African Journal of Microbiology Research Vol. 5(16), pp. 2287-2296, 18 August, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.613
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Biopreservative potential of marine *lactobacillus* spp

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Accepted 30 July, 2011

Bacteriocins are of special interest due to their potential value as natural preservative. The present study is a trial for production of such bacteriocin from a marine *Lactobacillus* sp. Lactic acid bacteria (LBA) was isolated from fish gut (*Mugil cephalus*) and prawn muscle (*Peneaus monodon*) samples and their density were found to be 5.2 x 10^7 and 6.4 x 10^7 CFU/g respectively. Various pathogens were isolated from ready to eat pickle samples (bottled). The LAB strains were tested against 10 different commercial antibiotics. Among them Vancomycin was the only antibiotic that showed a minimum of 40% resistance to the LAB strains tested. LAB strains were optimized at different parameters and maximum bacteriocin production was at pH 6, temperature of 35°C, 3.5% of salt concentration, 24th h of incubation period. Bacteriocin produced by these strains were precipitated from the culture filtrate using methanol and TCA which was further dialyzed, centrifuged and lyophilized. *Lactobacillus fermentum* was selected as the most potential strain for both bacteriocin production as well as antimicrobial activity. sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis proved that the obtained protein is homologous with a molecular weight of 18 kDa. FT-IR spectrum also confirmed the obtained protein as a bacteriocin. The study revealed that *Lactobacillus* strains of marine origin are having the potential to be used as biopreservatives especially in seafood industries. The production of bacteriocin from *L. fermentum* was found to be ideal for industrial scale production and commercial utilization.

Key words: Bacteriocin, *Lactobacillus fermentum,* biopreservative, fourier transform infrared (FT-IR).

INTRODUCTION

Food fermentation has a great economic value and the products obtained through this process put in improving human health. In this way LAB has contributed in a lot to fermented foods worldwide. The major genera of LAB is of importance in the food industry are *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus* and *Tetragenococcus.* They are significant in production and preservation of milk and dairy products, meat and meat products, vegetable products and as forage crops for animals (Lee, 2004).

Mankind had exploited lactic acid bacteria (LAB) for the production of fermented foods because of their ability to produce desirable changes in taste, flavor and texture as well as to inhibit pathogenic and spoilage microbes. Since they are involved in numerous food fermentations for millennia, it is assumed that most representatives of this group do not pose any health risk to man and are designated as GRAS (generally recognized as safe) organisms (Holzapfel et al., 1995). Different antimicrobials, such as lactic acid, acetic acid, hydrogen peroxide, carbon dioxide and bacteriocins produced by these bacteria, can inhibit pathogenic and spoilage micr-oorganisms, extending the shelf-life and enhancing the safety of food products (Aymerich et al., 2000).

One important attribute of LAB is their ability to produce antimicrobial compound called bacteriocin. Bacteriocins are proteinaceous compound, inhibitory effects towards sensitive strains produced by both Gram-positive and Gram-negative bacteria (Tagg et al., 1976). Bacteriocins producing lactic acid bacteria are used in food fermentations especially in dairy products. In USA, only nisin produced by *Lactobacillus lactis* has been permitted as a food preservative (FDA, 1988). It has also been used in health care products and cosmetics for treatment of acne. They are also being used in toothpaste and mouthwash for the inhibition of dental caries and periodontal diseases

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(Harlander, 1993). Bacteriocins can be exploited to inhibit undesirable microorganisms in the fermentation of wine (Navarro et al., 2000), beer (Ogden et al., 1985), vegetables (Daeschel and Fleming, 1984) and dairy products (Castla et al., 1996; Ross et al., 1999). The present study is on bacteriocin production by LAB of marine environment and its use as a biopreservatives in sea foods.

MATERIALS AND METHODS

The fin fish and prawn samples were collected from the Annan koil landing centre (Latitude 11° 29 'N; Longitude 79° 46' E) for the isolation of lactic acid bacteria. The pickle samples were collected from Tuticorin. Tuna and Prawn pickles were analyzed in the present study. Homogenized extract of the fish and prawn samples were serially diluted up to 10^{-5} dilutions and were plated on MRS agar (HI-MEDIA) by spread plate technique and incubated at 28 ± 2°C for 24 h. The isolated potential strains were identified based on Bergey's manual of systematic bacteriology (Buchanan et al., 1974). Approximately 10 g of sample (pickle) was homogenized in a sterile mortar and pestle using 90 ml of sterile 50% sea water and then serially diluted. About 0.1ml of the serially diluted samples was inoculated into the selective media to isolate the specific pathogens. The isolated pathogens were identified based on Bergey's manual of systematic bacteriology (Buchanan et al., 1974).

Primary screening for antibacterial activity

A preliminary test for inhibitory assay of LAB against food borne pathogens, isolated from the seafood pickles was done.

Well diffusion method (Reinheimer et al., 1990)

After swabbing the pathogens on the Muller Hinton agar plates, 0.1 ml of cell free culture broth of Lactic acid bacteria (LAB) centrifuged at 10,000 rpm for 20 min, poured into the wells and plates were incubated at 37°C for 24 h. The bacterial culture filtrate inhibiting the growth of pathogen was assessed based on the inhibition zone around the well and the results were recorded.

Test for antibiotic resistance of LAB

Muller Hinton Agar was used to check the antibiotic resistance of LAB against antibiotics like Ampicillin (A), Bacitracin (B), Cephotaxime (Ce), Ciprofloxacin (Cf), Erythromycin(E), Nalidixic acid (Na), Novobiocin (Nv), Penicillin-G(P), Tetracyclin (T) and Vancomycin (Va). After swabbing the LAB on MHA plates, the selected antibiotic discs were placed onto the agar medium and the incubated plates were observed for antibiotic assay.

Screening of bacteriocin producing bacteria

Screening was done by well diffusion method using the crude extract. The inhibitory activity was tested against the seafood borne pathogens.

