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PRESERVATIVE POTENTIAL OF PURIFIED BACTERIOCIN PRODUCED FROM *BREVIBACILLUS BORSTELENSIS* AG1 ISOLATED FROM MARCHA – A TRADITIONAL WINE STARTER CULTURE CAKE IN TOMATO PASTE

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ABSTRACT

Purified bacteriocin produced from *Brevibacillus borstelensis* AG1 isolated from Marcha a local wine starter herbal cake, was used to enhance the shelf life of tomato paste. Preservative effect of purified bacteriocin was studied for nine days in tomato paste inoculated with food borne pathogens and was compared to commercial biopreservative – nisin and chemical preservative – sodium benzoate. The indicator strains i.e. *Listeria monocytogenes* MTCC839, *Bacillus subtilis* CRI and *Clostridium perfringens* MTCC1739 were used at the amount 8.16, 8.13 and 8.18 log CFU/ml. Viable cells were counted periodically and a consistent reduction in number of viable cells of each tested pathogen was observed. It was found antagonistic against *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringes* MTCC1739 which are the most challengeable and food borne pathogens found in processed vegetables products. Purified bacteriocin was found active over a wide pH range i.e. 3.0 to 11.0 and was able to withstand temperature up to 100°C. It showed a better preservative potential by reducing pathogenic load of the tested strains (by 2.02, 2.05 and 2.02 log cycles (CFU/ml) of *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringes* MTCC1739, respectively) in tomato paste as compared to control (without bacteriocin). This proves efficiency of bacteriocin produced by *B. borstelensis* AG1 as biopreservative to enhance the safety and shelf life of acidic foods.

Keywords: Biopreservation**,** *Brevibacillus borstelensis*, bacteriocin, test indicators, tomato paste

INTRODUCTION

Safety and stability of food are based on an application of preservative factors called hurdles. Most of the decontamination technologies such as the oldest one e.g. cooking and more recent mild technologies i.e. pulsed-light, high pressure, ozone, ultrasound, etc. are not efficient or not compatible with the delicate texture and flavor of food. Chemical preservatives have been used since ages but consumers require more natural products with lower chemical treatment. An alternative solution that is catching rapid attention is the biopreservation technology **(Rodger, 2001; Calo-Mata *et al*., 2008; Dortu and Thonart, 2009)**. Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life **(Ananou *et al*., 2007)**. Beneficial bacteria or the fermentation products produced by these bacteria are used in biopreservation to control spoilage and render pathogens inactive in food **(Yousef and Carolyn, 2003)**. It is a benign ecological approach which is gaining increasing attention globally **(Ananou *et al*., 2007)**. The capacity to produce antimicrobial peptides is widely spread among Gram-positive bacteria. These substances are directed against competitive microorganisms, and thereby generate a selective advantage for their producers.

Among biopreservatives, bacteriocin has caught attention of food scientists to be used as a natural food biopreservative due to its antimicrobial activity against serious pathogenic and food spoilage bacteria. Different bacteriocin producing strains of *Bacillus* species as well as lactic acid bacteria have been isolated for this purpose but the keen interest towards bacteriocin of lactic acid bacteria worldwide is due to their essential role in majority of food fermentation, flavor development and preservation of food products along with proving safer for health **(Gautam and Sharma, 2009)**. They display a high degree of target specificity against related bacteria, although many have a wider spectrum of activity **(Jack *et al*., 1995)**. The proteinaceous nature of bacteriocin implies a putative degradation in the gastrointestinal tract of humans and animals, suggesting their use as natural preservatives in foods **(Cleveland *et al*., 2001)**. Strains from the *Bacillus* genus produce a diverse array of antimicrobial peptides, with several different basic chemical structures **(Gebhardt *et al*., 2002; Stein, 2005)**. Consumer demands for minimally processed foods or 'fresh foods' with no chemical preservatives have stimulated research interest in natural antimicrobial agents such as bacteriocins. Application of bacteriocin-producing strains in these food substrates may offer new opportunities in food biopreservation. Bacteriocins from *Bacillus* species have a potential preservative application in different food substrates like in dairy products such as milk and cheeses **(Sharma *et al*., 2009a,b)**.

