RESEARCH PAPER | Biology | Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Study of the effect of enterocin produced from *Enterococcus faecium* on some food pathogenic bacteria

KEYWORDS

Nibras Nazar Mahmood

Al-Mustansiriya University College of Science Department of Biology

ABSTRACT *I used 45 samples of raw milk to isolate Enterococcus faecium. I obtained three specimens, Ef1, Ef2, and Ef3, to investigate the effect of bacteriocin produced by Enterococcus faecium on some pathogenic food bacteria found in milk and dairy products.Enterocin produced by Enterococcus faecium was detected by using two methods, cup and well diffusion. A wide spectrum effect was found on both gram-positive and gram-negative bacteria, including Listiriamonocytogenes and Salmonellatyphimurium.The enterocin produced from Ef3 was partially purified to 80%by ammonium sulfate precipitation, and gave the highest yield reaching (640Au/ml). Enzymatic effect of enterocin demonstrated that proteolytic enzymes completely inactivated the bacteriocin, while other enzymes, including lipase, lysozyme,α-amylase, and catalase, showed no effect .The enterocin was heat-resistant at even 121˚c for 15 minute, and the optimum pH was at(4-9).*

Introduction

Using lactic acid bacteria (LAB) and/ or natural products related to them for the food preservation (i.e., biopreservation) seems to be a good approachtodecrease growth of pathogens, as illustrated by the barrier technology strategy (1). Bacteriocins are ribosomally-synthesized antibacterialpeptides obtainedfrom bacteria, and in most cases, they are active against related species; those of similar genetics. And that are able to kill other bacteria by attacking target membranes with pores (2,3). Based on mode of action and structure, they have been grouped into four classes (4).

Many researches focused on the production of bacteriocins from members of the LAB group, specifically *Lactobacillus* sp., *Lactococcus* sp.,*Leuconostoc* sp., *Enterococcus* sp., and *Pediococcus* sp. (5). The LAB bacteriocins are active against a wide spectrum of foodspoilage and foodborne bacteria.*Enterococcus* spp. are also well-known to produce bacteriocins that have demonstrated antimicrobial activity against different foodborne pathogens (6,7).Gram-positivepathogenic bacteria, including *Listeria monocytogenes,Clostridium* sp., and *Bacillus* sp. *L. monocytogenes* can produce a broad variety of human disease ranging from a nonspecific flu-like illness to severe diseases, like sepsis and meningitis (8).

Several bacteriocins from Gram-positive bacteria have fairly wide inhibitory spectra, and these bacteriocins may therefore have an applied potential as antimicrobial agents. The *Enterococcus* is a gram positive bacteria belong to lactic acid bacteria (LAB) which are well known producers of bacteriocin (9,10).

Many bacteriocins produced by enterococci,especially by *Enterococcus faecium* strains of differentorigins (rumen contents, sausages, waste, olives) have been described (11).In general, most enterocins are related to the class IIa bacteriocins, which are thermostable, pediocin-likebacteriocins. The use of bacteriocinogenic enterococci to control contamination in food, to prevent the growth of spoilage and pathogenic microorganisms (12) feed and in the digestive tract of animals has been reported (13,14). (*Enterococcus faecium*) as a feed additive for calves for rearing, piglets (15)

Enterococci are commensals of the animal and human intestinal tract (16).

Bacteriocin is potentially considered as antimicrobial agent for improving the safety of food products; therefore,this study aimsat the extraction, partial purification, and characterization of bcteriocin produced by *Enterococcus* faecium, and how the product may play a promising role in the preservation of food.

Materials and methods

Bacterial isolates and cultural media

The bacterial isolates of*Enterococcusfaecium* were isolated from raw milk, and there were grown in Ej-medium (17), while the indicator bacteria (*Listiria monocytogens*, *Staphylococcus aureus*,*Bacillus cereus, Salmonella typhi, Brucella* spp, and *Pseudomonas aeruginosa*) were obtained from the Department of Biology at the College of Science in the Al-Mustansiriya University, Baghdad, Iraq, and they were grown in brain heart infusion broth (Hi-Media/India). Then, they were identified by physiological, biochemical, and morphological tests according to (18).