Optimization of cultural condition for bacteriocin production

The effect of incubation period on growth and bacteriocin production was studied for 0 - 52h with 12 h intervals. The pH ranges (3, 4, 5, 6 and 7) were tested. A temperature range 20, 25, 30, 35 and 40°C were optimized to find out the optimum temperature. The range of 3-5% of salt concentration was optimized. Based on the results observed in the optimization the mass scale culture was carried out with the ideal parameters.

Extraction and partial purification of bacteriocins

The precipitation was made by the solvent extraction method. To the filtrate obtained 70% Methanol and 10% of Tri chloroacetic acid (TCA) were added in equal proportion and kept for 48 h at room temperature. This precipitate was dissolved in deionized water and dialyzed through a 1000 molecular weight cut of dialysis membrane against deionized water. The dialysis was done for 24 h and then centrifuged. The precipitate was lyophilized and used for further analysis.

Protein estimation

The Folin-Ciocalteu phenol method (Lowry et al., 1951) was used for the estimation of the total protein content in the sample.

FT-IR analysis

In the study of molecular vibrations, Infrared spectroscopy has contributed more to this field than Raman due to the rapid developments in Infrared instrumentation (Merritt et al., 1986). The vibrational spectra can be utilized directly and simply as molecular "finger prints" to characterize and identify the molecule (Roberts et al., 1985). The lyophilized bacteriocin sample from LAB6 was subjected to FT-IR analysis. The IR spectrum of the bacteriocin was recorded with a perkin-Elmer model 297 IR spectrophotometer. One part of the extract was mixed with 99 part of dried potassium bromide and it was scanned between 600-4000 wave number (cm^{-1}) at a speed of 1 micron. and with a programmed slit opening 2x and air as reference.

RESULTS

The total heterotrophic and lactic acid bacterial count in fish gut was found to be 2.14×10^9 and 5.2×10^7 CFU/g respectively. Similarly, the total heterotrophic and lactic acid bacterial count in prawn muscle was found to be 2×10^9 and 6.4×10^7 CFU/g respectively. A total of 116 morphologically distinct strains were isolated from the serially diluted fish gut and prawn muscle samples. 4 strains from fish gut and 6 strains from prawn muscle samples were identified as potent strains for bacteriocin production. After the screening procedures the potential strains were identified. They are LAB1 *Corynebacterium bovis*, LAB2 *Corynebacterium xerosis*, LAB3 *Lactobacillus alimentarius*, LAB4 *Lactobacillus animalis*, LAB5 *Lactobacillus casei*, LAB6 *Lactobacillus fermentum*, LAB7 *Lactobacillus plantarum*, LAB8 *Micrococcus varians*, LAB9 *Staphylococcus epidermidiis*, and LAB10 *Streptococcus mitis.*

Selective media used for the isolation of seafood pathogens are EMB agar, MRS agar, *Listeria* isolation agar, SS agar, TCBS agar, *Yersinia* identification agar

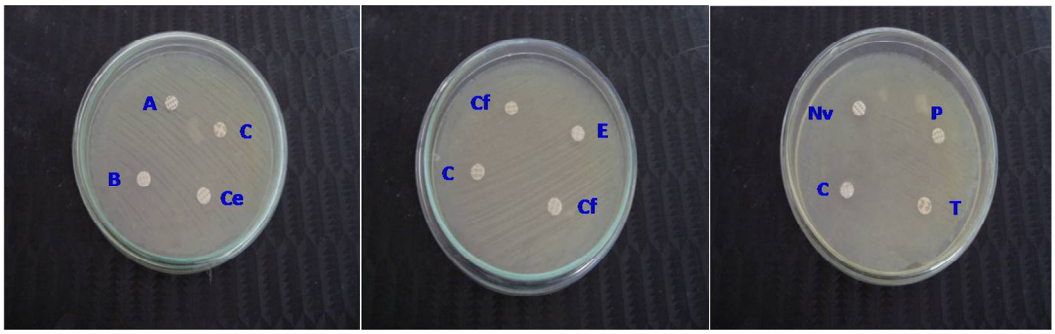


Figure 1. Antibiotic activity against LAB strains.

Table 1. Antibiotic activity of lactic acid bacteria.

LAB	A	B	Ce	Cf	E	Na	Nv	P	T	Va
LAB1	R	R	R	R	R	R	R	R	R	-
LAB2	R	R	R	R	R	R	R	R	R	-
LAB3	R	R	R	R	R	R	R	R	R	-
LAB4	R	R	R	R	R	R	R	R	R	-
LAB5	R	R	R	R	R	R	R	R	R	R
LAB6	R	R	R	I	R	R	R	R	R	R
LAB7	R	R	R	R	R	R	R	R	R	-
LAB8	R	R	R	R	R	R	R	R	R	-
LAB9	R	R	R	R	R	R	R	R	R	R
LAB10	R	R	R	R	R	R	R	R	R	R

*I- intermediate; R- resistant.

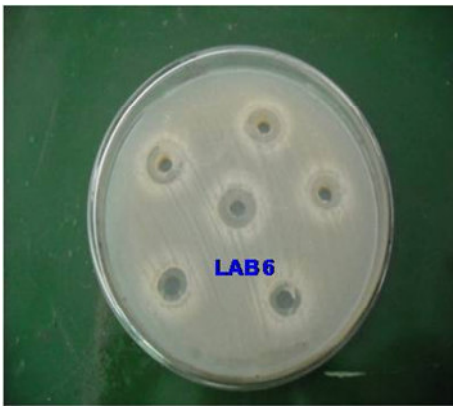


Figure 2. Antibacterial activity of bacteriocin against *Vibrio parahaemolyticus*

where in the density of *E. coli, Lactobacillus* spp, *Listeria* spp, *Salmonella, Vibrio* spp and *Yersinia* spp were enumerated to be in the fish pickle $1.9x10^4$, $2.7x10^4$, $2.1x10^4$, $3.2x10^4$, $1.5x10^4$ CFU/g. Likewise in the prawn pickle the pathogens were enumerated to be $1.3x10^4$, $1.7x10^4$, $2.2 x10^4$, $1.5 x10^4$, $2.8 x10^4$ and $1.0 x10^4$ CFU/g. Isolated pathogens were identified as FBP1 *E.coli,* FBP2 *Lactobacillus vulgaris*, FBP3 *Listeria monocytogenes*, FBP4 *Listeria* spp, FBP5 *Salmonella* spp, FBP6 *Shigella* spp, FBP7 *Staphylococcus aureus*, FBP8 *Vibrio cholera*, FBP9 *Vibrio parahaemolyticus* and FBP10 *Yersinia* spp.

