ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(2):342-347

ORIGINAL RESEARCH ARTICLE

Antibacterial Potential of Lactic Acid Bacteria and Its Metabolites against Food Borne Pathogens

P.K. Senthilkumar*[1], D.Reetha[1], N.Ramya[2] and D.Stella[1]

[1]*Department of Microbiology, Annamalai University. Chidambaram – 608 002, Tamil Nadu, India*
[2]*Department of Microbiology, Shree Raghavendra Arts and Science College, Keezhamoongiladi, Chidambaram – 608 102, Tamil Nadu, India*

Received 05 Dec 2011; Revised 15 Mar 2012; Accepted 24 Mar 2012

ABSTRACT

The nature of fermented products is different from one region to another. This is depending on the local indigenous microflora, which in turn reflected the climate condition of the area. Mankind has exploited lactic acid bacteria for thousands of years for the production of fermented foods because of their ability to produce desirable changes in taste, flavor and texture as well as inhibit pathogenic and spoilage microorganism. In this present study, the antibacterial potential of lactic acid bacteria and its metabolites was investigated against food borne pathogens. The lactic acid bacteria are isolated by pour plate method obtained from traditional fermented food products such as Curd, Buttermilk, Cheese and Yoghurt. Four lactic acid bacterial isolates *viz*., *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Pediococcus acidilactici* were identified. The antimicrobial activity of the purified lactic acid bacterial isolates against pathogenic microorganisms *viz*., *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus* were performed by Agar Well Diffusion method and the lactic acid bacterial isolates have the inhibitory activity against bacterial pathogens.

Key words: Milk products, Lactic acid bacteria, Food borne pathogens, Antibacterial activity and Well diffusion method.

1. INTRODUCTION

Lactic acid bacteria have been used for thousands of years in food and alcoholic fermentations. Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations. The bacteriocins from the lactic acid bacterial isolates generally recognized as safe (GRAS) lactic acid bacteria (LAB) have arisen a great deal of attention as a novel approach to control pathogens in food-stuffs. Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria. The antimicrobial effect of lactic acid bacteria has been appreciated by man for more than 10000 years and has enabled him to extend the shelf life of many foods through fermentation processes [1].

Bioactive compounds from plant by-products act as good preservatives. Antioxidants poly phenolic fraction from plant by-product are possible alternatives to synthetic antimicrobial agent can be easily degraded by living organisms. They are based on renewable raw materials (Protein, oils) and constitute eco-friendly alternatives to synthetic antimicrobial surfactants. Some of the microbes are best sources of bio-active compounds as they synthesize as secondary metabolites for their self defense against other competitive microorganisms [2].

The lactic acid bacteria (LAB) comprise a clade of Gram positive, acid tolerant, non-sporulation, non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics. These bacteria are usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end product of carbohydrate fermentation [3]. This trait has historically linked LAB with food fermentation as acidification inhibits the growth of spoilage agents. The LAB group comprises the genera *Lactobacillus, Streptococcus, Lactococus, Leuconostoc, Pediococcus, Aerococcus, Alloicoccus, Dolosigranulum, Enterococcus,*

***Corresponding Author:** P.K. Senthilkumar**, Email:** mailtopks@yahoo.com

Globicatella, Lactospaera, Oenococcus, Carnobacterium, Tetragenococcus, Vagoccus and Weissella. Historically, the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* form the core of the LAB group [4].

Members of these genera *Lactobacillus* plays an essential role in the fermentation of food and feed. The most important characteristics of the lactic acid bacteria are their ability to ferment sugars to lactic acid. This may desirable in making products and these organisms have been isolated and screened by using fermented foods such as curd, buttermilk, cheese and yoghurt. Different antimicrobials such as lactic acid, acetic acid, hydrogen peroxide, carbon-di-oxide and bacteriocins produced by these bacteria can inhibit pathogenic and spoilage microorganisms extending the shelf-life and enhancing the safety of food products [5].

