African Journal of Microbiology Research Vol. 7(6), pp. 436-439, 5 February, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.747
ISSN 1996 0808 ©2013 Academic Journals

Full Length Research Paper

Antimicrobial potential of *Lactococcus lactis* bacteriocin against *Salmonella typhi*

Farzana Rashid[1]*, Nadia Sharif [1], Ijaz Ali[2], Shagufta Naz[1], Saima Sharif[1] and Fakhar - un - Nisa[1]

[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan.
[2]Institute of Biotechnology and Genetic Engineering (IBGE), Khyber Pakhtunkhwa, Pakistan.

Accepted 24 January, 2013

Bacteriocins are natural antimicrobial peptides with potential applications in human health care and exhibit a bactericidal mode of action against different microorganisms. The present study was carried out to observe lactic acid bacteria prevalence in milk and to determine the antimicrobial activity of *Lactococcus lactis* bacteriocin against *Salmonella typhi*. Isolation and characterization of lactic acid bacteria was carried out on the basis of morphological, physiological and biochemical tests. Cell free extracts of bacteriocins were prepared by the ammonium sulphate precipitation. Antimicrobial activity was performed by Disc Diffusion method by using different organic solvents. It was observed that the genus *Lactococcus* was common (75%) in different milk samples. Antimicrobial activity of bacteriocin was increased in different solvents. Antimicrobial activity was maximum (17.0 ± 0.7 mm) at 90:10 bacteriocin and ethanol ratio, on the other hand it was minimum (8.5 ± 0.5 mm) at 25:75 bacteriocin and ethanol ratio at pH 6.0. It was stable at higher temperature (100°C for 60 min). Sensitivity test confirmed that bacteriocin lost its activity after treating with proteinase K (0.1 mg/ml).

Key words: Bacteriocin, lactic acid bacteria*, Lactococcus lactis, Salmonella typhi.*

INTRODUCTION

Milk is a composite biological fluid probably containing about 100 000 different molecular species in several dispersal states. It is the solitary source of food for the very young mammal. The role of milk is to nourish and provide immunological protection. The major component of milk is water; the remainder consists of lactose, protein (casein and whey proteins), and fat. It also contains smaller quantities of vitamins (vitamin A and vitamin C), minerals, enzymes (lactoperoxidase and acid phosphatase), specific blood proteins, and somatic cells (Richard, 2002). Milk supports the growth of a wide range of microorganisms. Various physio-chemical properties such as suspension of casein micelles, fat globules, solubilized lactose, whey proteins and some minerals influence the growth of micro-organisms in milk (Carr et al., 2002). Bacteriocins are produced by many Gram-positive and Gram-negative species, but those produced by Lactic acid bacteria (LAB) are of particular interest in pharmaceutical and food industry, since these bacteria have generally been regarded as safe (Giffel et al., 1998; Cleveland et al., 2001; Hemalatha and Shanthi, 2010). LAB are Gram positive, catalase-negative, rod or cocci shape, low GC content, acid tolerant, non-spore forming, and non-motile microorganisms (De Vuyst and Vandame, 1994).

LAB is used in food fermentations as natural or selected starters in which it performs acidification due to production of acetic acids and lactic flavors. The anti-

*Corresponding author. E-mail: nadiasharif.s7@gmail.com.

Abbreviations: CFS, Cell free supernatant; **CDC,** centre for disease control and prevention; **LAB,** lactic acid bacteria; **WHO,** World Health Organization; **MRS,** de Man Rogosa and Sharpe.

microbial compounds produced by these bacteria during lactic fermentation include bacteriocin, hydrogen peroxide, organic acids, reuterin and diacetyl (Holzafel et al., 2001; Hirano et al., 2003). These are known to strongly inhibit the growth of pathogens and are used for the control of bacterial diseases in humans and animals. LAB is valuable in preventing gastrointestinal disorders and in the recovery from diarrhoea of miscellaneous. *Lactococcus lactis* (*L. lactis*) is one of the important groups of LAB (Gilliand, 1990; Marteau et al., 1997). Bacteriocin could be the potential antimicrobial peptide against a wide range of microorganisms. In this paper, we observed the prevalence of lactic acid bacteria in milk, isolated crude bacteriocin from *L. lactis* and find its antimicrobial activity against *Salmonella typhi* (*S. typhi*).