All the 10 LAB strains isolated were found to be resistant to Ampicillin (A), Bacitracin (B), Cephotaxime (Ce), Erythromycin (E), Nalidixic acid (Na), Novobiocin (Nv), Penicillin-G(P), and Tetracyclin (T) (Figure 1). The resistance against Ciproflaxacin (Cf) was shown by all the LAB strains except LAB6, which showed an intermediate activity. Resistance to Vancomycin (Va) was shown by LAB 5, LAB 6, LAB 9 and LAB 10, these results are given in Table 1.

The bacteriocin obtained from LAB6 showed the maximum zone of inhibition compared to all the other strains. It showed a zone of inhibition of 7 mm against *V. parahaemolyticus* (Figure 2), 6 mm against *L. monocytogenes*, *Listeria* spp. 5 mm against *E. coli, Salmonella* spp. *S. aureus* and *Yersinia* spp and 4mm against *Shigella* spp. *Vibrio cholerae* and *Lactobacillus vulgaris*, whereas the supernatant of the same strain showed zone of inhibition of 5 mm against *Vibrio paraheamolyticus* (Figure 3), 4 mm against *L. monocytogenes, S. aureus* and *Yersinia* spp and 3 mm *against E. coli, Listeria* spp, *Salmonella* spp, *Vibrio*

Figure 3. Antibacterial activity of LAB strains against *Vibrio parahaemolyticus.*

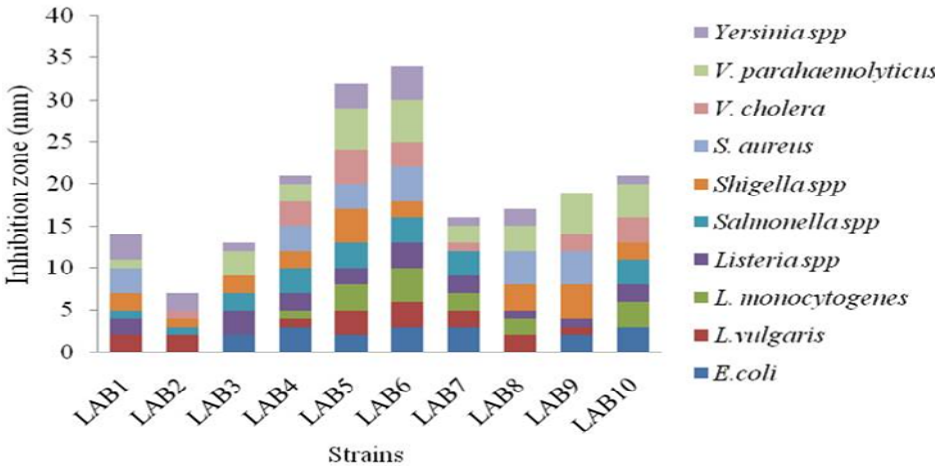


Figure 4. Antibacterial activity of LAB cell free extracts (Zone of clearance in mm).

cholerae and *Lactobacillus vulgaris.* These results are given in Figures 4 and 5 respectively.

The bacteriocin production was found maximum at an incubation period of 24 h. The results were depicted in (Figure 6). Among the various pH ranges studied, the growth of the potential strain was found to be highest at pH 6 (Figure 7). The optimum temperature for bacteriocin production was found to be 30 - 35°C (Figure 8). The optimum salt concentration for bacteriocin production was found to be 3.5% salt concentration (Figure 9).

The amount of protein was estimated to be 0.36 mg/ml. The FT-IR Spectrum of the protein revealed the presence of peaks at the wave numbers of 3288, 3090, 2958, 2924 and 2849 cm^{-1} which indicated the presence of NH, NH_3, CH_3, CH_2 and CH_2 groups respectively. The wave numbers 1660, 1632, 1589 and 1540 cm^{-1} indicated the presence of their bending mode of amide, methyl, and amide groups respectively. The asymmetric mode of the NH band occurred at the wave number 827 cm^{-1}. The molecular weight of bacteriocin from *L. fermentum* was found to be 18 kDa.

DISCUSSION

In the present study the isolation, partial characterization and activity of bacteriocin produced by *L. fermentum* was done. The bacteriocin producing lactic acid bacteria (LAB) were isolated from the fresh meat of marine fin fish and shell fish. It is interesting to note that majority of the *Lactobacillus* spp. that have been isolated from fresh and frozen fish/prawns were those species which were commonly found in animals and human beings (Kandler and Weiss, 1986). There are only a few reports available on isolation of LAB from fresh and seawater fish (Cone, 1982; Okafor and Nzeako, 1985). The LAB strains were evaluated for the production of inhibitory substances against various food borne pathogens. The pathogens used in the present were isolated from fish pickle. All the LAB strains showed a moderate inhibitory activity against the pathogens isolated from pickle samples. The use of bacteriocinogenic starter/protective cultures could improve the quality and increase safety by inhibiting the food-borne pathogens and spoilage microorganisms.

