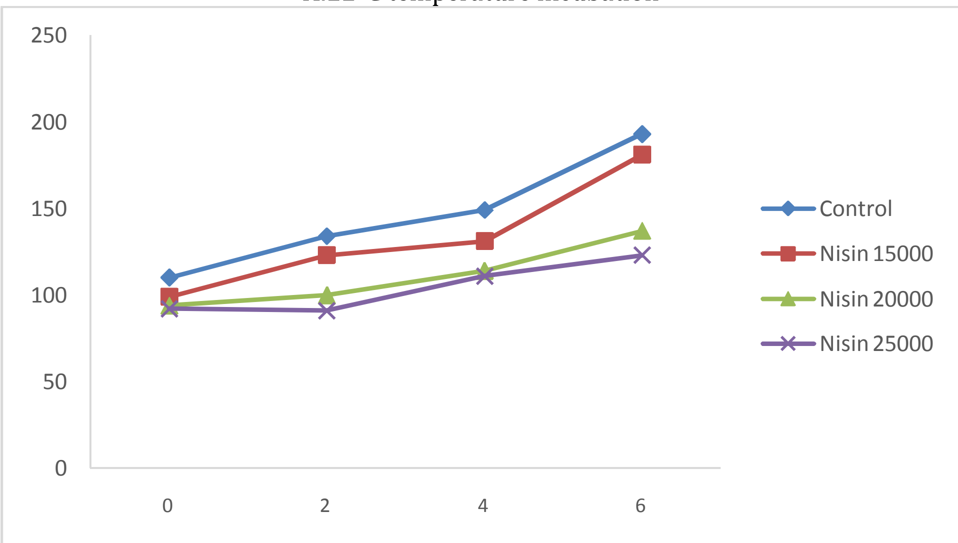
A- 22°C temperature incubation



B- Refrigeration temperature incubation
Fig 3-Effect of partially purified bacteriocins on total mold count in Peaches



