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Screening of lactic acid bacteria for their antibacterial activity against milk borne pathogens

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Abstract
Lactic acid bacteria were isolated from naturally fermenting food products (curd, cheese, yoghurt, butter, butter milk), by using specific medium. Totally 120 LAB were isolated and identified as *Lactobacillus fermentum*, *Lactobacillus delbrueckii* sub sp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Leuconostoc*, *Streptococcus thermophilus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactococcus lactis.* They can be broadly defined as Gram positive, anaerobic, microaerophilic or aerotolerant bacteria, either rod or coccus, catalase negative and fastidious in their growth. They were screened for their ability to produce bacteriocins and inhibit five pathogenic organisms *viz*., *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Staphylococcus aureus* and *Listeria monocytogenes.* The influence of antibacterial activity were obtained by using well diffusion assay and disc diffusion method. Among nine LAB isolates *Lactobacillus delbrueckii* sub sp. *bulgaricus* was found relatively dominating species of dairy products. These obtained results revealed the possibility of using bacteriocins of LAB as food bio preservatives to control food spoilage and pathogenic bacteria.

Keywords: Lactic acid bacteria, Pathogenic bacteria, Well diffusion assay and Disc diffusion method.

Introduction
Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, catalase negative cocci or rods which produce lactic acid as major end products from fermentation of carbohydrates. They are most widely used bacteria as starter cultures for the industrial processing of fermented dairy, meat, vegetable and cereal products. Numerous strains of Lactic Acid Bacteria (LAB) associated with food systems produce bacteriocin defined as proteinaceous substance that exhibit bactericidal activity against closely related organisms (Cassia Regina Nespolo and Adriano Brandelli, 2010) [6]. Many chemicals are being used for inactivation of food borne pathogens. The most important contribution of these bacteria to fermented products is to preserve the nutritive qualities of the raw material and inhibit the growth of spoilage and pathogenic bacteria (Matilla *et al.*, 1999) [16].

LAB can produce antimicrobial substances with the capacity to inhibit the growth of pathogenic and spoilage micro-organisms. Organic acids, hydrogen peroxide, diacetyl and bacteriocins are included among these compounds (Daeschel, 1989) [8]. Bacteriocins are special interest due to their potential uses as natural preservatives. Bacteriocins are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria (Tagg *et al.*, 1976) [19]. Nisin is the most extensively characterized bacteriocin of antimicrobial proteins produced by LAB (Jack *et al.*, 1991) [13]. Nisin is secreted by *Lactococcus lactis* has been used as a food preservative for more than 50 years and no significant bacterial resistance against nisin has been reported.

The ability of the Lactic acid bacteria to prevent and cure a variety of diseases has lead to the coining of the term probiotics or pro-life. LAB including microorganisms safe when added to food because it's not toxic and does not produce toxin, so-called food grade microorganisms and are designated as "Generally Recognized as Safe" (GRAS). 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.

The present research is a preliminary attempt to ascertain bacteriocin production by different strains of lactic acid bacteria and their efficacy against the pathogenic bacteria. Consumer

demand plays a major role in the modification of our food supply and their demand is currently driven towards foods that are ''natural'' and free of additives, but still safe and convenient to use (Rhodehamel, 1992) [18]. In general, several of the organisms isolated from a food product will be able to produce spoilage metabolites when allowed unlimited growth. The aim of this study was the screening of antibacterial activities of lactic acid bacteria from fermented dairy products.

Materials and Methods

Collection of samples

Five different samples of Curd, Cheese, Yoghurt, Butter, and Butter Milk, were collected randomly from local markets of Villupuram district, Tamil Nadu, India and the samples were aseptically brought to the laboratory of Division of Microbiology for further analysis.

Isolation and identification of LAB

Serially diluted samples of the fermented milk products (Curd, Cheese, Yoghurt, Butter and Butter Milk,) were inoculated on to De Man Rogosa Sharpe (MRS), and M17 agar medium. After inoculation the samples were incubated at 37 ˚C for 24-48 hours. Colonies that appeared on the agar plates were counted using colony counter and the results were recorded as colony forming units per milliliter (cfu/ml) or gramme (cfu/g) of sample. 20-30 colonies per sample were randomly taken from both M17 and MRS (30 °C and 45 °C) agar plates corresponding to the highest dilution at which growth occurred. Cell morphology of all isolates of LAB was determined by microscopy. After Microscopic observations, the colonies were sub-cultured to purity on MRS or M17 medium for rods and cocci, respectively. Pure isolates were characterized based on morphology, physiology and biochemical tests (Fawole and Oso, 1998) [10], (Oyeleke and Manga, 2008) [17].

Standard strains

The standard strains used in this study were *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Staphylococcus aureus*, and *Listeria monocytogenes*, the strains were obtained from MTCC.