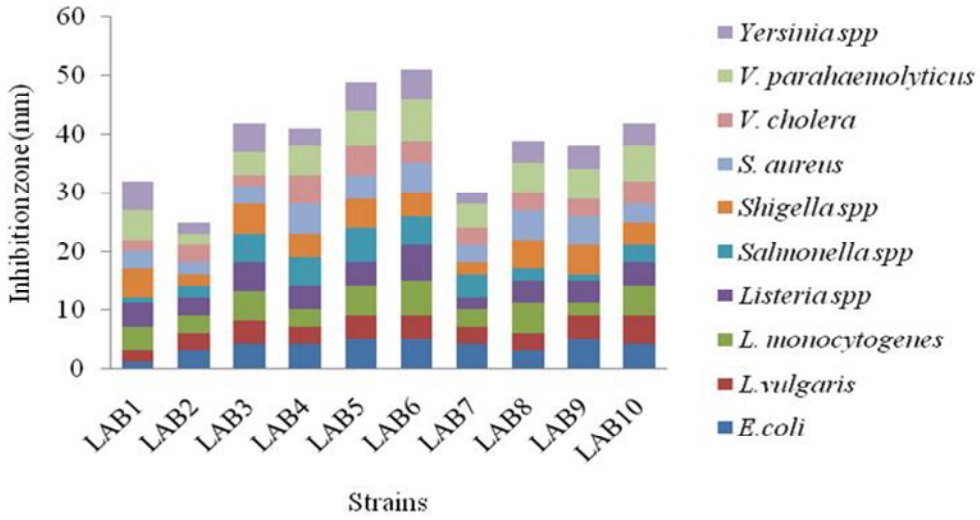
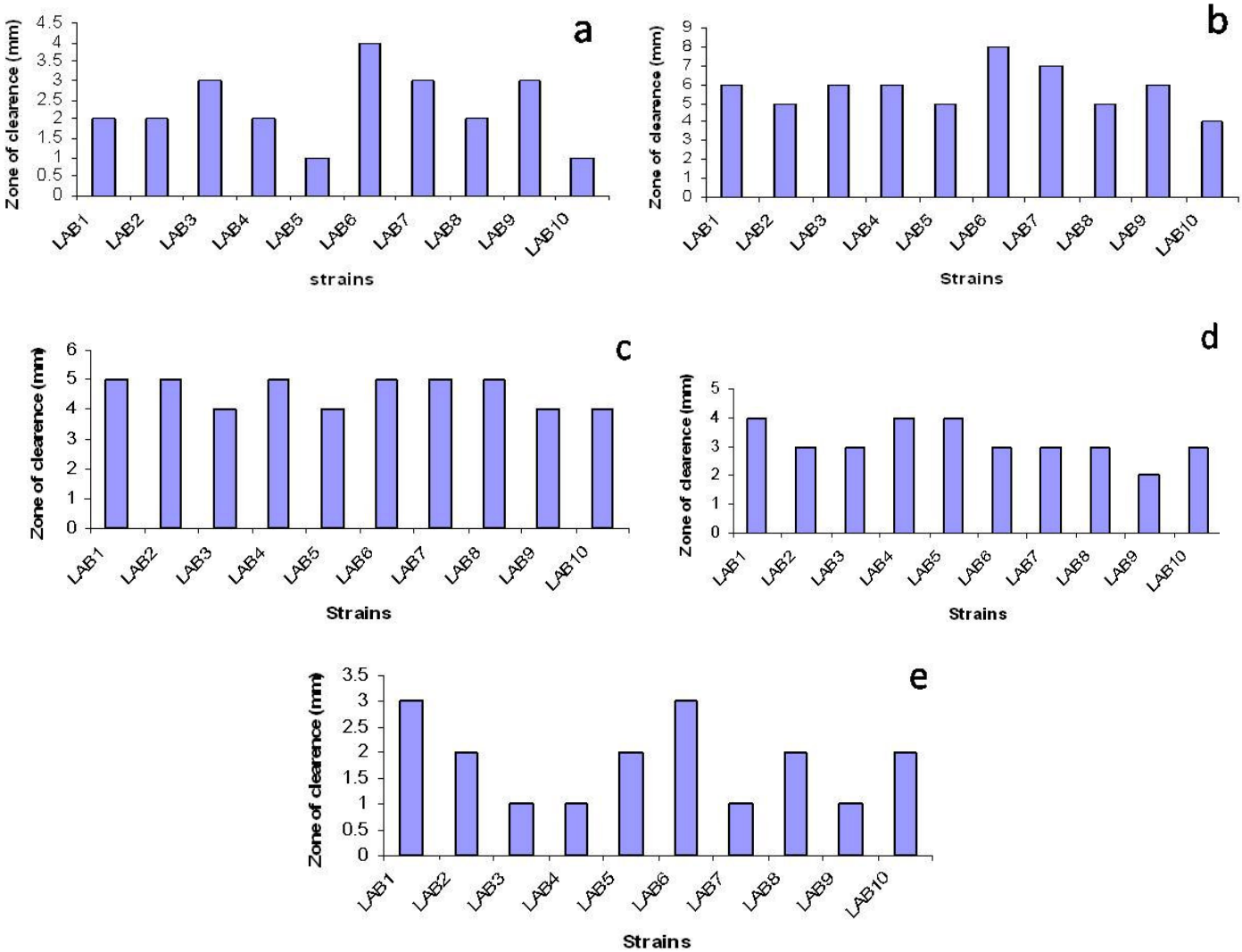
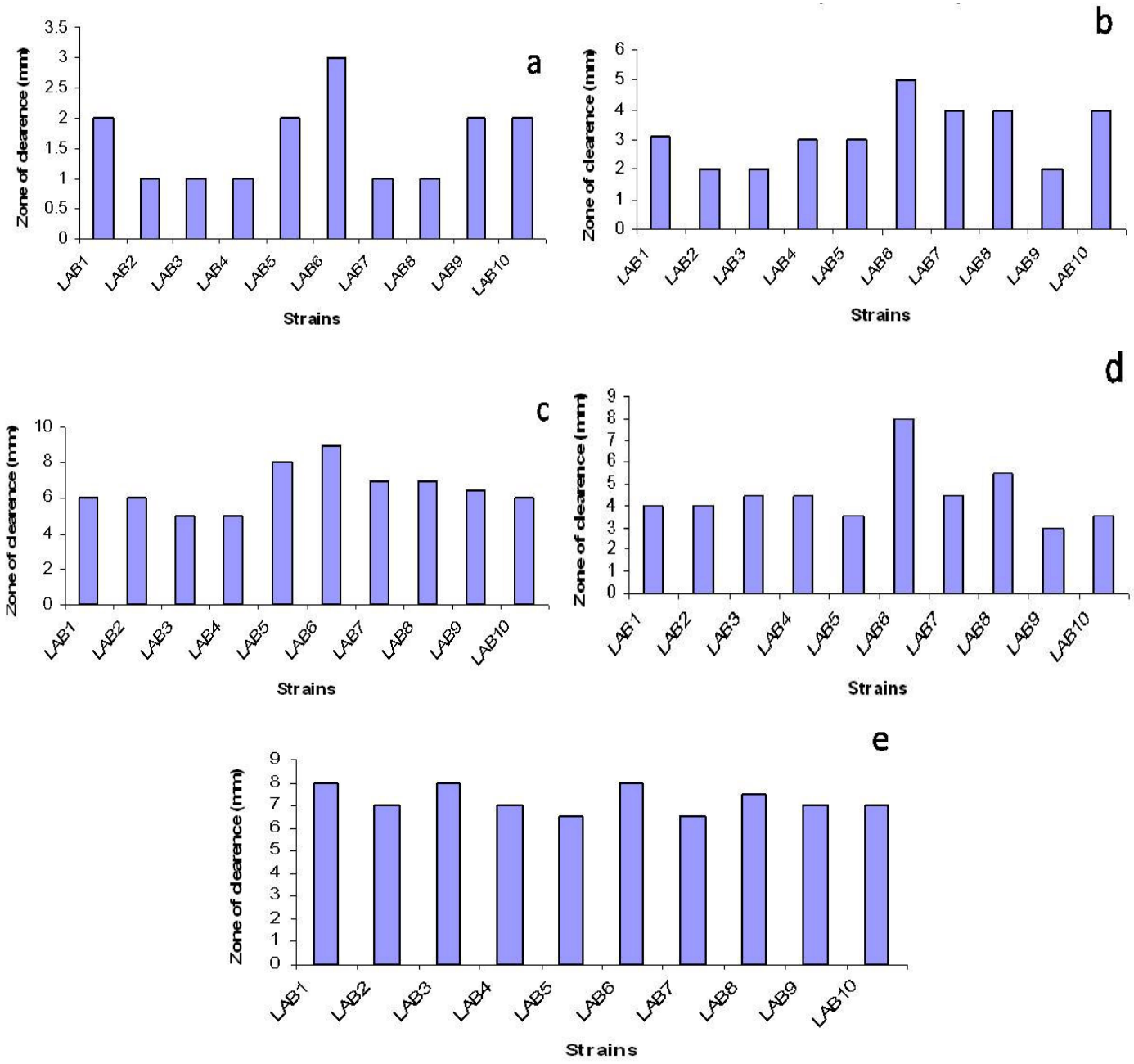