A:22°C temperature incubation



B-Refrigeration temperature incubation

Fig 4-Effect of Nisin on total mold count in Peaches

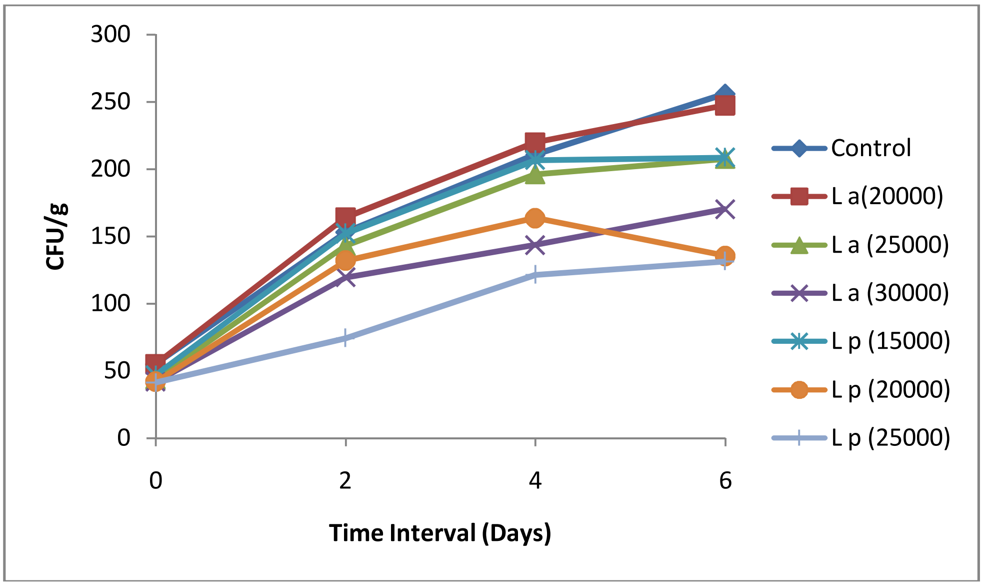