MATERIALS AND METHODS

Bacteriocin isolation and purification

Sampling

Fifty milk samples (four-pasteurized /heat treated and forty six non pasteurized / raw) from different localities of Lahore, Pakistan were collected in 100 ml capacity bottles. The samples were stored at 4°C for subsequent experiments. The test microorganism, *S. typhi* was obtained from post graduate research institute (PGMI) Lahore.

Sample preparation

Ten ml milk sample was transferred to 5 ml of sterile physiological saline (0.9% NaCl) as described by Kumari and Garg (2007) to obtain the countable bacterial population on agar plates.

Identification of bacterial strains

One milliliter sample was plated out on MRS (LAB) agar plates. The plates were incubated at 37°C for 24 to 48 h. After incubation, well-isolated colonies were picked up randomly and transferred to MRS broth for identification. Colony characteristics (morphology, shape, color and size) Gram-staining and catalase activity (3% H_2O_2) were studied for identification up to genus level as described by Harrigan and McCance (1990). Production of gas from glucose, temperature requirement (10, 37 and 45°C), NaCl tolerance (4.5 and 6.5%) and growth at pH 3.9 and 9.6 were performed in MRS broth. The isolates were tested for their carbohydrate fermentation ability. From the overnight grown MRS broth of 50µl carbohydrate source containing 2% glucose, mannitol, lactose and maltose was inoculated in 5 ml liquid MRS medium lacking glucose but containing Phenol red (0.04 g/L) as pH indicator and other test carbohydrates. The test media were incubated for 24 hrs at 28 °C. The acid production was observed between 24 to 48 h (Purama et al., 2008).

Production of crude bacteriocin

Lactococcus lactis bacteria were more common (75%) in all milk samples so it was selected for the bacteriocin production. The *L. lactis* strains were grown in MRS broth at 37°C for 24 h. The broth was separated from the cells by centrifugation at 10 000 rpm for 30 min. Purification of bacteriocin was carried out by adding the ammonium sulphate to the cell free supernatant at the concentration of 123 g/L. The solution was stirred on magnetic stirrer for 15 to 20 min and centrifuged at 10 000 *g* for 15 to 20 min. The pH of the cell free supernatant was adjusted (6.5) with 1N NaOH (Ogunbanwo et al., 2003; Rajaram et al., 2010).

Measurement of zone of inhibition

The discs (5.0 mm) were saturated with bacteriocin and placed on the plates, which were inverted and incubated at 37°C for 24 h. After incubation, the diameter of the zones of complete inhibition was measured by transparent mm scale. Chloramphenicol was used as positive control.

Effects of temperature, pH, organic solvents and proteinase K on bacteriocin activity

Bacteriocin activity was tested at different temperatures 100°C (10, 30 and 60 min) and 121°C (15 min). Residual activity of bacteriocin was also tested from pH range of 3 to 9. The proteinaceous nature of antimicrobial substances was tested by treating the cell free supernatant of 24 h culture (pH 6.0) with 0.1 mg/ml proteinase K (Bio LAB) for 2 h, heat inactivated cell free supernatant (100° C, 10 min) was also treated with proteinase K enzyme (1mg/ml) at 37°C for 1 h (Albano et al., 2007). Enzyme-treated samples were incubated for 1 h at 37°C (42°C in the case of proteinase K), and solvent-treated samples were incubated for 1 and 5 h at 25°C before being tested for antimicrobial activity. Effects of heat stability and pH were analyzed by assaying the bactericidal activity after 15 min of incubation at 45, 60, 75, and 90°C.

RESULTS AND DISCUSSION

The LAB which was isolated from milk included 75% *lactococcus*, 16% *lactobacilli* and 9% *leuconostic* suggest that the samples include highest number of *lactococcus* sp. followed by *lactobacillus*. The acid production as a result of carbohydrate fermentation indicates that the growth rate on glucose, lactose and maltose are the same, while mannitol growth rates are lower.