Figure 5. Activity of bacteriocins against pathogens (Zone of clearance in mm).



Figures 6. Bacteriocin activity at (a)12th h (b) 24th h (c) 36th h (d) 48th h (e) 60th h of incubation period.

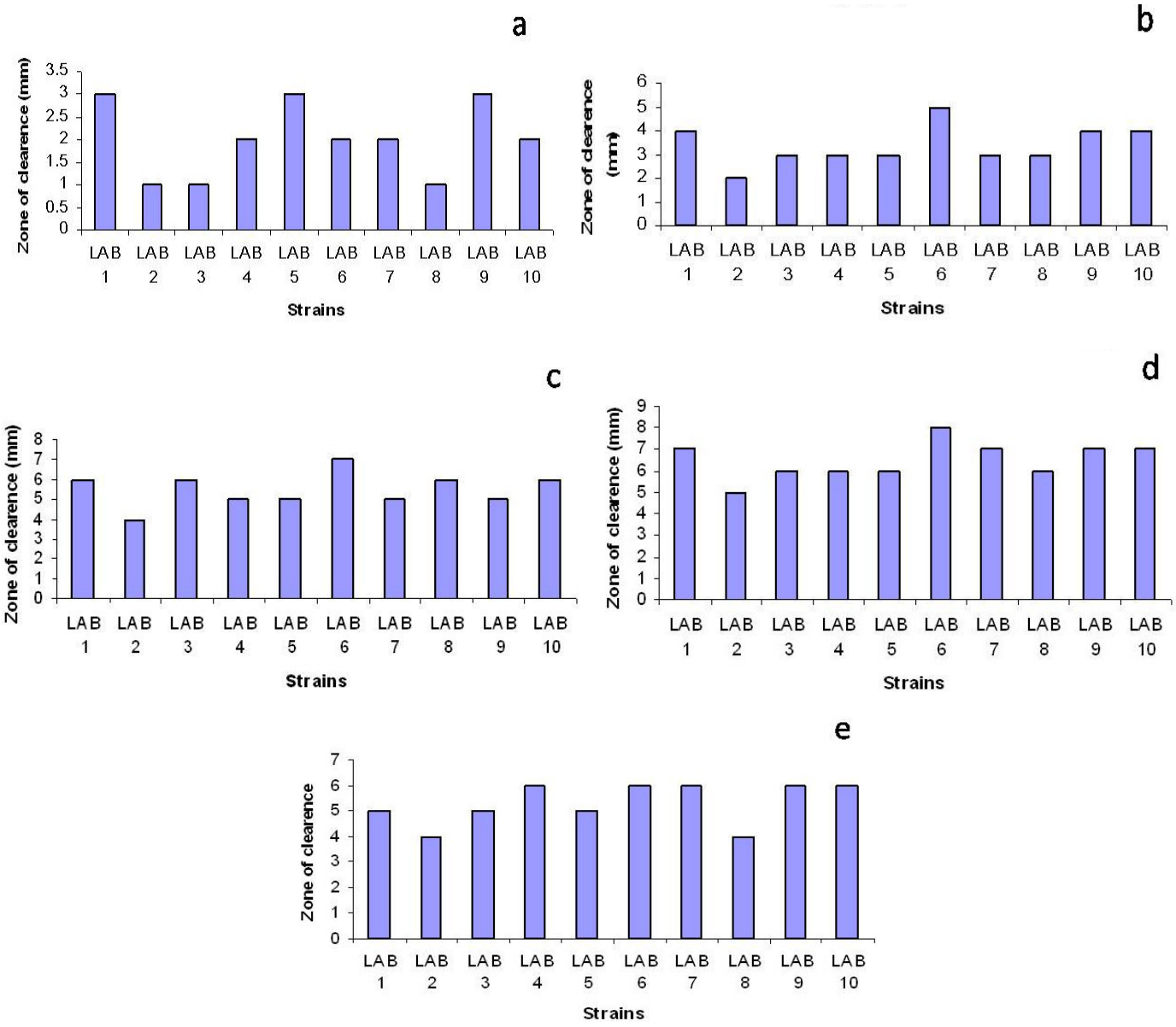


Figures 7. Bacteriocin activity between pH range of (a) 3, (b) 4, (c) 5, (d) 6 and 7 (e).