Detection of *Enterococcus faecium* produced enterocin

1- Cup agar method

The isolates were grown in MRS broth (Hi-Media/India) pH-6.5, seeded with 1% inoculum of overnight culture, and incubated anaerobiclly at 37˚c for 24h.After incubatio n,*Enterococcusfaecium*were cultured in MRS agar by streking method and incubation at 37˚c for 24h, and then were made like discs by using cork borer of 5mm diameter, and placed on the surface of nutrient agar media that were prepared previously and spread by 0.1 ml of indicator bacteria (10^6cell/ml), and incubated at 37˚c for 24h including control time (MRS agar without inoculated bacteria)(19). And zones of inhibition were measured in mm diameter.

2-Well diffusion method

Nutrient agar plates were spread with 0.1ml (10^6cell/ml) of indicator bacteria. Wells(5mm in diameter) were cut into these agar plates, and 100µl of the culture*Enterococcusfaecium*of supernatants were placed into each well.The plates were then incubated at 37˚c for 24h and zones of inhibition were measured in mm diameter(20).

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Enterocin activity assessment

Extraction of crud enterocin from a selected isolate (Ef3) was carried out as described by (21) with some modification. *Enterococcus faecium* was cultivated overnight in MRS broth at 37˚C for 48hr. After centrifugation at 6000 rpm for 15 min at 4˚C, the cell- free supernatant was filtered through 0.22µm filters and neutralized to pH 6.5, to eliminate the inhibitory effect caused by the decrease of pH by using NaOH (1N) to avoid the effect oforganicacids. Pour plates were prepared from nutrient agar seeded with 10^6cell/ml indicator bacteria. Enterocin activity measured by two fold dilution, using Physiological saline solution and Wells, were cut into pour plates with 5mm cork borer and filled with 100µl of the cell supernatant. The plates were incubated at 37˚Cfor 24h. Inhibition was detected by zone of clearing sterile round the supernatant well. The highest dilution is the one that produced a definite zone of milliliter (AU/ml) (22)

AU was calculated as:

(1000/100) ×D, were 1000 :constant, 100: volum of supernatant in well (µl) and D: the dilution factor (23).

Partial purification of enterocin

Cell –free supernatant was obtained by centrifugation (6000 rpm for 15 minat,4˚C) of MRSbroth inoculated with *E. faecium* and incubated at 37˚C for 24h.Ammonium sulfate was added to the cell-free supernatant at (40,50,60,70,80)% saturation (w/v),the mixtures were stirred for 2h at 4˚C and later centrifuged at 8000 rpm for 20 min at 4˚C ,the precipitates were re-suspended in 50 Mm potassium phosphate buffer (pH6.5) and dialyzed against the same buffer for 24h at 4˚C (24(.

Study the effect ofenzymes, heat stability and pHon thepartiallypurifiedenterocin

Effect enzymes

Partially purified enterocin was treated with enzymes at a final concentration of 1mg/ml as the following: α-amylase, Lipase,Lysozyme, Trypsin,Pepsin, Papain,Catalase, Protinase-K (Sigma-USA). All samples were adjusted to pH7 except that treated with pepsin,which was adjusted to pH 3. Samples were filtered-sterilized using filter membrane 0.22µm, and then incubated at 37˚C for 2h. Residual enzyme activity was finally stopped by boiling for 5 min (25).

Heat stability of enterocin

Partially purified enterocin samples were adjusted to pH 5.5 and then heated to(40,60 ,100)˚C respectively. Enterocin activity was assessed after (10,15,30)min at each of these temperatures.Activity was also assessed after 15 min at 121˚C (26).

Effect of different pH

For pH stability, partially purified enterocin samples were adjusted to pH range of (2-11), using 5N NaOH or 5N HCl. All samples were incubated at 37 ˚C for 24h. (26).

Results and discussion

Isolation and identification of strain *E. faecium*

The enterocin producing strain was isolated from the raw milk and they were identified as ***E. faecium*** by the morp hological,cultural,physiological,and biochemical characteristics such as catalase negative, Gram-positive, facultatively anaerobic coccus with the ability to grow at 45°C and pH 9.6, and in the presence of both 4% bile salts and 0±04% sodium azide. It did not produce gas from glucose; it gave a positive Voges Proskauer reaction and produced ammonia from arginine. The final pH in glucose broth was 4±2,and acid was produced from l-arabinose,and non motile,and isolated ***E. faecium*** fromraw non-pasteurized milk, where this bacteria close in this food, as a result of the digestive canal of different animals, spread in water and soil at milking machinery and tools in different manufacturing conditions of these foods (27).