Bacteriocins are generally defined as extracellular released peptide or protein that shows a bactericidal activity against more distantly related species. The inhibitory spectrum of some bacteriocins also include food spoilage and for food borne pathogenic microorganisms. The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative dates back of to the first half of last century but research on bacteriocin of lactic acid bacteria has expanded in the last two decades, searching for novel bacteriocin producing strains from dairy, meat and plant products, as well as traditional fermented products. Among the gram positive bacteria, bacteriocins produced by many lactic acid bacteria used in food fermentation and dairy products, including strains in the genera *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc*. In USA, only nisin produced by *Lactobacillus lactis* is permitted as a food preservative [6].

2. MATERIALS AND METHODS

2.1. Collection of milk product samples

The milk product samples *viz*., Curd, Buttermilk, Cheese and Yoghurt were collected individually in suitable sterile containers from the place of their availability and brought to the laboratory for further study.

2.2. Isolation of lactic acid bacteria

The Lactic acid bacteria were isolated from Curd, Buttermilk, Cheese, and Yoghurt by using the MRS Agar medium by Pour plate method. Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e*., By the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests.

2.3. Maintenance of lactic acid bacterial isolates

The Lactic acid bacterial isolates were maintained by sub-culturing routinely in Nutrient Agar slants at every 15 days and stored in refrigerator at 4^0C.

2.4. Preparation of bacteriocin from lactic acid bacterial isolates

After primary screening potential strains were inoculated in MRS broth of 30 ml and kept in a shaker at 150 rpm at 38°C. After 24 hours cultures were centrifuged at 3000 rpm for 20 minutes. Supernatant was decanted into sterile test tubes, adjust to pH 6.5-7.0 with a NaOH to remove organic acid effect. H_2O_2 was neutralized by the addition of catalase from bovine liver at 200µ/ml. The mixture of the supernatant was filtered with a 0.2µm Millipore filter membrane [7].

2.5. Partial purification of Bacteriocin by Ammonium sulphate precipitation method

To the filtrate, 80% Ammonium sulphate precipitation was done (i.e) 14.52 g of Ammonium sulphate was added to dissolve in the culture filtrate and kept in refrigeration overnight for precipitation. This precipitate was dissolved in 2ml of deionized water and dialysed through a 100 molecular weight cut-off dialysis membrane for 48 hours and tested for antimicrobial activity.

2.6. Partial purification of Bacteriocin by solvent extraction method

To the filtrate, obtained 70% methanol and 10% Trichloroacetic acid were added in equal proportion and were kept for 48 hours to precipitate at room temperature. This precipitate was dissolved in 2 ml of deionized water and dialysed through a 1000 molecular weight cut-off dialysis membrane for 48 hours and tested for its antimicrobial activity.

2.7. Antimicrobial activity method

2.7.1. Test Organisms

Four pathogenic bacteria *viz*., *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus* were used during the present study and were obtained from SGS India laboratories – Thoraipakkam, Chennai – 96. The cultures were sub-cultured and maintained on Nutrient agar slants and stored at 4°C.

2.7.2. Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards.

2.7.3. Well diffusion method

Sterile Muller Hinton Agar plates were seeded with the indicator organisms by swabbing the entire surface of the culture plates. The holes 6mm in diameter were aseptically punched out of the agar plates, and then 250 – 1000ml modified MRS broth cultures of the putative organisms were separately introduced into the holes or spotted on to the surfaces of pre poured culture plates and incubated aerobically at 35°C for 24 hours. After overnight incubation, inhibition observed by clear zones extending laterally from the border of the putative isolates were noted and recorded in mm diameters.

