International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.3, pp 1281-1283, April-June 2013

IPACT-2013[14th – 15th March 2013]
National Conference on Industrial Pollution And Control Technology-2013

Characterization of heat stable and inhibitory activity of bacteriocin produced by Lactobacillus acidophilus

S.Elamathy* and D.Kanchana

Department of Microbiology, Annamalai University,Annamalai Nagar, Chidambaram, Tamilnadu,India

***Corres. Author : ela.micro86@gmail.com**

Abstract: Bacteriocins from lactic acid bacteria (LAB) are natural antimicrobial peptides or proteins with interesting potential application in food preservation and health care. The present study was aimed to isolate LAB were isolated and screened for bacteriocin production by agar well diffusion method. Among them *L. acidophilus* isolated from meat showed maximum zone of inhibition in *Staphylococcus* sp (16mm) followed by *Klebsiella*. sp (14mm). The bacteriocin of *L. acidophilus* was found to be heat stable (75^{o}C for 15min). Addition of surfactants (Tween 80, EDTA and SDS) up to 1% to crude bacteriocin showed increase in antibacterial activity. Our present study demonstrates the possibility of using *L. acidophilus* or its bacteriocin as a preservative in food industry.
Keyword: *Lactobacillus acidophilus*, Antibacterial activity, Bacteriocin, Lactic acid bacteria.

Introduction

Bacteriocins are proteinaceous substances produced by many bacterial strains and exhibit bactericidal activity against the closely related organisms. They have been the subject of extensive studies in recent years because of their prospective use as natural food preservatives[1]. Lactic acid bacteria (LAB) are traditionally used as starter cultures for the fermentation of foods and beverages because of their contribution to flavour and aroma development and to spoilage retardation. The preservative effect is mainly due to the acidic condition developed by the bacteria in food, but they are capable of producing and excreting inhibitory substances other than lactic and acetic acid. These include bacteriocins, hydrogen peroxide, ethanol, diacetyl and carbon dioxide[2] . The first bacteriocin to appear on both the European food additive list and the United States FDA list was intended for the use in the production of pasteurized processed cheese[3]. In addition to acids, hydrogen peroxide, diacetyl and bacteriocins or bactericidal proteins produced during lactic fermentations may also play inhibitory roles against pathogenic microbes[4,5]. The main objective of the present study is to evaluate growth and the nature of the antibacterial compound (bacteriocin) from *L. acidophilus*, their inhibitory effect on food borne pathogens and also the partial characterization of the produced bacteriocin.

Materials And Methods

Isolation Of Bacteriocin Producers

Bacteriocin producing organisms were isolated from raw meat. Samples were diluted and plated on MRS (de Man, Rogosa and Sharpe) medium and bacteriocin activity was assayed by Agar Diffusion Test (ADT)[6], using *E.coli* as an indicator organism because of its sensitivity to all type of bacteriocins. Bacteriocin positive colonies with a large diameter of zone of inhibition were identified according to Bergey's manual of determinative bacteriology (1994) and selected for the further bacteriocin production and characterization studies.

Screening For Antibacterial Activity

All the isolates of LAB were screened for the production of antibacterial compound by testing them against food borne pathogen (*E.coli, Staphylococcus* sp, *Bacillus* sp, *Pseudomonas* sp, *Klebsiella* sp) by agar well diffusion method. Strain was spot inoculated on MRS agar, incubated at 35^0C for 22 – 24h.

Effect Of Heat

Crude bacteriocin was exposed to various heat treatments (15, 30, 45, 60, 75^0C) in boiling water bath for 60 min and every 15 min of time interval, an aliquot of crude bacteriocin was removed to determine the activity[7].

Effect Of pH

Active Crude bacteriocin from selected LAB was adjusted to different pH (2.0 – 10.0), incubated for 4hrs and aliquots were removed to determine the bacteriocin activity[7].

Effect Of Surfactants

Crude bacteriocin was treated with different detergents (Tween–20, SDS, Ethylene Diamine Tetra Acetic acid (EDTA), concentration ranging from 0.05 – 1.0% and incubated at 35^0C for 3h, and activity was determined[8].