Fig 5-Effect of partially purified bacteriocins on total mold count in Kinnow

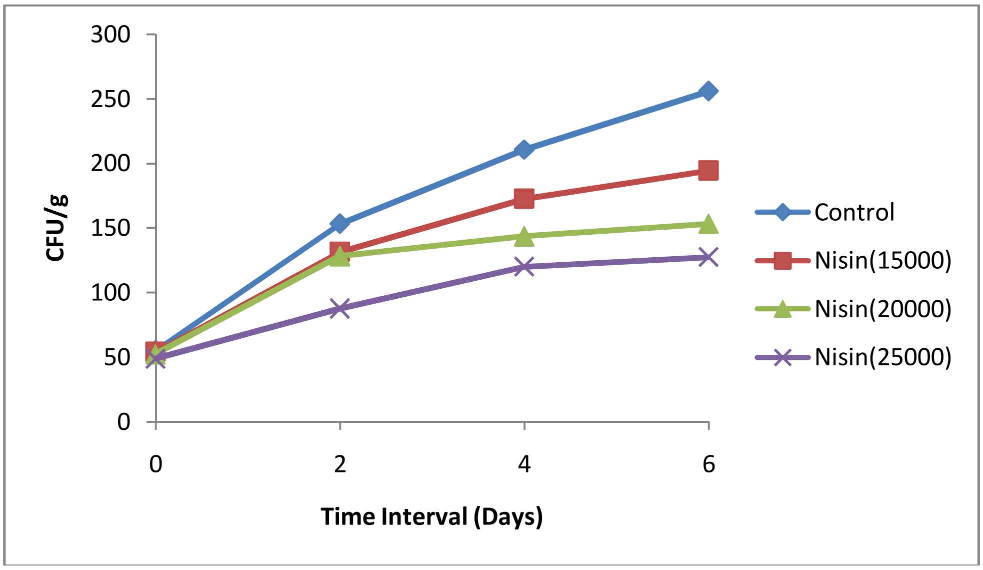
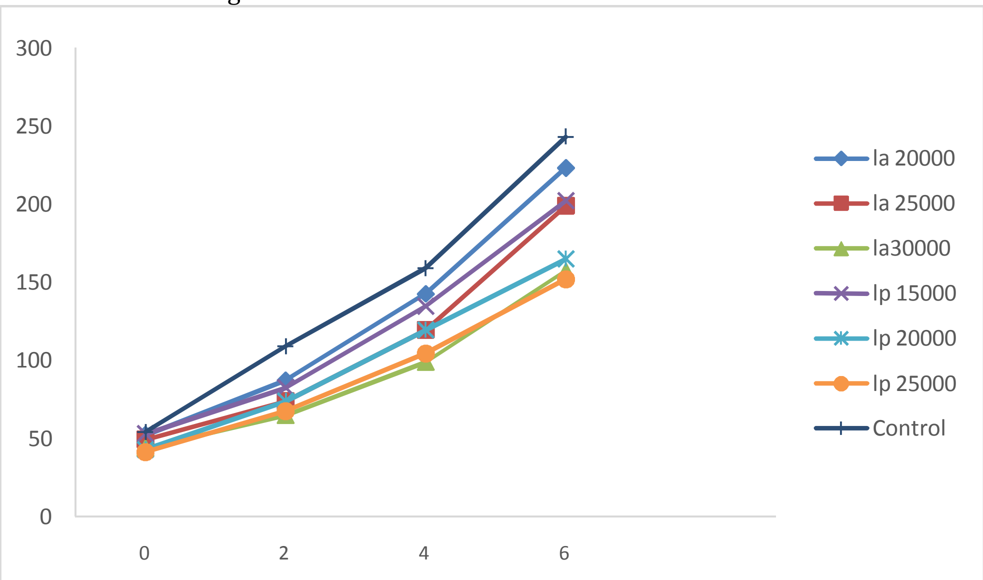
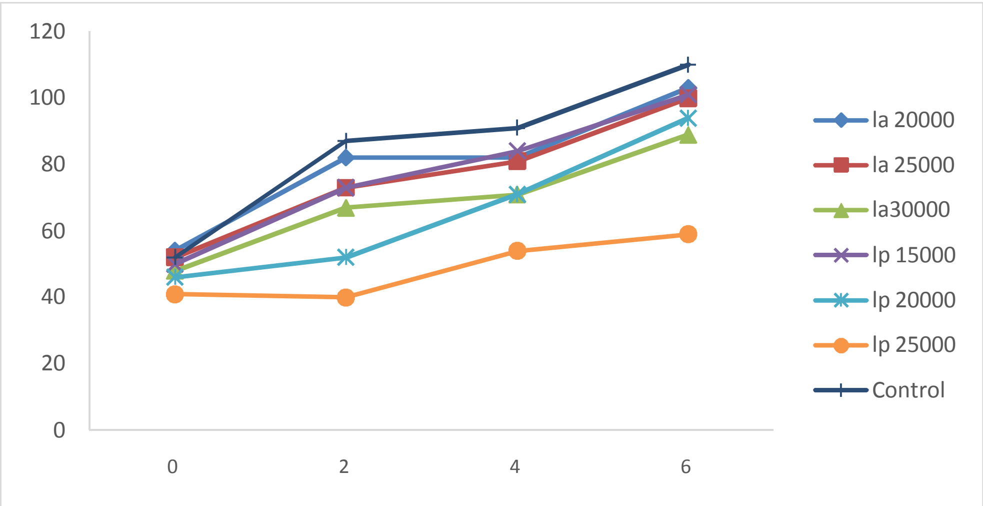