3. RESULTS AND DISCUSSION

LAB was first discovered by Scheele from sour Buttermilk. Pasteur discovered in 1857, that the souring of Buttermilk was caused by the microorganisms. Lactic acid was first produced commercially by M/S Clinton processing company Clinton, Lowa (USA). The universal standard literature accepted for the isolation and characterization of any microorganisms in general and lactic acid bacteria in particular is the Bergey's manual of systemic bacteriology. Lactic acid bacteria found to be associated with various substances *via*., fermented silages. Isolation and screening of microorganisms from naturally occurring processes have always been the most powerful means for obtaining useful cultures for scientific and commercial purposes. This certainly holds true for Lactic acid bacteria (LAB), which are used throughout the world for manufacture of a wide variety of traditional fermented foods.

Marilingappa Hamuna and Kadirvelu Jeevarathnam [8] were isolated and characterized lactic acid bacteria from traditional fermented foods such as appam, batter and Yoghurt. Two *Lactobacillus* were isolated and used for the extraction of bacteriocin and used as biopreservatives. In the present study, the lactic acid bacteria were isolated from traditional fermented foods such as Curd, Buttermilk, Cheese and Yoghurt and the isolates were *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Pediococcus acidilactici*. In this study, the lactic acid bacteria were characterized from the traditional fermented foods. The lactic acid bacteria were enumerated by pour plate and spread plate method. By the method of Gram staining the morphological characterization were observed under microscope.

The conversion of carbohydrates to lactic acid may be well considered as the most important fermentation process employed in food technology. However the microbiology and biochemical foundations of lactic fermentation become know only in the course of the last century. As a result, it becomes possible to control this process scientifically and to apply it to modern food technology [9]. The isolated strains were identified on basis of their morphological, physiological and biochemical characteristics, the lactic acid isomer produced, the ability to ferment sugars and 16S rDNA analysis. The genus was *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, *Leuconostoc* and *Lactococcus* [10].

After morphological identification the identified strains were treated for physiological, carbohydrate and biochemical test. In physiological various temperatures *viz*., 15, 37 and 45°C in broth were determined by visual turbidity after 72 hours incubation and different pH 4.4 and 8.6 were given for their growth. In carbohydrate various sugars Arabinose, Lactose, Maltose, Mannitol, Mannose, Sucrose and Xylose were given to the isolated strains to conform the particular genus and the biochemical test also analyzed for the isolated strains and Oxidase test given the positive result.

Lactobacillus bulgaricus, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Streptococcus lactis* and *Streptococcus feacicum* had antimicrobial activity. The most sensitivity strains are *Staphylococcus aureus* was set as a target microorganism. The cultures of *Streptococcus facium* and *Lactobacillus plantarum* gave the most intense antimicrobial activity by adding $CaCO_3$ to the medium (to bind accumulated lactic acid) increased the antibiotic activity of the lactic acid bacteria [11].

The bacteriocin of *Lactobacillus acidophilus* isolated from the milk product which was purified by Ammonium sulphate precipitation was tested for antimicrobial activity. The test strains are *Vibrio cholerae*, *Shigella sonnei, Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 23 mm, in *Shigella sonnei* the inhibition zone was 21 mm, in *Bacillus cereus* the inhibition zone was 20 mm and in *Staphylococcus aureus* the inhibition zone was 22 mm. among these results *Vibrio cholerae* was the best result that was 23 mm and *Bacillus cereus* shows the lowest result that was 20 mm (**Fig 1**). The antibacterial activity of *Lactobacillus bulgaricus*. The test strains are *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholera*, the inhibition zone was 22 mm, in *Shigella sonnei* the inhibition zone was 20 mm. In *Bacillus*

cereus, the inhibition zone was 18 mm and in *Staphylococcus aureus* the inhibition zone was 20 mm. Among these, *Vibrio cholerae* shows the best result that was 22 mm, and *Bacillus cereus* showed the lowest result that was 18mm (**Fig 2**).