Recent outbreaks of emerging pathogens such as *L. monocytogenes* that has caused severe illness through food ingestion have prompted the scientific community to focus their studies on the anti-*Listeria* activity of bacteriocins produced by *Lactobacillus* and *Pediococcus* strains (Todorov et al., 1999; Aymerich et al., 2000; Messens et al., 2002).

In the present investigation 100% of the isolated LAB strains were resistance to Ampicillin, Bacitracin, Cephotaxime, Erythromycin, Nalidixic acid, Novobiocin, Penicillin-G and T etracyclin. However only 40% of the strains showed resistance to Vancomycin. Generally many LABs are resistant to antibiotics. This resistance attributes are often intrinsic and non transmissible (Curragh and Collins, 1992). Among antibiotics, Vancomycin is one of the last antibiotics broadly efficacious against clinical infections caused by multidrug resistant pathogens (Johnson et al., 1990).

The effect of incubation period, pH, temperature and salinity of medium on the production of bacteriocin was also investigated in all LAB strains. The pH 6, temperature of 35°C, salinity of 35 ppt, 24 h of incubation was

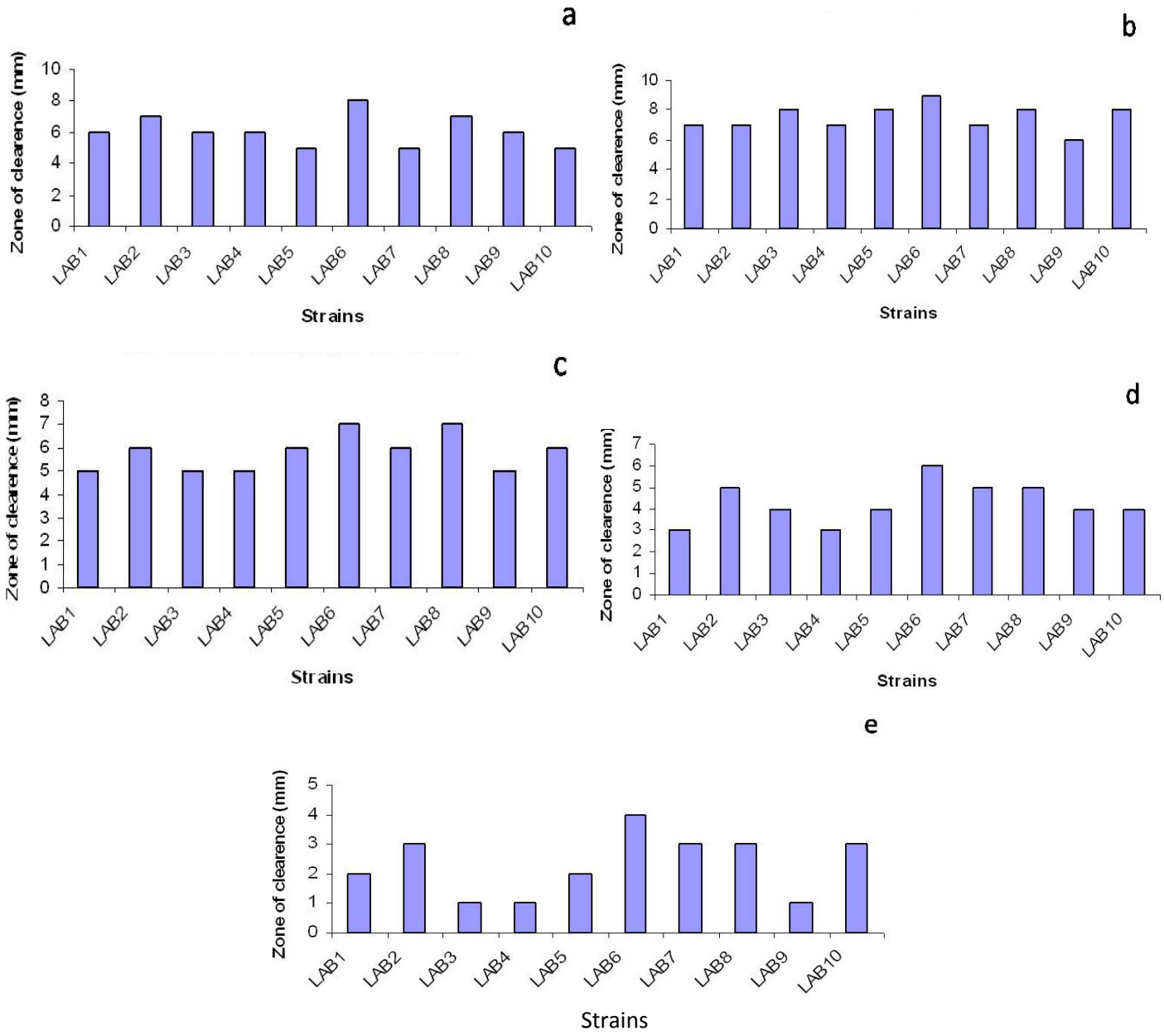


Figures 8. Bacteriocin activity between (a) 20°C, (b) 25°C, (c) 30°C, (d) 35°C, and (e) 40°C of temperature ranges with 5°C interval.

found to be the optimal parameters for the most potential producers of bacteriocin (that is) *L. fermentum*. According to Ogunbanwo et al. (2003) the use of constituted medium at 30°C of incubation temperature, initial pH of 5.5 and 48 to 60 h fostered the best production of bacteriocin by *Lactobacillus brevis* OG1 which seemed to differ from the results of the present study.