Most studies concerning the food applications have focused on bacteriocins produced by lactic acid bacteria (LAB), mainly nisin and a few others **(Galvez *et al*., 2008)**. Although nisin is the only bacteriocin currently licensed as a biopreservative, its applications are restricted due to its very low activity at a neutral or an alkaline pH. Therefore, the search for new bacteriocins with improved physico-chemical properties (stability in a wide range of pH and temperature) and also a broad antimicrobial spectrum is of great interest for their application in foods.

MATERIAL AND METHODS

Bacterial isolate and bacteriocin preparation

A bacteriocin producing strain *Brevibacillus borstelensis* AG1 isolated from Marcha (a traditional starter culture for the production of various indigenous sweet-sour alcoholic beverages of North Eastern States of India) was used to evaluate the antagonistic potential of bacteriocin produced by it against spoilage bacteria and/or food borne pathogens i.e. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739. The inhibitory spectrum was checked by bit/disc and well diffusion assay **(Barefoot and Klaenhammer, 1983; Kimura *et al*., 1998)**. Bacteriocin produced was purified to homogeneity by ammonium sulfate precipitation (50% salt saturation) followed by gel exclusion chromatography. Purity of bacteriocin was checked by SDS-PAGE **(Sharma *et al*., 2011)**. The activity of culture supernatant, partially purified and purified bacteriocin was calculated by serial two fold dilution method. Two-hundred µl of sample was poured into the wells cut on the lawns of indicators in nutrient agar plates. The plates were then incubated at 35°C for 24 h and the zones of inhibition formed around the wells were measured. After incubation at

35°C for 24 h clear inhibition zones were evaluated and expressed in arbitrary units (AU/ml) **(Barefoot and Klaenhammer, 1983)**.

Application of bacteriocin

The tomato paste which was prepared by boiling and blenching of tomatoes (*Lycopersicon esculentum*) in sterile conditions was taken to evaluate biopreservative potential of bacteriocin. Tomatoes (2.5 kg) were washed thoroughly and peeled by scalding followed by hand peeling. Scalding was accomplished by conveying the tomatoes through boiling water for 3 minutes. Then these were immersed in cold water to crack the skin. The tomatoes were peeled and converted into pulp using mixer grinder. Clean sterile 50 ml test tubes were taken and tomato paste sample (40 ml) was put in each set of sterile test tubes. Tomato paste after blanching was pasteurized by keeping in hot water at 72°C for 2 min. The paste was then brought to room temperature. Total Soluble Solids (TSS) and pH of the tomato paste were checked. The test strains viz. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739 were used for inoculating different sets of tomato paste sample at the amount 8.13, 8.16 and 8.18 log10 CFU/ml, respectively (Tab 1) and the activity of partially purified bacteriocin, purified bacteriocin, nisin and chemical preservative was studied against them. These tubes were properly plugged, sealed with adhesive tape and hot wax. Biopreservatives i.e. partially purified bacteriocin, purified bacteriocin and nisin were added at the rate of 2000 **parts per million (ppm)** while chemical preservative i.e. sodium benzoate was added at the rate of 600 ppm in pathogen treated tomato paste for comparative study of different preservatives against indicator microorganisms while control was kept as such i.e. without addition of any preservative. The storage stability was tested at an interval of 0, 1, 2, 3-9 days at 4°C and the change in log CFU/ml was noted.