Result And Discussion

Bacteriocins of lactic acid bacteria have the potential as food bio preservatives to control several pathogenic and spoilage bacteria. For economical and regulatory purposes, these bacteriocins should be produced in large amounts and preferably in a medium compound of food gradients. Attempts have been made to produce bacteriocin from different organisms such as *Lactobacillus* sp., *Leuconostoc* sp., *Lactococcus* sp., *Pediococcus* sp., *Streptococcus* sp. etc[9,6]. In this study, an attempt was made to produce bacteriocin in MRS medium and characterization of bacteriocins produced by *L. acidophilus* isolated from meat samples, respectively. The organism were tested for their inhibitory activity over some food borne pathogens such as *E.coli, Pseudomonas* .sp, *Bacillus* .sp, *Staphylococcus* .sp, *Klebsiella* .sp. Almost all the tested pathogens were inhibited by these bacteriocin producers (Table 1). The maximum zone of inhibition showed in *Staphylococcus* .sp (16mm). In our findings observing the activity of bacteriocin in *L. acidophilus* heat treated at 75 ^{o} C for15mins Therefore, it could be grouped under stable low molecular weight bacteriocin (Table 2). The phenomenon of heat stability of bacteriocin from LAB up to 121^0 C for 15min has been reported earlier in Lactocin RN 78[10] and in *L.brevis* OGI[7]. Exposure of bacteriocin sample to surfactants like anionic and cationic resulted in an increase in bacteriocin titers. We observed that the addition of nonionic detergents such as Tween 80 reduced the bacteriocin activity from *L. acidophilus* (Table 3). Sahar *et al*.[11] who reported increase in activity with tween 20. Cationic detergent hexadecyl trimethylammonium bromide increased bacteriocin activity.

Table: 1 Inhibition of various food pathogens by bacteriocins of *L. acidophilus*

S.No	Organism	Inhibition zone (mm)
		L. acidophilus
1	*E. coli*	13
2	*Pseudomonas* .sp	10
3	*Bacillus* .sp	12
4	*Staphylococcus* .sp	16
5	*Klebsiella* .sp	14

Table: 2 Effect of heat on stability of bacteriocin

Time interval (min)	Zone of inhibition in mm				
	15°C	**30 °C**	**45 °C**	**60 °C**	**75 °C**
15	12.00	11.33	10.66	9.66	8.00
30	11.66	11.00	10.00	8.33	7.66
45	11.00	10.33	9.00	8.00	7.33
60	10.00	9.66	8.33	7.00	7.00

Table: 3 Effect of surfactants on stability of bacteriocin

Conc. Detergent (%)	TWEEN 80	SDS	EDTA
0.2	7.00	9.00	11.66
0.4	8.00	10.33	12.00
0.6	6.33	11.66	11.00
0.8	5.30	12.33	10.33
1.0	5.00	10.00	14.00

Conclusion

The Bacteriocin identified from *L. acidophilus* isolated from meat significant characters such as heat resistance, acid and alkaline tolerance and storage stability at room temperature for a longer period of time. *L. acidophilus* can be recommended as a bio-preservative in food industry

References

1. Villiani, F., Apone, M., Blaitta, G., Maureillo, G., Pepe, O., Moschetti, G. (2001), Detection and characterization of a bacteriocin, garviencin L-15, produced by *Lactococcus garvieae* isolated from cow's milk, *Journal of Applied Microbioogy*, 88, 877- 886.
2. Rajaram G, Manivasagan P, Thilagavathi B and Saravanakumar A (2010) Purification and Characterization of a Bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. Adv. J. Food Sci. Technol. 2(2), 138-144.
3. Forouhandeh H, Zununi Vahed S, Hejazi MS, Nahaei MR and Akbari Dibavar M (2010) Isolation and phenotypic characterization of lactobacillus species from various dairy products. Curr. Res. Bacteriol. 3(2), 84-88.
4. Lindgren, S.W., Dobrogosz, W.J. (1990) Antagonistic activities of lactic acid bacteria in food and feed fermentation", *FEMS Microbiology Reviews*, 87, 149- 164.
5. Zhu, W.M., Liu, W., Wu, D.Q. (2000), Isolation and characterization of a new bacteriocin from *L. gasseri* KT7, *Journal of Applied Microbiology*, 88, 877-886.
6. Yang, A.R., Yanling, M. (1999), Bacteriocin production from lactic acid bacteria in a dairy based medium, *Indian Journal of Microbiology*, 43, 267-293.
7. Ogunbanwo ST, Sanni A and Onilude AA (2003) Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr. J. Biotechnol. 2 (8), 219-227.
8. Mojgani N, Sabiri G, Ashstiani M and Torshizi M (2009) Characterization of bacteriocins produced by *Lactobacillus brevis* NM 24 and L. fermentum NM 332 isolated from Green olives in Iran. J. Microbiol. 6, S2- 5.
9. Aktypis, A., Kalantzopoulos, G., Ten Brink, B. (1998), Purification and characterization of thermophilin T, a novel bacteriocin produced by *Streptococcus thermophilus* ACA – DC 0040, *Journal of Applied Microbiology*, 84, 568 – 576.
10. Mojgani N and Amirinia C (2007) Kinetics of growth and bacteriocin production in *L. casei* RN 78 isolated from a dairy sample in Iran. Int. J. Diary Sci. 2, 1-12.
11. Sahar F Deraz, Martin Hedstrom, Eva Nordberg Karlsson, Sara Linse, Ashraf A Khalil and Bo Mattiasson (2007) Production and physicochemical charaterization of acidocin D20079, a bacteriocin produced by *Lactobacillus acidophilus* DSM 20079. World J. Microbiol. Biotechnol. 23, 911–92.

*****