Antibacterial activity of enterocin from *E. faecium*

The susceptibilities of various Gram-positive andnegative bacteria to growth inhibition by the cup agar method (Table 1) and the supernatant of *E.faecium*by well diffusion method(Table2)shows inhibitory activity against(*Listiria monocytogens* , *Staphylococcus aureus* , *Bacillus cereus*, *Salmonella typhi*,*Brucella* spp *and Pseudomonas aeruginosa*,)maximum activity observedagainst*Listiria monocytogens* ,*Staphylococcusaureus*and *Salmonella typhi.*

Table 1:Antibacterial activity from *E. faecium* against indicator bacteria by used cup agar method on MRS agar

Indicator bacteria Inhibition zone diameter(mm)						*E.faecium*
Pseudomonasaeruginosa	*Brucella* spp	Salmonellatyphi	Bacilluscereus	Staphylococcusaureus	Listiria monocytogens	
13	-	15	10	15	17	Ef1
12	-	15	11	16	17	Ef2
14	8	17	13	19	20	Ef3

Table 2:Antibacterial activity from *E. faecium*supernatants against indicator bacteria by used well diffusion method on MRS broth

Indicator bacteria Inhibition zone diameter(mm)						*E.faecium*
Pseudomonasaeruginosa	*Brucella* spp	Salmonellatyphi	Bacilluscereus	Staphylococcusaureus	Listiria monocytogens	
14	-	13	10	12	12	Ef1
12	-	13	9	11	15	Ef2
14	7	13	11	17	18	Ef3

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

According the tables above we notice that the cup agar method was in better production of enterocin compared with productivity in the liquid media in spite of the presence of variation in the inhibition of these isolates. And came in line with what indicated by (28) to the presence of variation in the effectiveness of the bacteria producing bacteriocin in solid and liquid media,microorganism product have on the solid media does not necessary to be a producer of liquid media.This was also confirmed by (29) who has pointed out to the inefficiency of some isolates producing bacteriocin in demonstrating their effectiveness in the liquid media. Or, it may be exposed to certain bacteria mutations for one reason or another, which may result in loss of bacteriocin receptors and the indicator bacteria become resistant. (30) noted that the occurrence of modulating receptors for colicin resulted from mutations lead to be resistant isolates.The best selected local isolates was the one belonging to bacteria *E.faecium*, which gave greater inhibition zones against bacteria gram positive and negative (Ef3).

Extraction and partial purification of enterocin

Partial purification of enterocin was performed by precipitation with ammonium sulfate. Ammonium sulfate precipitation is the most commonly method used to purify protein from broth culture (**31**). It is the best , first –choice salt because it has high solubility and is relatively inexpensive (32). The enterocin was recovered following the 80%ammonium sulfate saturation of the cell –free supernatant with an increase activity unit of 320 AU/ml for cell-free supernatan 640 AU/ml for partially purified entrocin .

The activity ratio between various bacteriocinsdiffered from species to species and even from strain to srain(33). Even during controlled fermenter experiments, considerable differences in activity yields are obtained,and an influence of the environmental process conditions on obtained bacteriocin activity can be seen. In general, the cultivation conditions directly affect bacteriocin production as such (specific bacteriocin production in particular) and, indirectly, through biomass production (34).(31) showed that some bacteriocin activity was lost in the supernatant, probably due to the saturation of adsorbing sites on bacterial cells. During the purification producers,each step resulted in considerable loss of protein concentration while specific activity increased(35).

Effect enzymes on enterocin

Enterocin was totally inactivated by treatment with pepsin, trypsin, papain and proteinae-K,whereas treatment with α-amylase,lipase,lysozyme and catalase did not affect the activity of it (Table 3).The sensitivity of enterocin to proteolytic enzymes indicates that it is proteinaceous in nature.

Table (3): Effect of enzymes on the partial purified enterocin activity produced by *E. faecium*

Enzyme	Enterocin activity (AU/ml)
Pepsin	0
Trypsin	0
Papain	0
Proteinase –K	0
α-amylase	640
Lipase	640
Lysozyme	640
Catalase	640

These results are consistent with many of the studies that have relied on the test sensitivity toward enterocin purified enzymes (26) and (36) which pointed to the loss of enterocin produc to bacteria *E.faecium* to its effectiveness after digestion enzymes protein such as PapainandPepsinandTrypsinandProteinase -K;which confirms the nature ofthe protein. Enterocin not affected bythe product of *E.faecium* also notes when treated with enzymes, Lipase Catalase, α-amylase, Lysozyme (37,38).