Table 1 Application of purified bacteriocin (2000 ppm) as a biopreservative

	Tomato paste		
Set A	$T+I_L$+PBac	$T+I_B$+PBac	$T+I_C$+PBac
Set B	$T+I_L$+Bac	$T+I_B$+Bac	$T+I_C$+Bac
Set C	$T+I_L$+nisin	$T+I_B$+nisin	$T+I_C$+nisin
Set D	$T+I_L$+sodium benzoate	$T+I_L$+sodium benzoate	$T+I_C$+sodium benzoate
Set E	$T+I_L$	$T+I_B$	$T+I_C$

Where, i) $T+I_L$ tomato paste inoculated with *L. monocytogenes* MTCC839
ii) $T+I_B$ tomato paste inoculated with *B. subtilis* CRI
iii) $T+I_C$ tomato paste inoculated with *C. perfringens* MTCC1739
iv)) $T+I_I$ is control without preservative

Statistical Analysis

The experimental data were analyzed by using factorial completely randomized design (CRD) at 5 % level of significance. Factorial CRD was used for comparative study of biopreservative with nisin and sodium benzoate against the indicators.

RESULTS

Bacteriocin production

The culture supernatant of *B. borstelensis* AG1 showed inhibition zones of 16 mm, 14 mm and 13 mm against *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringenes* MTCC1739, respectively indicating appreciable bacteriocin production (Figure 1).

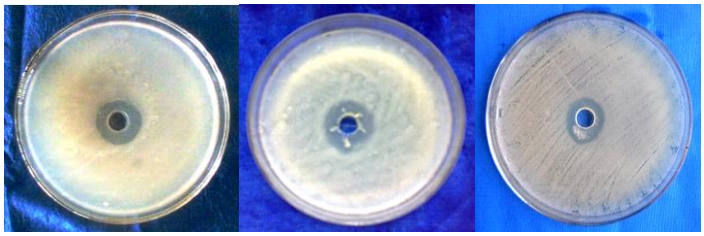

Figure 1 Antimicrobial activity of bacteriocin produced by *B. borstelensis* AG1 against *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739 (from the right to the left)

After purification, the crude bacteriocin exhibited high potential to inhibit the test indicators. The size of inhibition zones after partial and complete purification was found to increase by 20% to 80% against different test strains i.e. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC 1739 (Tab 2).

Table 2 Percentage increase in inhibition zone size against indicators due to partially purified and purified bacteriocin of *B. borstelensis* AG1 as compared to culture supernatant

Indicator	Bac^{*}	Bac^{**} - Bac^{*}		Bac^{***} - Bac^{*}	
	Zone size (mm)	Zone size (mm)	% increase	Zone size (mm)	% increase
L. monocytogenes MTCC 839	10	12	20	18	80
B. subtilis CRI	10	12	20	14	40
C. perfringens MTCC 1739	8	11	37.5	13	62.5

Bac^{*} - Culture supernatant
Bac^{**} - Partially purified bacteriocin
Bac^{***} - Purified bacteriocin
% increase Bac^{**} $\frac{Bac^{**}-Bac^{*}}{Bac^{*}}$
%increase Bac^{***} $\frac{Bac^{***}-Bac^{*}}{Bac^{*}}$

Purification of crude bacteriocin (culture supernatant) produced by *B. borstelensis* AG1 was achieved by salt saturation technique (ammonium sulphate precipitation) followed by gel exclusion chromatography. The precipitation was attained at 50 % level of saturation. After precipitation, bacteriocin produced by *B. borstelensis* AG1 had produced 30,000,00 AU/ml. The precipitates dissolved in buffer were subjected to gel exclusion chromatography resulted in purified bacteriocin with molecular mass of 12 KDa. The activity units of purified bacteriocin were increased to 40,00,000 AU/ml. It was found active over a wider pH range i.e. 3.0-11.0 and was thermostable upto 100°C.

Tomato paste used in the present study has been found to have Total Soluble Solids (TSS) and pH 9 °Brix (°B) and 4.7, respectively. As the bacteriocin was stable at low pH, it was tested to be used as biopreservative in acidic foods having low pH viz. tomato paste with pH 4.7. After treating test indicators i.e. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739 inoculated tomato paste with bacteriocin, it was found that with increasing time there was a decrease in the number of viable cells as compared to control. In case of tomato paste inoculated with *L. monocytogenes* MTCC839, initially log cycle (CFU/ml) was 8.13 for partially purified bacteriocin, purified bacteriocin, nisin, chemical preservative and for control having no preservative (Tab 3).