Results of antimicrobial activity of bacteriocin showed that its activity was maximum (17.0 ± 0.7 mm) at 90% concentration and minimum (8.5 ± 0.5 mm) at 25% concentration in ethanol extract (Table 1). Bacteriocin activity was highest at pH 6.0 (Figure 1). Bacteriocin activity was negatively affected by increasing the temperature; however, it still remains active after heating at 121°C for 15 min (Table 2). Activity of crude extract was higher as compared to cell free supernatant (CFS) after ammonium sulphate precipitation. Bacteriocin was sensitive to Proteinase K enzyme and it lost its activity by the treatment with 1.0 and 0.1 mg. SDS had also a positive effect on its activity (Table 3).

Bacteriocin activity is positively affected by the solvent extracts. The bactericidal activity of bacteriocin was sensitive to organic solvents and proteinase K, partially stable to heat, and active over a wide range of pH values. Maximum inhibitory activity was observed at pH 6.0 (Rajaram et al., 2010; Khalid et al., 1999). Bacteriocin was heat stable at 100°C for 10, 30 and 60 min and

Table 1. Antimicrobial activity of bacteriocin with organic solvents against *S. typhi* .

Bacteriocin concentration (%)	Zone of inhibition (mm)				
	Distilled water	Acetone	Chloroform	Ethanol	Methanol
25	9.0 ± 0.6	8.0 ± 0.5	10.0 ± 0.4	11.0 ± 0.3	11.5 ± 0.5
50	10.5 ± 0.5	10.8 ± 0.9	11.5 ± 0.8	12.0 ± 0.6	12.5 ± 0.4
75	11.0 ± 0.6	11.0 ± 0.4	12.5 ± 1.0	15.0 ± 0.5	15.0 ± 0.7
90	14.0 ± 0.8	12.5 ± 0.8	16.0 ± 0.4	17.0 ± 0.7	16.5 ± 0.6

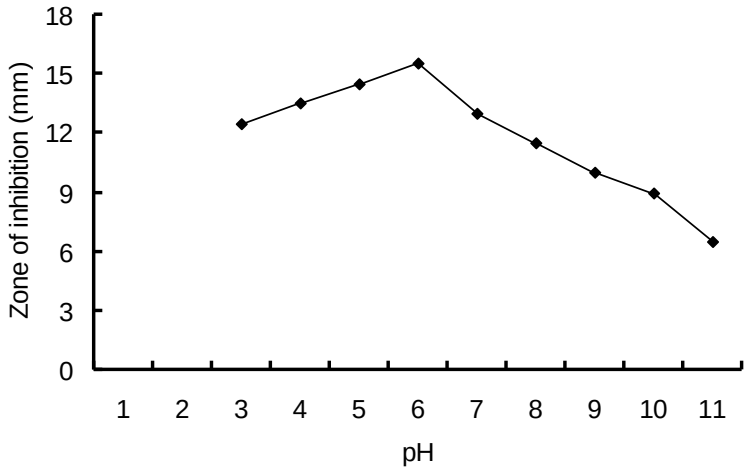

Figure 1. Effect of pH on bacteriocin activity.

Table 2. Effect of different temperatures on Bacteriocin activity.

Temperatures (°C)	Time (min)	Zone of inhibition (mm)
100	10	9.5 ± 0.5
100	30	7.5 ± 0.7
100	60	7.0 ± 0.4
121	15	5.5 ± 0.9
160	30	–

-, No activity.

Table 3. Effect of ammonium sulphate, SDS and Proteinase K on Bacteriocin activity.

Treatment	Quantity	Zone of inhibition (mm)
Crude extract of bacteriocin	100%	16.5 ± 0.7
Ammonium sulphate	20%	16.0 ± 0.5
	40%	15.0 ± 0.8
SDS	5 $mgml^{-1}$	14.5 ± 0.5
	2 $mgml^{-1}$	15.0 ± 0.3
	1 $mgml^{-1}$	15.5 ± 0.9
	0.1 $mgml^{-1}$	13.0 ± 0.3
Proteinase K	20%	–
	40%	–

-, No activity.