Fig 1: Antimicrobial activity of crude bacteriocin from *Lactobacillus acidophilus* by ammonium sulphate precipitation method

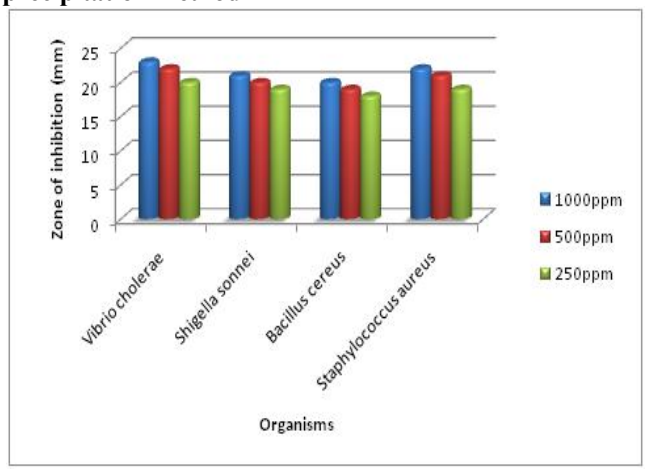
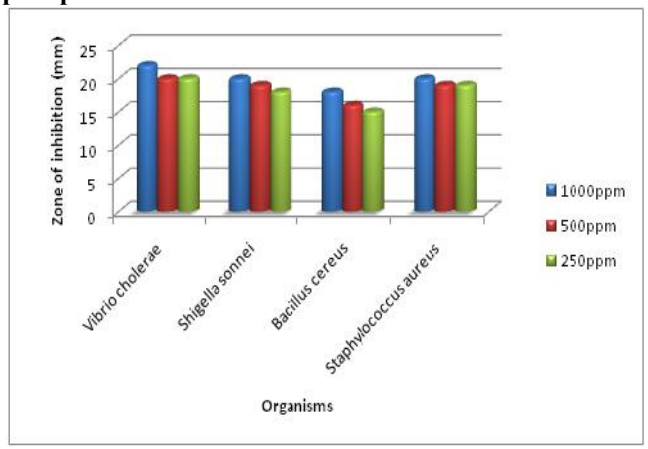


Fig 2: Antimicrobial activity of crude bacteriocin from *Lactobacillus bulgaricus* by ammonium sulphate precipitation method



The antibacterial activity of bacteriocin obtained and purified by Ammonium sulphate precipitation from *Streptococcus thermophilus* was investigated. The test strains are *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 20 mm, in *Shigella sonnei* the inhibition zone was 19 mm, *in Bacillus cereus* the inhibition zone was 18 mm and in *Staphylococcus aureus* showed the best result that is 22 mm and *Bacillus cereus* showed the lowest result that was 18 mm (**Fig 3**). The antibacterial activity of *Pediococcus acidilactici*. The test strains are *Vibrio cholerae*, *Shigella sonnei, Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae* the inhibition zone was 21 mm, in *Shigella sonnei*, the inhibition zone was 20mm, in *Bacillus cereus* the inhibition zone was 18 mm and in *Staphylococcus aureus* the inhibition zone was 20 mm. Among these results, *Vibrio cholerae* showed the best result that was 21 mm, and *Bacillus cereus* showed the lowest result that was 18mm (**Fig 4**).

Lactobacillus was isolated from MRS and LAPTg (Lactose Propyl Thiogalactosidase) broth and their antimicrobial activity was tested against the test strains using the disc diffusion method. All isolated, except *Escherichia coli* 0157:H7, showed additional 3 to 4 mm of inhibition zone. This was < 3 mm for *Escherichia coli* 0157: H7. *Lactobacillus* isolates were the inhibitor to the test strains followed by *Pediococcus*, *Streptococcus* and *Leconostoc*. *Escherichia coli* 0157:H7 was the least sensitive in all cased [12]. The antimicrobial activity was determined for the identified strains such as *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Pediococcus acidilactici*. Bacteriocins activity was precipitated by ammonium sulfate extraction method showed maximum activity compared to solvent extraction method.