Table 3 A comparative study to use biopreservative (partially purified bacteriocin and purified bacteriocin), nisin and sodium benzoate against *L. monocytogenes* MTCC839 to enhance shelf life of tomato paste

Days	Control (no preservative) (log CFU/ml)	Partially purified bacteriocin (log CFU/ml)	Purified bacteriocin (log CFU/ml)	Nisin (log CFU/ml)	Chemical preservative (log CFU/ml)	Mean
0	8.13	8.13	8.13	8.13	8.13	8.13
1	9.19	6.11	6.03	6.17	6.07	6.7
3	10.30	7.22	7.16	7.29	7.18	7.8
5	10.40	9.31	9.27	9.39	9.25	9.5
7	12.53	12.50	12.41	12.52	12.31	12.45
9	13.59	13.55	13.47	13.47	13.39	13.51
Mean	10.69	9.47	9.41	9.51	9.39	

A decrease of 2.02, 2.1, 1.96 and 2.06 log cycle (CFU/ml) of *L. monocytogenes* MTCC839 was observed at first day in tomato paste treated with partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively. While in control, there was an increase of 1.06 log cycle (CFU/ml).

On third day increased by 1.11, 1.13, 1.12 and 1.11 log cycle (CFU/ml) in samples with a partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate was observed while in control an increase of 1.11 log cycle (CFU/ml) was observed. At day 3, an increase 0.1, 2.09, 2.11, 2.10, 2.07 log cycle (CFU/ml) was noticed for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively. At day 9 increase by 5.46, 7.44, 7.44, 7.30 and 7.32 log cycle (CFU/ml) was observed for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively. The Critical Difference (CD) value was 0.05. The T value was 0.0018 and D value was 0.0019 and the TxD value was 0.0044. Among all the preservatives used, purified bacteriocin was found statistically at par with sodium benzoate (9.39 and 9.41 mean log cycle (CFU/ml), respectively) followed by partially purified bacteriocin and nisin (9.47 and 9.51 mean log cycle (CFU/ml), respectively). Maximum spoilage was observed in control having highest mean value.

Similar preservation studies for tomato paste inoculated with *B. subtilis* CRI (8.16 log cycle (CFU/ml) and 1.0 OD) were also carried for 0, 1, 3, 5, 7 and 9 day. Initially log cycle (CFU/ml) was 8.16 for control, partially purified bacteriocin, purified bacteriocin nisin and sodium benzoate. The means for control, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate were found to be 10.71, 9.48, 9.41, 9.52 and 9.42, respectively (Tab 4). A decrease in log cycle (CFU/ml) was observed at day 1 (by 2.05, 2.13, 1.97 and 2.05 log cycle (CFU/ml) for partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively; in case of control it was increased by 1.06 log cycle (CFU/ml). An increase of 1.07, 1.13, 1.14, 1.12 and 1.09 log cycle (CFU/ml) was observed at day 3 for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively. Then increase by 0.14, 2.07, 2.08, 2.09 and 2.07 log cycle (CFU/ml) was found for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate.

Table 4 A comparative study to use biopreservative (partially purified bacteriocin and purified bacteriocin), nisin and sodium benzoate against *B. subtilis* CRI to enhance shelf life of tomato paste

Days	Control (no preservative) (log CFU/ml)	Partially purified bacteriocin (log CFU/ml)	Purified bacteriocin (log CFU/ml)	Nisin (log CFU/ml)	Chemical preservative (log CFU/ml)	Mean
0	8.16	8.17	8.16	8.16	8.16	8.16
1	9.22	6.12	6.03	6.19	6.11	6.73
3	10.29	7.25	7.17	7.31	7.20	7.84
5	10.43	9.32	9.25	9.40	9.27	9.53
7	12.54	12.49	12.41	12.53	12.35	12.46
9	13.60	13.55	13.46	13.57	13.41	13.52
Mean	10.71	9.48	9.41	9.52	9.42	

Increase by 4.38, 7.43, 7.43, 7.38 and 7.30 log cycle (CFU/ml) for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively was found on day 9 which was much higher than the previous one. The CD value was 0.05. The T value was 0.0021 and D value was 0.0023. The TxD value was 0.0052. Among applied preservatives, preservative effect purified bacteriocin was found to be statistically at par with sodium benzoate followed by partially purified bacteriocin and nisin, respectively. Maximum spoilage was found in control having highest mean value.