121°C for 15 min, its activity was inhibited by the addition of Proteinase K, reflecting the proteinaceous nature (Khalid et al. 1999).

Conclusion

Bacteriocins produced by LAB cover a very broad field of applications in medicine, as well as, in food industry. In medicine sector bacteriocins produced by LAB might play a role during *in vivo* interactions occurring in the gastrointestinal tract and contribute gut health. It required further research to unravel the role of LAB bacteriocins as a food preservative and antimicrobial drug.

ACKNOWLEDGEMENT

We are thankful to Post Graduate Medical Institute (PGMI) for their cooperation in providing the *S. typhi* strain.

REFERENCES

Albano H, Todorov, SD, Van Reenen CA, Hogg T, Dicks LMT, Teixeira P (2007). Characterization of two bacteriocins produced by *Pediococcus acid-ilactici* isolated from “Alheira”, a fermented sausage traditionally produced in Portugal. Int. J. Food Microbiol. 116:239-247.

Carr FJ, Hill D, Maida N (2002). The lactic acid bacteria: A literature survey. Crit. Rev. Microbiol. 28:281-370.

Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001). Bacteriocins: safe, natural, antimicrobials for food preservation. Int. J. Food Microbiol. 71:1-20.

De Vuyst L, Vandamme EJ (1994). Nisin, an Antibiotic Produced by *Lactococcus lactis* subsp. *Lactis*: Properties, Biosynthesis, Fermentation and Applications. In: Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications. Blackie Academic and Professional Inc, London, England, pp. 151-221.

Giffel MCT, Benmer RR, Bonestroo MM, Rombouts FM (1998). Incidence and characterization of *Bacillus cereus* in two dairy processing plants. Int. J. Food Microbiol. 50:479-492.

Gilliand SE (1990). Health and nutritional benefits from lactic acid bacteria. FEMS J. Microbiol. Rev. 87:175-178.

Harrigan WF, McCance ME (1990). Laboratory Methods in Food and Dairy Microbiology, 8th ed. Academic Press Inc., London. pp. 7-23, 286-303.

Hemalatha S, Shanthi S (2010). *In vitro* characterization of bacteriocin producing *Bacillus subtilis* from milk samples. Afr. J. Microbiol. Res. 4:2004-2010.

Hirano J, Yoshida T, Sugiyama T, Koide N, Mori I, Yokochi T (2003). The effect of *Lactobacillus rhamnosus* on enterohemorrhagic *Escherichia coli* infection of human intestinal cells *in vitro.* Microbiol. Immunol. 47:405-409.

Holzafel WH, Habere P, Geisen R, Bjorkroth J, Ulrich S (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin. Nutr. 73:365-373.

Khalid F, Siddiqi R, Mojgani N (1999). Detection and characterization of heat stable Bacteriocin (Lsctocin LC-09) produced by a clinical isolate of *lactobacilli.* Med. J. Islam. Acad. Sci. 12:67-71.

Kumari A, Garg AP (2007). A Bacteriocin from *Lactococcus lactis* CCSUB94 isolated from milk and milk products. Res. J. Microbiol. 2:375-380.

Marteau P, Minekus M, Havenaar R, Huiss JHJ (1997). Survival of lactic acid bacteria in a dynamic model of stomach and small intestine: Validation and the effects of bile. J. Dairy Sci. 80:1031-1037.

Ogunbanwo ST, Sanni AI, Aonilude A (2003). Characterization of Bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OGI. Afr. J. Biotechnol. 2:219-227.

Purama RK, Agrawal M, Majumder A, Ahmed S, Goyal A (2008). Antibiotic sensitivity, carbohydrate fermentation characteristics and plasmid profiles of glucansucrase producing four *Leuconostoc* strains. J. Pure Appl. Microbiol. 2:139-146.

Rajaram G, Manivasagan P, Thilagavathi B, Saravanakumar A (2010). Purification and characterization of Bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. Adv. J. Sci. Technol. 2:2010.

Richard KR (2002). Diary Microbiology Handbook. Wiley Interscience, Inc. London, UK, pp. 91-121.