The (**Fig 5**) showed the results of antibacterial activity of *Lactobacillus acidophilus* which was obtained by solvent extraction method. The test strains are *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 21 mm, in *Shigella sonnei*, the inhibition zone was 19 mm, in *Bacillus cereus* the inhibition zone was 18 mm and in *Staphylococcus aureus* the inhibition zone was 19 mm. Among these results *Vibrio cholerae* showed the best result that is 21 mm, and *Bacillus cereus* showed the lowest result that is 18mm. The (**Fig 6**) showed the results of antimicrobial activity *Lactobacillus bulgaricus* which was obtained by solvent extraction method. The test strains are *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 20 mm, in *Shigella sonnei* the inhibition zone was 19 mm, in *Bacillus cereus* the inhibition zone was 16 mm and in *Staphylococcus aureus* the inhibition zone was 19 mm. Among these results, *Vibrio cholerae* shows the best result that is 20 mm, and *Bacillus cereus* shows the lowest result that was 16mm.

The antibacterial activity of *Streptococcus thermophilus* which was obtained by solvent extraction method was showed in (**Fig 7**). The test strains are *Vibrio cholerae*, *Shigella sonnei*, *Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 19 mm, in *Shigella sonnei* the inhibition zone is 18 mm, in *Bacillus cereus* the inhibition zone was 15 mm and in *Staphylococcus aureus* the inhibition zone was 18 mm. Among these results, *Vibrio cholerae* shows the best result that was 20 mm, and

Bacillus cereus showed the lowest result that was 15mm. The results of antibacterial activity of *Pediococcus acidilactici* which was obtained by solvent extraction method was showed in (**Fig 8**). The test strains are *Vibrio cholerae, Shigella sonnei, Bacillus cereus* and *Staphylococcus aureus*. In *Vibrio cholerae*, the inhibition zone was 19 mm, in *Shigella sonnei* the inhibition zone was 18 mm, in *Bacillus cereus* the inhibition zone was 14 mm and in *Staphylococcus aureus* the inhibition zone was 18 mm. Among these results *Vibrio cholerae* shows the best result that was 19 mm, and *Bacillus cereus* shows the lowest result that was 14mm. From this study, it is concluded that the bacteriocin produced by Lactic acid bacteria can be applied both in, food industry and medical sector. They can be used in cheese, fermented sausage, beer, etc., yielding food quality and food safety advantages. Bacteriocins of Lactic acid bacteria can play a role in human gastrointestinal track, hence contributing to human health.

Fig 3: Antimicrobial activity of crude bacteriocin from *Streptococcus thermophilus* by ammonium sulphate precipitation method

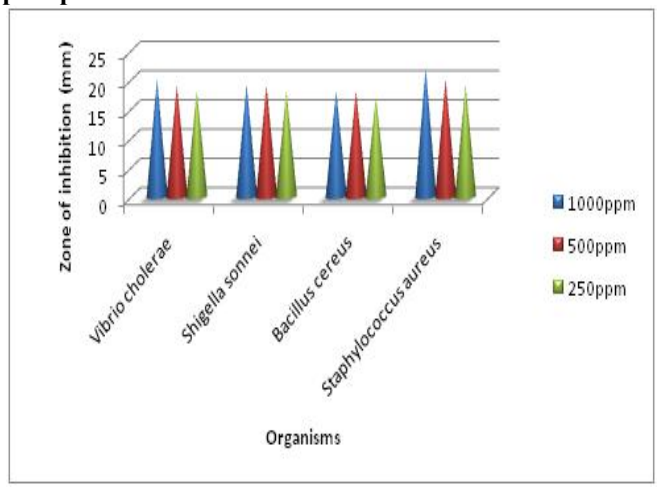


Fig 4: Antimicrobial activity of crude bacteriocin from *Pediococcus acidilactici* by ammonium sulphate precipitation method