Effect of preservatives on tomato paste inoculated with *C. perfringens* MTCC1739 (8.18 log cycle (CFU/ml) and 1.0 OD) was also observed for 0, 1, 3, 5, 7 and 9 day. Initial log cycle (CFU/ml) was 8.18 for control, partially purified bacteriocin, purified bacteriocin nisin and sodium benzoate. The means for control, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate were 10.72, 9.47, 9.42, 9.54 and 9.45, respectively (Tab 5). A decrease in viable count of *C. perfringens* MTCC1739 was observed on day 1 (2.02, 2.12, 1.98 and 2.03 log cycle (CFU/ml) for partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate treated tomato paste, respectively) while an increase 1.07 log cycle (CFU/ml) was found in case of control.

Table 5 A comparative study to use biopreservative (partially purified bacteriocin and purified bacteriocin), nisin and sodium benzoate against *C. perfringens* MTCC1739 to enhance shelf life of tomato paste

Days	Control (no preservative) (log CFU/ml)	Partially purified bacteriocin (log CFU/ml)	Purified bacteriocin (log CFU/ml)	Nisin (log CFU/ml)	Chemical preservative (log CFU/ml)	Mean
0	8.17	8.18	8.18	8.18	8.17	8.18
1	9.24	6.16	6.06	6.20	6.14	6.76
3	10.32	7.24	7.15	7.33	7.20	7.85
5	10.44	9.29	9.21	9.40	9.28	9.52
7	12.55	12.44	12.40	12.53	12.42	12.47
9	13.60	13.54	13.53	13.58	13.47	13.55
Mean	10.72	9.47	9.42	9.54	9.45	

Increase by 1.08, 1.08, 1.09, 1.13 and 1.06 log cycle (CFU/ml) was noted for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate at day 3. Then there was a continuous increase by 0.12, 2.05, 2.06, 2.07 and 2.08 log cycle (CFU/ml) for control sample, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate. Increase in microbial count was higher at day 9; the increase by 4.36, 7.38, 7.47, 7.38 and 7.33 log cycle (CFU/ml) was found for control, partially purified bacteriocin, purified bacteriocin, nisin and sodium benzoate, respectively. The CD value was 0.05. The T value was 0.0115 and D value was 0.0016. The TxD value was 0.0036. During the preservation studies, purified bacteriocin was found to possess better preservative effect having minimum mean value compare to sodium benzoate followed by partially purified bacteriocin and nisin in toamato paste inoculated with *C. perfringens* MTCC1739. Maximum spoilage was found in case of control having highest mean value. The inoculation dose of tested strains in the present study was purposely being kept on the higher side {> 8 log cycle (CFU/ml)} to evaluate efficiency of the bacteriocin against the tested pathogens and to ensure the microbial safety (Table 3, 4 and 5).

DISCUSSION

The bacteriocin produced by *B. borstelensis* AG1 has been effective against Gram- positive indicators viz. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739. It is observed that purified bacteriocin is quite effective in preserving and enhancing shelf life of foods having low pH viz. tomato paste. Its wide range antagonism and higher activity units were comparable to commercially accepted biopreservative – nisin. Reduction of pathogenic bacteria is due to the bactericidal action of the bacteriocin. Thermostable nature is one of the desirable characteristic for application of bacteriocin as biopreseravative. The bacteriocin produced by *B. borstelensis* has also been found to be thermostable i.e. it is able to resist heat and thus can be used as a potential biopreservative.