Fig 6-Effect of Nisin on total mold count in Kinnow

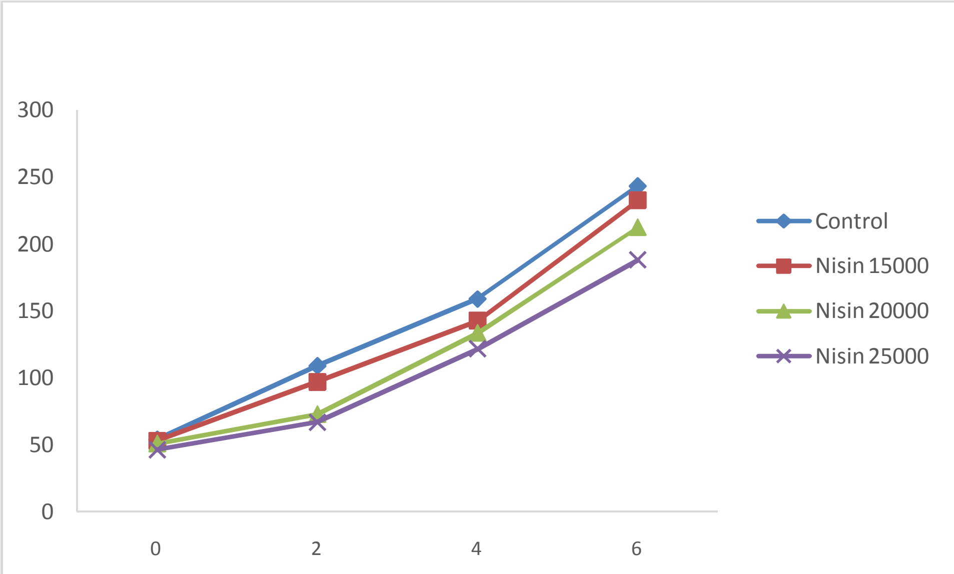


A- 22°C temperature incubation

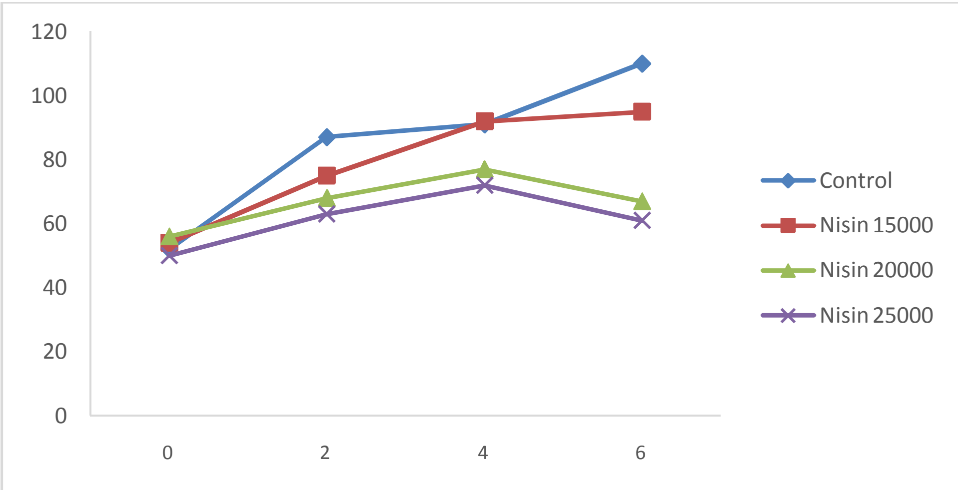


B- Refrigeration temperature incubation

Fig 7-Effect of partially purified bacteriocins on total mold count in Tomato



A- 22°C temperature incubation



B- Refrigeration temperature incubation

Fig 8-Effect of Nisin on total mold count in Tomato

DISCUSSION

One of India`s greatest achievement post-independence has been attaining self-sufficiency in food production. India is the world`s largest producer of food next to China, and has the potential of being the largest in the food and agriculture sector in future. India produces 176.3 million tonnes of milk (highest in the world) 259.3 million tonnes of fruits and vegetables (second largest) 284 million tonnes food grain (third largest) and 88.1 billioneggs [14].India processes 2.2 % of its fruits and vegetables compared to 65% in USA, 23% in China, 83% in Malaysia, 80% in South Africa, 78% in Philippines and 70% in Brazil. In the West, bulk of the fruits and vegetables are processed and the scale of the processed food industry is so large that it can sell processed foods much cheaper than fresh fruits and vegetables [15].

Majority of farmers in India are small scale (20% with 1-2 ha land holdings) to marginal scale (56.7 % with less than 1 ha land holding) who produce, harvest and also market on their own [16]. They are generally poor, with low level of literacy, capital and management constraints. Fruit and vegetable items are generally marketed as raw with farmers preferring to sell their produce in the local market immediately after harvest due to inadequate storage facilities. Fresh food being highly perishable deteriorates rapidly. These losses are estimated to the extent of 20-30% which amounts to Rs 70,000 crores annually [17] due to inadequate cold storage facility.

LAB (Lactic acid bacteria) can play an important role to reduce this loss. These organisms have major potential for use in food bio-preservation. They are safe to consume and naturally dominate the microflora of foods. Antimicrobials produced by them are preservatives which can be used to control the growth of spoilage and pathogenic bacteria in food and prevent post-harvest losses. Bacteriocins, antimicrobials from LAB, are suitable for food preservation and recent studies conducted suggest that their use offers a lot of advantages such as a) extend shelf life b) provide protection especially during times of temperature abuse c) decrease the risk of transmission of food borne pathogens d) decrease the losses due to food spoilage e) reduce the application of chemical preservatives f) permit the application of less severe heat treatment without compromising food safety (Hurdle Concept). Moreover, they have any therapeutic application and are not known to cause allergies. Being of LAB origin they are probiotic in nature also and help in restoring the normal gut microflora. They have been given GRAS (generally regarded as safe) status. Bacteriocins can be used in three main ways-i) bacteriocin preparation can be added to both fresh as well as processed food ii)inoculation of the bacteriocin producer strain under conditions that favor production of the bacteriocin in the food and iii) also use of an ingredient (fermentate of a bacteriocinogenic strain) in food. Whole cell LAB have been reported to inhibit growth of *Listeria monocytogenes* and *Salmonella typhimurium* in vegetables, *Listeria monocytogenes* in meat [18], cheese [19] and *Bacillus*sp in milk [20].