Heat stability of enterocin

The enterocin was resistant to treatments of (40,60 ,100)°C for (10 ,15, 30)min, maintained its full effectiveness with the exception of retaining 50% of its effectiveness when treated with a degree of 121 °C for 15 minutes as in table 4.The thermostability feature might be related to the molecular structure of enterocin,other studies where indicated characterize enterocin produced from bacteria *E.faecium*and *E.faecalis* with small molecular weights thermal stability and its ability to withstand temperatures of up to 121 °C for 15 minutes or more(39,40).

Table (4):Effect of temperature on the partial purified enterocin activity produced by *E. faecium*

Temperature(°C)	Time(minutes)	Bacteriocin activity(AU/ml)
40	10	640
	15	640
	30	640
60	10	640
	15	640
	30	640
100	10	640
	15	640
	30	640
121	15	320

Effect of different pH

Full activity of enterocin was retained in samples at pH 4-9, at pH 10 activity was reduced to the half, and at pH 2,3,11 no activity was observed table (5). The increased inhibition activity under alkaline pH conditions could be due to alterations in peptide diffusion in such conditions(41) . It retained activity even in the pH range of 2-10 (42). Growth and bacteriocin production at low pH may be advantageous for survival in the upper intestinal tract in view of probiotic use. (43)

Table (5):Effect of pH on the partial purified enterocin activity produced by *E. faecium*

pH	Bacteriocin activity (AU/ml)
2	0
3	0
4	640
5	640
6	640
7	640
8	640
9	640
10	320
11	0

Conclusion

In all, enterocin may have a practical application in the food industry to control listerial

contamination.The results produced in the present study highlighted the remarkable potential as food biopreservation agents exhibited by these strains and the enterocins

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

they produced. This was observed in the light of the fact that the enterocins and the strains analyzed showed little sensitivity to high temperatures and pH variations. Also, the capacity to inhibit *Listeria monocytogenes* and *Salmonella typhi* two very important pathogens, associated with food poisoning, reinforced the use of *E. faecium* in food preservation strategies.