Inactivation of purified bacteriocin with proteolytic enzyme reflects its protein nature and thus suggests its break down in digestive tract of human beings rendering it completely harmless **(Sharma *et al*., 2011)**. Purified bacteriocin has been found active in low pH range i.e. up to 3.0, so it can be used as a biopresevative in acidic food items. Since, in the present study, tested pathogens had been added in very higher concentration to observe antimicrobial potential of different preservatives and therefore samples under study could be kept safe for short period only. But in nature, contamination of processed food items takes place at very low pace; therefore, it can be stated strongly that bacteriocin produced by *B. borstelensis* AG1 would prove very effective to enhance shelf life of acidic foods i.e. tomato paste for long time because of its strong preservative attributes.

From the above study, it is observed that purified bacteriocin is quite effective in preserving and enhancing shelf life of foods having low pH viz. tomato paste. Its wide range antagonism and higher activity units were comparable to commercially accepted biopreservatives - nisin. Since, in the present study, test pathogens had been added in very higher concentration to observe the antimicrobial potential of different preservatives and therefore samples under study could be kept intact for short period only. But in nature, contamination of processed food items takes place at very low rate, thus it can be presumed safely that bactericoin produced by *B. borstelensis* AG1 would prove very effective to enhance shelf life of acidic foods i.e. tomato paste for long time because of its strong preservative attributes.

Similar trend has been observed by **Lucas *et al.* (2006)** on a commercial tomato paste, inoculated with strains of *B. coagulans* CECT 12 and CECT 561 and stored at temperature of 37, 22 and 4°C. At 37°C, the concentration of viable cells for strain CECT 12 was reduced significantly within the first 24 h by 2.3 and 3.0 log units for 3 and 6 µg/ml AS-48 respectively. Similar reductions were obtained in sample stored at 22°C and 4°C but the surviving fraction decreased more slowly during prolonged storage at both temperatures **(Lucas *et al.*, 2006)**. Endospore forming bacteria especially *Bacillus* spp. represents the main bacterial population of vegetable purees **(Carlin *et al.*, 2000)**. **Garcia *et al.* (2004)** studied the effectiveness of enterocin EJ97 to inhibit the growth of strains *B. macroides* and *B. maroccanus* in zucchini puree. The bacteriocin had a bactericidal effect on *B. maroccanus* after several incubation conditions (4 h at 37°C, 24 h at 15°C and 48 h at 4°C).

CONCLUSION

The purified bacteriocin of *B. borstelensis* AG1 inhibited the growth of food borne pathogens i.e. *L. monocytogenes* MTCC839, *B. subtilis* CRI and *C. perfringens* MTCC1739 that required to be controlled in any food industry. The use of purified bacteriocin as food biopreservative in tomato paste was found very effective. Antimicrobial effect shown by purified bacteriocin was found statistically at par with commercial chemical preservative i.e. sodium benzoate leading to a conclusion that bacteriocin produced by *B. borstelensis* AG1 was an effective natural preservative to enhance safety and shelf life of acidic food items, however, the other more detail studies are necessary.

REFERENCES

ANANOU, S., MAQUEDA, M., MARTÍNEZ-BUENO, M., VALDIVIA, E. 2007. Biopreservation, an ecological approach to improve the safety and shelf life of foods (Ed.) *Communicating Current Research and Educational Topics and Trends in Applied Microbiology* Formatex, ISBN 978-84-611-9423-0.

BAREFOOT, S.F., KLAENHAMMER, T.R. 1983. Detection and activity of Lactacin B-a bacteriocin produced by *Lactobacillus acidophilus. Applied and Environmental Microbiology*, 45, 1808-1815.

CALO-MATA, P., ARLINDO, S., BOEHME, K., DE MIGUEL, T., PASCOAL, A., BARROS-VELAZQUEZ, J. 2008. Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products. *Food and Bioprocess Technology*, 1, 43-63. http://dx.doi.org/10.1007/s11947-007-0021-2

CARLIN, F., GUINEBRETIERE, M.H., CHOMA, C., PASQUALINI, R., BRANEONIER, A., NGUYEN, T.C. 2000. Spore forming bacteria in commercial cooked, pasteurized and chilled vegetable purees. *Food Microbiology*, 17, 153-165. http://dx.doi.org/10.1006/fmic.1999.0299

CLEVELAND, J., MONTVILLE, T.J., NES, I.F., CHIKINDAS, M.L. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology* 71, 1–20.