Bacteriocins can be an important tool in food preservation using multiple hurdles. In Indian context, it can be utilized at the farm level to prolong shelf life and facilitate marketing of the fresh produce at the farm level.It can be coupled with use of cold storage facility created in some niche pockets to enhance the shelf life of fresh produce which will facilitate phased marketing. Moreover, bacteriocin can also be used by the food industry as an additive in processed food instead of chemical preservatives.

Bacteriocins generally have a narrow spectrum of activity, mainly effective against Gram positive organisms. However, various species of *Lactobacillus* genera are known to produce bacteriocins which are effective against Gram negative organisms as well. They have even been reported to inhibit the growth of food spoilage fungi[21,22,23,24]. Most of the published work on antimicrobial efficacy of bacteriocins centers around *in vitro* studies. *In vivo*studies need to be conducted to ascertain its antimicrobial efficacy in various food systems. Nisin is the only bacteriocin worked on extensively and used commercially in food systems as cheese and meat products. However, there is little scientific information available on *in vivo* efficacy of bacteriocins especially in fresh produce. It is expected to be lower as compared to *in vitro* efficacy which can be attributed to intrinsic factors of a food system like pH, a_w, redox potential, presence/absence of other antimicrobials if any and microbial ecology of the food.

The present study will aim at elucidating the *in vivo* antimicrobial efficacy of *Lactobacillus* bacteriocins against fungi/bacteria which are known to cause food spoilage of fresh produce. *Lactobacillus plantarum* MTCC 9503 and *Lactobacillus acidophilus* NCDC 291 were chosen as bacteriocin producers in this study. Their bacteriocin producing ability had been demonstrated in our previous laboratory studies. *L plantarum* produced 10,000 AU of partially purified bacteriocin whereas *L acidophilus* NCDC 291 produced 1000 AU of partially produced bacteriocin. Four molds namely *Aspergillus, Penicillium, Fusarium* and *Alternaria* were chosen as food spoilage agents for fresh produce, fruits- Pears, Peaches, Kinnow and Tomato. Bacteriocin preparation obtained from both the lactics was applied to the fresh produce after inoculating them with known number of mold spores. Application of partially purified bacteriocin preparation did not adversely affect the physico-chemical quality parameters of these fresh produce. It was successful in prevent mold growthon their surface and thereby extending its shelf life by four days at refrigeration temperature and by two days at 22°C.

Bacteriocins can be used for value addition at farm level in all kinds of fresh foods as well as processed foods to prevent spoilage by bacterial and fungal agents [25,26,27]. Further work on its fermentative production using natural media can be undertaken to make their industrial production economically viable.

ACKNOWLEDGEMENT

Funding provided by University Grant Commission, New Delhi in the form of Major Research Project is gratefully acknowledged.

REFERENCES

1. Khader, A.A. [2003]. A perspective on post-harvest horticulture. Hort. Sci. 38: 1004-1008.
2. Ross, R.P., Morgan, S. and Hill, C.[2002]. Preservation and fermentation: past, present and future. Intl. J. Food Microbiol. 79:3-16.
3. Wood, B.J.[1992]. The lactic acid bacteria in health and disease. Appl Sci. 1:151-170 Federal Register 1988.
4. Federal Register.(1988). Nisin preparation: affirmation of GRAS status as a direct human food ingredient. Federal Register.54:11247-11251.
5. McAulife, O., Ross, R.P. and Hill, C. [2002]. Lantibiotics: structure, biosynthesis and mode of action. Microbiol Review. 25: 285-308.