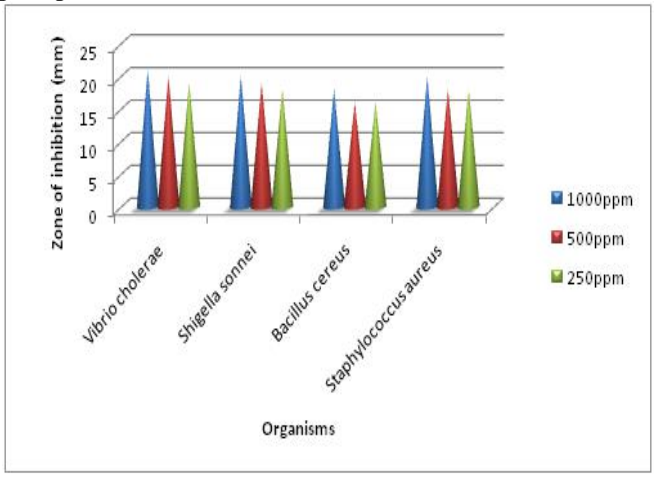


Fig 5: Antimicrobial activity of crude bacteriocin from *Lactobacillus acidophilus* by solvent extraction method

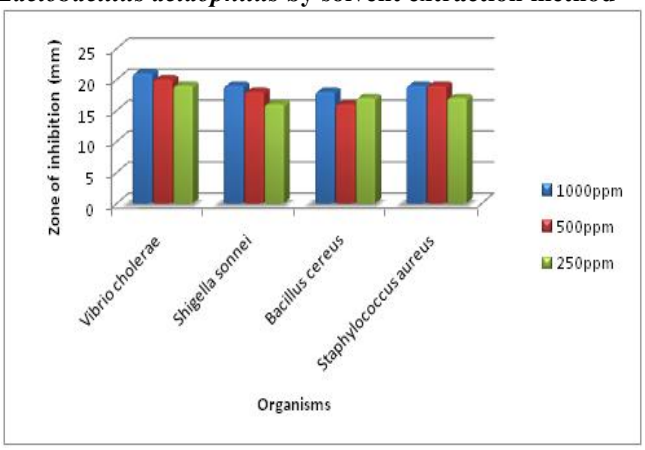


Fig 6: Antimicrobial activity of crude bacteriocin from *Lactobacillus bulgaricus* by solvent extraction method

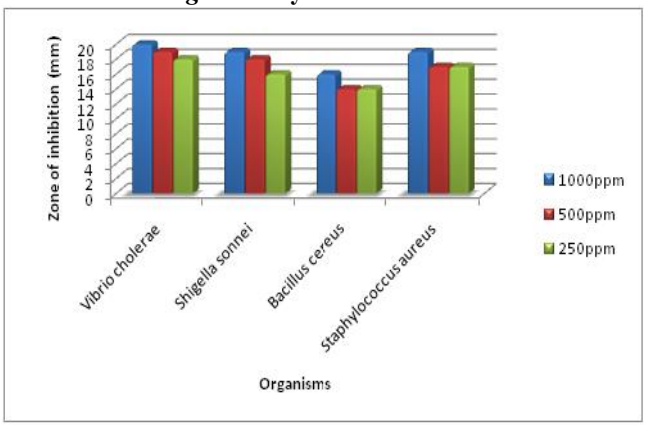


Fig 7: Antimicrobial activity of crude bacteriocin from *Streptococcus thermophilus* by solvent extraction method