L. fermentum produced bacteriocin in high level and the strain showed the maximum inhibitory activity against *Vibrio parahaemolyticus, L. monocytogenes , Listeria* sp*. and S. aureus.* Besides, the productions of bacteriocins having a wide spectrum of antibacterial activity against seafood borne pathogens like *Listeria*, *Clostridium* and even Gram-negative pathogens like *Pseudomonas* and *E. coli* to employ as biopreservatives. Accordingly *L. acidophilus* and *L. casei* (Stiles and Holzapfel, 1997) may be of great interest as probiotics strains because of their ability to adhere to intestinal epithelial cells and being of human origin.

The protein purification was done by methanol and trichloroacetic acid precipitation followed by dialysis against deionised water. Extraction of bacteriocin using organic solvents indicated that bacteriocin was removed from the aqueous phase and could be recovered from the organic phase. This suggested that part of the bacteriocinmolecule has a hydrophobic character, and shares this property with most other bacteriocins (Klaenhammer, 1993). In the present study also Trichloroacetic acid



Figures 9. Bacteriocin activity between (a) 3%, (b) 3.5%, (c) 4%, (d) 4.5% and to (e) 5% of salt concentration with 0.5% of interval.

precipitation at the rate of 70% methanol and 10% tri chloroacetic acid aggregated bacteriocin from cell free broth and the antibacterial activity was also observed which the same in the bacteriocin R from *L. fermentum.*

In the present study FT-IR Spectrum of the protein revealed the presence of peaks at the wave numbers of 3288, 3090, 2958, 2924 and 2849 cm^{-1} which indicated the presence of NH, NH_3, CH_3, CH_2 and CH_2 groups respectively. The wave numbers 1660, 1632, 1589 and 1540 cm^{-1} indicated the presence of their bending mode of amide, methyl, and amide groups respectively. Comparing the results with the nisin standard, the protein in the sample was confirmed as a bacteriocin. The peak at 1546 cm^{-1} indicated a secondary amide was reported (Silverstein et al., 1991; Yakimov et al., 1995). The FT-IR spectrum offered concrete evidence that the substance contained a peptide in its structure. Acidocin 8912 (Tahara et al., 1992) and lactacin B (Barefoot and Klaenhammer, 1984) were reported to be 5.4 and 6.5 kDa, respectively. In the present study the molecular weight determination thorough SDS-PAGE showed that the molecular weight of bacteriocin as 18 kDa protein. Compared to many other studies the molecular weight obtained in the present study seemed to be high.

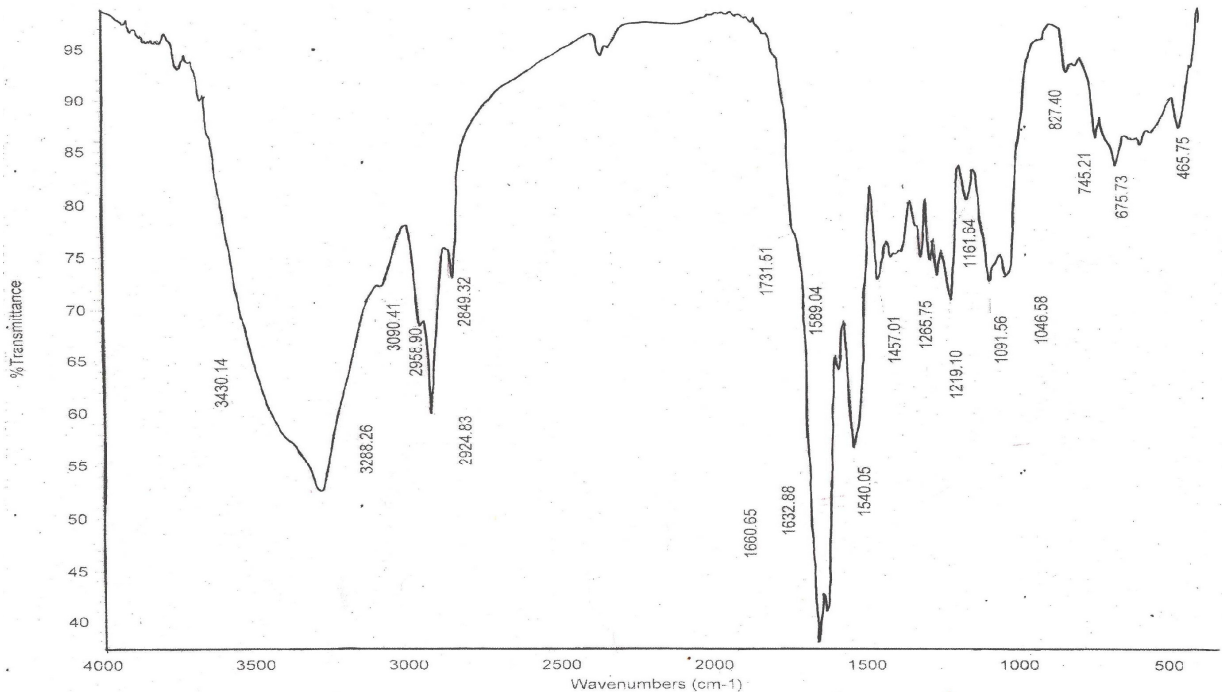
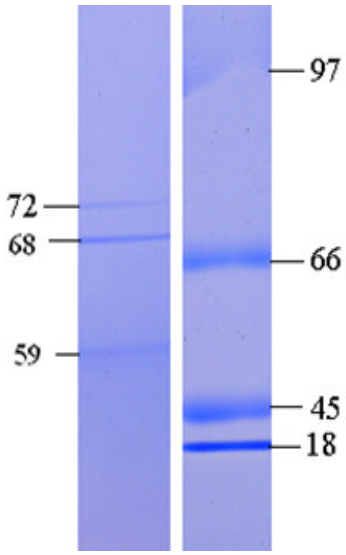


Figure 10. The FT-IR spectrum of Bacteriocin from *L. fermentum.*



Lane 1 Lane 2

Figure 11. SDS-PAGE of partially purified protein Lane 1-Marker, Lane 2-Partial purified Bacteriocin.