REFERENCE

1-Henning, C., Gautam, D.and Muriana ,P.(2015). Identification of Multiple Bacteriocins in Enterococcus spp.Using an Enterococcus-Specific Bacteriocin PCR Array.Microorganisims.3:1-16. | | 2-Héchard,Y.;Sahl, H.-G.Mode of action of modified and unmodified bacteriocins from gram-positive bacteria. Biochimie 2002, 84, 545–557. | | 3- Zacharof, M.P.; Lovitt, R.W. Bacteriocins produced by lactic acid bacteria a review article. APCBEE Procedia 2012, 2, 50–56. | | 4-Heng NCK, Wescombre PA, Burton JP, Jack RW and Tagg, JR. (2007) . In: Riley, MA, Chavan MA (Eds.), The Diversity of Bacteriocins in Gram positive Bacteria Bacteriocins: Ecology and Evolution. Springer- Verlag, Berlin Heidelberg. | | 5- Ghrairi T, Frere J, Berjaud JM, Manai M (2008).Purification and characterization of bacteriocins produced by Enterococcus faecium from Tunisian rigouta cheese. Food Control, 19: 162-169. | | 6- Amalaradjou, M.A.R.; Bhunia, A.K. Chapter five—Modern approaches in probiotics research to control foodborne pathogens. Adv.Food Nutr. Res. 2012, 67, 185–239. | | 7-Vera Pingitore, E.; Todorov, S.D.; Sesma, F.; de Melo Franco, B.D.G. Application of bacteriocinogenic Enterococcus mundtii CRL35 and Enterococcus faecium ST88Ch in the control of Listeria monocytogenes in fresh minas cheese .Food Microbiol .2012,32: 38–47. | | 8- Beuchat LR (1996). Pathogenic microorganisms associated with fresh produce. J. Food Prot., 59: 204-216. | | | 9-Schleifer, KH.and Kilpper-Balz,R. (1987). Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. System. Appl. Microbiol. 10: 1-19. | | 10- Hardie, JM.and Whiley, RA.(1997).Classification and overview of the genera Creptococcus and Enterococcus. Soc. Appl. Bact. Symp.Series. 26:1S-11S. | | 11- Aymerich, T.; Holo, H.; Havartein, LS. ; Hugas, M.; Garriga, M.and Nes,IF.(1996). Biochemical and genetic characterization of enterocin A from Enterococcus faecium, a new antilisterial bacteriocin in the pediocin family of bacteriocins.Appl.Environ. Microbiol . , 62: 1676-1682. | | 12-Khan, H ., Flint,S. and Yu,P.-L.(2010). Enterocins in food preservation.Inter.J.Food Microbiol.141:1-10. | 13- Cotter PD, Hill C, Ross RP (2005). Bacteriocins: developing innate immunity for food. Nature Rev. Microbiol., 3: 777-788. | | 14- Galvez A, Abriouel H, Lopez RL Omar NB (2007).Bacteriocin-based strategies for food biopreservation. I. J.Food Microbiol., 120: 51-70. | | 15- EFSA (European Food Safety Authority), 2014. Scientific Opinion on the safety and efficacy of Oralin® (Enterococcus faecium) as a feed additive for calves for rearing, piglets, chickens for fattening, turkeys for fattening and dogs. The EFSA Journal.2014;12(6):3727. | | 16- Devriese, LA.; Pot, B. and Collins, MD.(1993) Phenotypic identification of the genus Enterococcus and differentiation of phylogenetically distinct enterococcal species and species groups. J Appl Bacteriol 75: 399–408. | | 17- Suzzi, G.; Caruso, M.; Gardini, F.; Lombardi, A.; Vannini, L.; Guerzoni, M. E.; Andrighetto, C. and Lanorte, M. T. (2000). A survey of the enterococci isolated from Italian cheese . J. Appl. Microbiol., 89:267-274. | 18- Holt, J.G.; Krieg, N.R.;Sneath,P.H.; Staley, J.T. and Williams, S.T. (1994). Bergy' sManual of Determinative Bacteriology.9th edition, Williams & Wilkins Co., Baltimore, Maryland ,USA. PP: 528-1063. | | 19-Al-Qassab,A.O. and Al-Khafagi,Z.M.(1992).Effect variabil conditions on inhibitory activity for intestinal lactobacilli against coliform bacteria caused diarrhoea. J.Agric.Scien.Iraqi. vol. 3(1):18-26. | | 20- Gupta, U.; Rudramma; Rati, E.R. and Joseph, R.(1998). Nutritional quality of lactic acid fermented bitter gourd and fenugreek kleaves. International Journal of Food Science and Nutrition, 49(2):101-108. | | 21-Annamalai,N.; Manivasagan ,P. ; Balasubramanian ,T. and Vijayalakshmi ,S.(2009).Enterocin from Enterococcus faecium isolated from mangrove environment. African Journal of Biotechnology. 8(22):6311-6316. | 22- Ivanova I, Miteva V, Stefanova TS, Pantev A, Budakov I, Danova S,Montcheva P, Nikolova I, Dousset X, Boyaval P (1998).Characterization of a bacteriocin produced by Streptococcus thermophilus 81.Int. J. Food Microbiol. 