DORTU, C., THONART, P. 2009. Bacteriocins from lactic acid bacteria: interest for food products biopreservation. *Biotechnol. Agron. Soc. Environ.* 13, 143-154.

GÁLVEZ, A., LUCAS, L.R., ABRIOUEL, H., VALDIVIA, E., OMAR, B.N. 2008. Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical Reviews in Biotechnology*, 28, 125–152.

GARCIA, M.T., LUCAS, R., ABRIOUEL, H., OMAR, B.N., PERAZ, R., GRANDE, M.J., MARTINEZ-CANAMERO, M., GALVEZ, A. 2004. Antimicrobial activity of enterocin EJ97 against *Bacillus marcroides/Bacillus maroccanus* isolated from zucchini puree. *Journal of Applied Microbiology*, 97, 731-737. http://dx.doi.org/10.1111/j.1365-2672.2004.02351.x

GAUTAM, N., SHARMA, N. 2009. Review-Bacteriocin: Safest approach to preserve food. *Indian Journal of Microbiology*, 49, 204-211. http://dx.doi.org/10.1007/s12088-009-0048-3.

GEBHARDT, K., SCHIMANA, J., MÜLLER, J. 2002. Screening for biologically active metabolites with endosymbiotic bacilli isolated from arthropods. *FEMS Microbiology Letters*, 217, 199–205. http://dx.org/10.1111/j.1574-6968.2002.tb11475.x

JACK, R.W., TAGG, J.R., RAY, B. 1995. Bacteriocins of Gram-positive bacteria. *Microbiology Reviews*, 59, 171–200.

KIMURA, H., SASHIHARA, T., MATSUKI, H., SONOMOTO, K., ISHIZAKI, A. 1998. Novel bacteriocin of *Pediococcus* sp. ISK – 1 isolated from well aged bed of fermented rice bran. *Annuals of New York Academy of Science*, 864, 345-348. http://dx.doi.org/10.1111/j.1749-6632.1998.tb10336.x

LUCAS, R., GRANDE, M.J., ABRIOUEL, H., MAQUEDA, M., OMAR, B.N., VALVIDIA, E., MARTINEZ-CANAMERO, M., GALVEZ, A. 2006. Application of the broad spectrum bacteriocin enterocin AS-48 to inhibit *Bacillus coagulans* in canned fruit and vegetable foods. *Food and Chemical Toxicology*, 44, 1774-1781. http://dx.doi.org/10.1016/j.fct.2006.05.019

RODGERS, S. 2001. Preserving non-fermented refrigerated foods with microbial cultures: a review. *Trends in Food Science and Technology*, 12, 276-284. http://dx.doi.org/10.1016/S0924-2244(01)00093-0

SHARMA, N., ATTRI, A., GAUTAM, N. 2009a. Purification and characterization of bacteriocin like substance produced from *Bacillus lentus* with perspective of a new biopreservative for food preservation. *Pak. J. Sci. Ind. Res.* 52, 191–199.

SHARMA, N., GUPTA, A., GAUTAM, N. 2011. Application of purified bacteriocin- basicin produced from *Bacillus sp.* AG1 to improve safety and shelf life of dairy food products. *International Journal of Food and Fermentation Technology*, 1(1), 119-127.

SHARMA, N., KAPOOR, G., GAUTAM, N., NEOPANEY, B. 2009b. Characterization of partially purified bacteriocin of *Bacillus* sp. MTCC 43 isolated from rhizosphere of radish (*Raphanus sativus*) and its application as a potential food biopreservative. *J. Sci. Ind. Res.* 68, 881–886.

STEIN, T. 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular Microbiology*, 56, 845–857. http://dx.doi.org/10.1111/j.1365-2958.2005.04587.x

YOUSEF, A.E., CAROLYN, C.C. 2003. Food microbiology: a laboratory manual Wiley. pp. 226 ISBN 978-0-471-39105-0