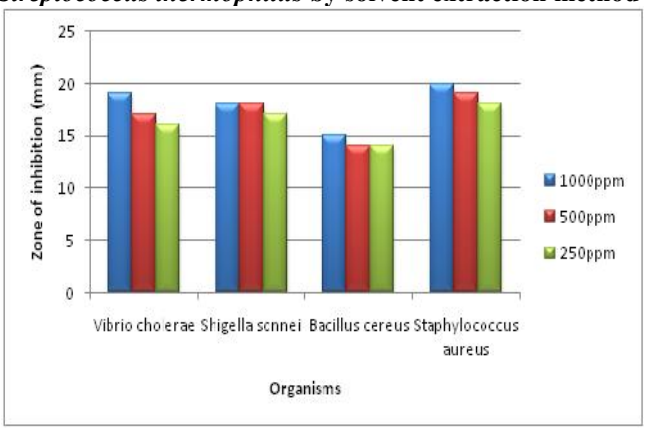
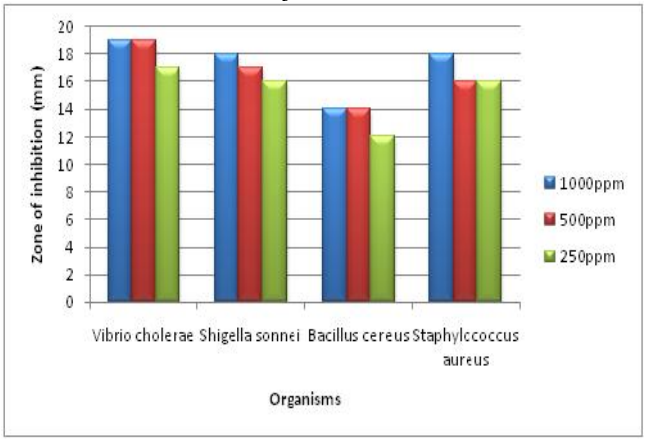


Fig 8: Antimicrobial activity of crude bacteriocin from *Pediococcus acidilactici* by solvent extraction method



REFERENCES

1. Padmanabha Reddy, V, M.D. Christopher and I. Sankara Reddy. 2006. Antimicrobial activity of *Lactobacillus acidophilus*. *J. Veterinary and Animal Sciences*, 2(4): 142-144.
2. Michel Bakar Diop, Robin Dubois-Dauphin, Emmanuel Tine, Abib Ngom, Jacqueline Destain, Philippe Thonart, 2007. Bacteriocin producers from traditional food products. *Biotechnol. Agron. Soc. Environ*., 11:275-281.
3. Nowroozi J, M. Mirzaii and M. Norouzi. 2004. Study of *Lactobacillus* as probiotic bacteria. *Iranian J. Public Health*, 33: 1-7.
4. Ko, S.H and C. Ahn. 2000. Bacteriocin production by *Lactococcus lactis* KCA 2386 isolated from white kimchi. *Food Sci. Biotechnol*., 9: 263-269.
5. Gobben, G.J, I. Cain-Joe, V.A. Kitzen, I.C. Boels, F. Boer, J.A.M. De Bont. 1998. Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii* sub sp. *bulgaricus* NCFB 2772 with a simplified defined medium. *Appl. Environ. Microbial*., 64(4): 1333-1337.
6. Eva Rodriguez, Juan L. Arques, Manuel Nunez, Pilar Gaya and Margarita Medina. 2005. Combined effect of High-Pressure treatments and bacteriocin producing LAB on inactivation of *E. coli* in raw milk cheese. *Applied Environmental Microbiology*, 3399-3404.
7. Adetunji, V.O and G.O. Adegoke, 2007. Bacteriocin and cellulose production by Lactic acid bacteria isolated from West African soft Cheese. *African J. Biotechnol*., 6: 2616-2619.
8. Marilingappa Hamuna and Kadirvelu Jeevarathnam. 2004. Mode of action of Lactococcin B, a thiol activated bacteriocin from *Lactococcus lactis. Appl. Environ. Microbial*., 59(4) 1041-1048.
9. Kandler O. 1983. Carbohydrates metabolism in Lactic acid bacteria. *Antonic Van Leewenhook*, 49: 209-224.
10. Harun – Ur – Rashid, Kaname Togo and Minoru Ueda, Taku Miyamoto. 2007. Identification and characterization of dominant Lactic acid bacteria isolated from traditional fermented milk Dahi in Bangaladesh. *J. Microbial biotechnol*., 23 125-133.
11. Frederic Lerory and Luc De Vuyst. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends food sci. Technol*., 15: 67-68.
12. Girum Tadesse, Eden Ephraim and Mogessie Ashenafr. 2003. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamitta traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. *International Journal of Food Safety*, 5: 13-20.