42: 147-158. | | 23-Parente, E.; Brienza, C.; Moles, M.and Ricciardi, A.(1994).A comparison of methods for the measurement of bacteriocin activity. J. Microbiol.Meth., 22: 95-108. | | 24-Burianik,LL. andYousef.,AE.(2000) Solvent extraction of bacteriocins from liquid cultures.Lett. Appl. Microbiol., 31: 193-197. | | 25- Pilasombut, K., Sakpuaram, T., Wajjwalku, W., Nitisinprasert, S., Swetwiwathana, A., Zendo, T., Fujita, K., Nakayama, J. and Sonomoto, K. (2005). Purification and amino acid sequence of a bacteriocins produced by Lactobacillus salivarius K7 isolated from chicken intestine. Songklanakarin J.Sci.Technol. 28(1):121-131. | | 26-Bayoub,K. ; Mardad , I .;Ammar ,E .; Serrano ,A .; Soukri, A.(2011). Isolation and purification of two bacteriocins 3D produced by Enterococcus faecium with inhibitory activity against Listeria monocytogenes.Curr. Microbiol.62:479-485. | 27-Hugas,M.; Garriga, M.and Aymerich, M. T. (2003).Functionality of enterococci in meat products. Int. J.Food Microbiol.88:223-233. | 28-Tagg,S.R.; Dajani ,A.S.and Wannamaker,L. W. (1976). Bacteriocin of gram –positive bacteria .Bacteriol .Rev .40: 722-756. | 29- Pugsley, A.P.(1983).Auto induced synthesis of colicin E2.Mol.Gen.Genet.90 : 379–383. | | 30-Cursino, L.;Smarda , J. ; Chartone, E. and Nascimento, A .(2002) .Recent updated aspects of colicins of Enterobacteriaceae. Braz.J.Microbiol. 33 : 196–217.(Abstract). | | 31-Sivakumar,N.,Rajamani andAl-Bahry,S. (2010).Partial Characterization of bacteriocins produced by Lactobacillus acidophilus and Pediococcus acidilactici. Braz. Arch. Biol. Technol. 53 (5):1177-1184. | | 32-Jakoby,W. B.(1971).Crustallization as a purification technique. Enzyme purification and related techniques.Methods in Enzymology, vol. 22, Academic Press. | | 33-Eijsing,V.G. H., Skeie, M., Middelhoven, P. H., Brurberg, M. B. and Nes, I. F. (1998). Comparative studies of class II a bactfricins of lactic acid bacteria.Appl.Environ.Microbiol. 64 (9): 3275- 3281. | | 34- DeVuyst, L.and Leroy, F. (2007). Bactriocins from lactic acid bacteria: production, purification, and food application. J. Mol. Microbiol.Biotechnol. 13: 194-199. | | 35-Ogunbanwo,S.T.,Sanni,A.I.and Onilude,A.A.(2003).Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactbacillus brevis OG1. Afr. J. Biotechnol. 2(8): 219-227. | | 36-Kang,J.H. and Lee, M.S.(2005).Characterization of a bacteriocin produced by Enterococcus faecium GM-1 isolated from an infant.Journal of Applied Microbiology.98(5):1169-1176. | 37-Line,J.E.; Svetoch , E.A . ; Erslaanov , B.V.; perelygin ,V.V.; Mitsevich ,E.V.;Mistevich ,I.P.; Levechuk ,V.P.;Sevetoch ,O.E. ;Seal ,B.S. ;Siragusa ,G.R. and Stern ,N.J. (2008). Isolation and purification Enteriocin E-760 with broad antimicrobial activity against Gram - positive and Gram negative bacteria .Antim. Agents and Chemoth.52(3) :1094- 1100 . | 38- Khay, E.; Idaomar,M. ; Castro , L.M.P.; Bernardez,P.F.;Senhaji ,N.S. and Abrini,J.(2011).Antimicrobial activities of the bacteriocin-like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk.African J.of Biotechnology.10(51):10447-10455. | 39-Galvez,A.; Valdivia, E. ; Camafeita ,H. ; Mendez, E. ; Martinez, E. and Maqeda,M . (1998).Isolation and characterization of enterocin EJ97,a bacteriocin produced.Arch. Microbiol.171 : 59–65. | 40- Balla , E. ; Dicks, L.T. ; Toit, M.D. ; Van–Dermewe, M.J. and Holzapfel, W. H. (2000).Characterization and cloning of the gene encoding enterocin 1017A and enterocin 1017B, two antimiciobial peptides produced by Enterococcus faecalis BFE 1071. Appl. Environ.Microbiol. 66(4): 1298-1304. | | 41-Ferreira,A.E.; Canal,N.; Morales,D.; Fuentefria,D.B.and Corção,C.(2007). Characterization of Enterocins Produced by Enterococcusmundtii Isolated from Humans Feces.Brazilian Archives of Biology and Technology.50(2):249-258. | | 42-Badarinath,V.and Halami,P.M.(2011).M0lecular characterization of classIIa,heat-stable enterocin produced by Enterococcus faecium MTCC 5153.Indian J.Biotech.10:307-315. | | 43-Toit,M.D.;Franz,C.M.A.P.;Dicks,T.M.L.and Holzapfel,W.H.(2000).Preliminary characterization of bacteriocins produced by Enterococcus faecium and Enterococcus faecalis isolated from pig faeces.J.Appl.Microbiol.88:482-494. |