Conclusion

The present study showed that the bacteriocin of *L. fer* effects on some clinically important food borne pathogens. This revealed the potential application of bacteriocin produced by *L. fermentum* as a biopreservatives for the improvement of the microbial safety of fermented foods and reduction in food contamination which causes illness to human beings. The study revealed that *Lactobacillus* strains of marine origin are having the potential to use as biopreservatives especially in seafoods. The production of bacteriocin from *L. fermentum*, seems to be ideal for industrial scale production and commercial utilization.

ACKNOWLEDGMENT

We thank Dr. T. Balasubramanian, Dean, Faculty of Marine Sciences, Annamalai University, Parangipettai for providing facilities.

REFERENCES

Aymerich MT, Garriga M, Monfort JM, Nes I, Hugas M (2000). Bacteriocin-producing *lactobacilli* in Spanishstyle fermented sausages: Characterization of bacteriocins. Food Microbiol., 17: 33–45.

Barefoot SF, Klaenhammer TR (1984). Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob. Agents Chemother., 26: 328–334.

Buchanan RE, Gibbons NE, Cowan ST, Holt TG, Liston J, Murry RGE, Niven CF, Ravin AW, Stainer RY (1974). Bergey's Manual of Determinative Bacteriology. Eds: Williams and Wilkins Co: Baltimore.

Castla D, Requena T, Gomez R (1996). Antimicrobial activity of lactic acid bacteria isolated from goats milk and artisanal cheeses: characteristics of a bacteriocin produced by *Lactobacillus curvatus*.

IFPL 105. J. Appl. Bacteriol., 81: 35–41.
Cone DK (1982). A *Lactobacillus* spp. from diseased female rainbow trout, Salmo gairdneri Richardson, in Newfoundland, Canada. J. Fish Dis., 5: 479-485.
Curragh HJ, Collins MA (1992). High levels of spontaneous drug resistance in *Lactobacillus*. J. Appl. Bacteriol., 73: 31-36.
Daeschel MA, Fleming HP (1984). Selection of lactic acid bacteria for use in vegetable fermentations. Food Microbiol., 1: 303–313.
Food and Drug Administration (1988). Nisin preparation: Affirmation of GRAS status as direct human food ingredient. Federal Register., 53: 11247.
Harlander SK (1993). Regulatory aspects of bacteriocin used. In: Bacteriocin of LAB, ed by DG Hoover and LR Steinson San Diego, California. Academic Press Inc.
Holzapfel WH, Geisen R, Schillinger U (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. Inter. J. Food Microbiol., 24: 343-362.
Johnson AP, Uttley AHC, Woodford N (1990). Resistance to Vancomycin and Teicoplanin: an emerging clinical problem. Cl Microbiol Rev., 3: 280-291.
Kandler O, Weiss N (1986). In: Bergey's Manual of Systematic Bacteriology Vol. 2, Baltimore: Williams and Wilkins., pp.1209–1234.
Klaenhammer TR (1993). Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev., 12: 39-86.
Lee YK (2004). Microbial Biotechnology principles and applications., pp.124.
Lowry OH, Rosenberg HJ, Farr AL, Randall RF (1951). Protein measurement with Foiln phenol reagent. J. Bio. Chem., 193: 265-275.
Merritt WM, Dean JA, Settle FA (1986). Instrumental method of analysis. CBS, New Delhi.
Messens W, Neysens P, Vansieleghem W, Vanderhoeven J, De Vuyst L (2002). Modeling Growth and Bacteriocin Production by *Lactobacillus amylovorus* DCE 471 in Response to Temperature and pH Values Used for Sourdough Fermentations. Appl. Env. Microbiol., 68: 1431-1435.
Navarro L, Zarazaga M, Saenz J, Ruiz-Larrea F, Torres C (2000). Bacteriocin production by lactic acid bacteria isolated from Rioja red wines. J. Appl. Microbiol., 88: 44–51.
Ogden K, Waites MJ, Hammond JRM (1985). Nisin and brewing. J. Inst. Brew., 94: 233–238.
Ogunbanwo ST, Sanni AI, Onilude AA (2003). Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr. J. Biotechnol., 2: 219-227.
Okafor N, Nzeako BC (1985). Microbial flora of fresh and smoked fish from Nigerian fresh waters. Food Microbiol., 2: 71-75.
Reinheimer JA, Demkow MR, Candioti MC (1990). Inhibition of coliform bacgeria by lactic cultures. Austr. J. Dairy Technol., 5-9.
Roberts RM, Gilbert JC, Waldb BR, Wingrove AS (1985). Evidence for cyclic bromonium ion transfer in electrophilic bromination of alkenes: Reaction of ω-alkenyl glycosides with aqueous N-bromosuccinimide. Mod. Exp. Organic Chem., 52: 7663-7678.
Ross RP, Galvin M, McAuliffe O, Morgan SM, Ryan MP, Twomey DP, Meaney WJ, Hill C (1999). Developing applications for lactococcal bacteriocins. Antonie van Leeuwenhoek., 76: 337–346.
Silverstein RM, Bassler GC, Morrill TC (1991). Spectrometric identification of organic compounds, 5th ed., John Wiley and Sons, New York., 512.
Stiles ME, Holzaphel WH (1997). Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol., 36: 1 –29.
Tagg JR, Dajani AS, Wannamaker LW (1976). Bacteriocins of Gram positive bacteria. Bacteriol. Rev., 40: 722-756.
Tahara T, Kanatani K, Yoshida K, Miura H, Sakamoto M, Oshimura M (1992). Purification and some properties of acidocin 8912, a novel bacteriocin produced by *Lactobacillus acidophilus* TK8912. Biosci. Biotechnol. Biochem., 56: 1212–1215.
Todorov S, Onno B, Sorokine O, Chobert JM, Ivanova I, Dousset X (1999). Detection and characterization of a novel antibacterial substance produced *by Lactobacillus plantarum* ST 31 isolated from sourdough. Int. J. Food Microbiol., 48: 167-177.
Yakimov MM, Timmis KN, Wray V, Fredrickson HL (1995). Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. Appl. Environ. Microbiol., 61: 1706–1713.