6. Sonomoto, A. and Yokota,A. [2011]. Lactic acid bacteria and Bifidobacteria. Current Progress in Adv Research, CaisterAcad Press, ISBN 978-1-904455-82.
7. Messi, P., Bondi, M., Sabia, C., Battini, R., and Manicardi, G. [2001]. Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a *Lactobacillus*
8. *plantarum* strain. Intl. J. Food Microbiol. 64: 193-198.
9. Lade, H.S., Chitanand, M.P., Gyananath, G. and Kadam, T.A.[2006]. Studies on some properties of bacteriocins produced by *Lactobacillus* species isolated from agro-based waste. The Internet J. Microbiol. 2(1).
10. Torodov, S.D. and Dicks, L.M.T. [2005]. *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram negative bacteria. Enzyme Microbiol. Technol. 36:318-326.
11. Ennahar, S., Aoude-Warner, D., Sorokine, O., Van Dorsselaer, A., Bringel, F., Hubert, J.C. and Hasselmann, C. [1996]. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. Appl. Environ. Microbiol. 62:4381-4387.
12. Enan, G., el Essaway, A.A., Uyttendaele, M. and Debevere, J. [1996]. Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Intl. J. Food Microbiol. 30:189-215.
13. Vera, E.P., Salvucci, E., Sesma, F. and Nader-Macias, M.E. [2007]. Different strategies for purification of antimicrobial peptides from Lactic acid bacteria. In: Communicating Current Research and Educational Topics and Trends in Applied Microbiol, Memdez-Vilas (Ed), Formatex, Spain, pp 557-568.
14. Monilos, A.C., Abriouel, H., Omar, N.B., Lucas, R., Valdivia, E., and Galvez, A. [2006]. Inactivation of *Listeria monocytogenes* in raw fruits by Enterocin AS-48. J. Food Protect. 71: 2460-2467.
15. Anon.[2018]. Annual Report, Ministry of Food Processing Industries, p 4.
16. Anon. [2011]. Annual Report, Ministry of Food Processing Industries, p6.
17. Jain, A.K.[2010]. Country Status report on India, ICAR, New Delhi, p 2.
18. Mangaraj, S. and Singh, R.[2008]. Status of food processing industries in India and its future outlook. Ind. Food Industry 5: 35-42.
19. Schillinger, U. and Lucke, F.K. [1989]. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Mirobiol. 55:1901-1906.
20. Buyong, N., Kok, J. and Luchansky, J.[1998]. Use of genetically enhanced, pediocin producing starter culture, *Lactococcus lactis* MM217, to control *Listeria monocytogenes* in cheddar cheese. Appl Environ. Microbiol. 64:4842-4845.
21. Rossland, E., Borge, G.I.,Langsrud, T., Sorhaug, T. [2003]. Inhibition of *Bacillus cereus* by strains of *Lactobacillus* and *Lactococcus* in milk. Intl. J. Food Microbiol.89: 205-212.
22. Batish, V.K., Roy, U., Lal, R. and Grover, S. [1997)]. Antifungal attributes of lactic acid bacteria- a review. Critical Rev. Biotechnol. 17: 209-225.
23. Roy, U., Batish, V.K., Grover, S. and Neellkantan, S.[1996]. Production of antifungal substance by *Lactococcus lactis* subspp*lactis* CHD-28.3. Intl. J. Food Microbiol.32**:** 27-34.
24. Batish, V.K., Lal, R. and Grover, S. [1990]. Studies in environmental and nutritional factors on production of antifungal substance by *Lactobacillus acidophilus R*. Food Microbiol. 7: 199-206.
25. Batish, V.K., Lal, R. and Grover, S. [1989]. Screening lactic acid starter cultures for antifungal activity. Cul Dairy Prod J. 24: 22-25.
26. Settani, L. and Corsetti, A. [2008]. Application of bacteriocins in vegetable food biopreservation. Intl. J. Food Microbiol. 121: 123-138.
27. Gourama, H. and Bullerman, L.B. [1997]. Anti-aflatoxigenic activity of *Lactobacillus caseipseudoplantarum*. Intl. J. Food Microbiol. 34: 131–143.
28. Gourama, H. and Bullerman, L.B. [1995]. Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. J. Food Protect.58: 1249-1256.

CITATION OF THIS ARTICLE

Seema Garcha. *In vivo* Biopreservation of Fresh Produce Using Lactic Bacteriocins. Bull. Env. Pharmacol. Life Sci., Vol 8 [9] August 2019: 117-126