Preparation of cell-free supernatants

LAB strains to be tested for antimicrobial activity were incubated in MRS broth for 48 hrs at 37 °C. Bacterial cells were removed by centrifuging the culture at 10000 rpm for 15 min at 4 °C. The pH values of supernatant were adjusted to pH 6.5-7.0 by the addition of 1 N NaOH, the supernatant were membrane filtered (Millipore, 0.22µm) and stored at 4 °C.

Well diffusion assay

The antimicrobial activity of the isolated LAB (cell free filtrate) against the pathogenic bacteria (*Escherichia coli*, *Salmonella typhi*, *Shigella*, *Staphylococcus aureus*, and *Listeria monocytogenes)*. The test bacteria were incubated in Nutrient broth at appropriate temperature for 24 hrs. Petridishes containing 20 ml of Muller Hinton agar were prepared previously and inoculated with 0.1 ml of 24 hrs broth culture of pathogenic bacteria. Once solidified the dishes were stored for 2 hrs in a refrigerator. Four wells were made and filled using different concentration like 25µl, 50 µl, 75 µl, 100 µl of cell-free filtrate and the petridishes were incubated at 37 °C for 24 hrs. Then the diameter of the inhibition zone was measured with calipers in mm. The antimicrobial activity was determined by measuring the clear zone around the wells.

Disc diffusion method

Disc diffusion method proposed by Bauer (Bauer *et al.*, 1996) [5] was followed for susceptibility test. The surface of a plate containing Mueller Hinton agar (Hi media) was swabbed with tryptic soy broth containing indicator strains of Gram – positive (*Listeria monocytogenes, Staphylococcus aureus*) and Gram – negative (*Escherichia coli, Salmonella typhi*, *Shigella*) organisms. A paper disc (diameter, 6 mm) was soaked with filtered supernatant of lactic acid bacteria and the paper disc were placed on the surface of each plate. The plates were incubated at 37 °C for 24 hrs. Each plate was examined for clear inhibition zones around the discs.

Results and Discussion

Totally 9 lactic acid bacteria were identified from the 120 isolates obtained from Curd, Cheese, Yoghurt, Butter, and Butter Milk. The species identified were, *Lactobacillus fermentum*, *Lactobacillus delbrueckii* sub sp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Leuconostoc*, *Streptococcus thermophilus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus and Lactococcus lactis*. Among the nine LAB, *Lactobacillus delbrueckii* sub sp. *bulgaricus* was found abundantly dominating species of dairy products. The isolated Lactic Acid Bacteria (LAB) from dairy products on MRS and M17 media depending on their positive Gram stain, negative for indole, catalase, motility and spore forming with rods shape, these characters were according to (Axelsson, 1998) [3]. LAB particularly those belonging to beneficial and non- pathogenic bacteria. They also play an essential role in dairy industry due to the tremendous level of human consumption of several important fermented products, mainly cheese and acidified or fermented milks (Farkye, 2004) [11].

The identified Lactic acid bacteria show antagonistic activity against five food pathogens by production of bacteriocins. Maximum production of bacteriocin was obtained in MRS broth containing at least 1.2% glucose or xylose. Also, MRS medium with 1% NaCl found that the antibacterial activity increased. The antibacterial activity of zone inhibition of five food pathogens against supernatant of LAB by well diffusion assay and disc diffusion method was measured. The extracts of nine-isolated LAB gave zone of inhibition against the indicator food pathogenic strains such as *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Staphylococcus aureus* and *Listeria monocytogenes.*

Among the nine LAB, the strongest diameter zones (14 -16 mm) obtained with the extract of *Lactobacillus delbrueckii* sub sp. *bulgaricus, Leuconostoc* and *Lactobacillus acidophilus* against *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Staphylococcus aureus* and *Listeria monocytogenes*. The intermediate diameter zones (10-13 mm) of LAB are *Lactobacillus fermentum* and *Pediococcus pentosaceus* and the smallest or weak diameter zones (6-9 mm) of LAB are *Lactobacillus plantarum* and *Streptococcus thermophilus.* Resistant pathogen growth was obtained with the extracts of *Pediococcus acidilactici* and *Lactococcus lactis* (table 1 to table 5). The strain which showed the largest and smallest zone of growth inhibition was selected for further strain development studies.

Lactic acid bacteria were the predominant microbial group in fermented dairy products, which is playing an important role in fermentation process. All of these essential activities contribute to the texture, flavor and frequently attributes of fermented products (El-Soda *et al*., 2003), (Abd El Gawad *et al*., 2010) [1]. Nine lactic acid bacteria were selected and allowed for antibacterial activity against five different bacterial pathogens which are usually present in food and can cause food borne illness in human being. All LAB were subjected to inhibitory activity test using agar well diffusion method and disc diffusion method. The strongest antagonistic activity of *Lactobacillus delbrueckii* sub sp. *bulgaricus* against selected pathogens. *Lactobacillus* are important in food fermentation as well as the production of bacteriocin (Adam and Moss, 1995) [2]. Bacteriocin are antimicrobial agent produced by bacteria which are active against closely related bacteria as claimed by Klaenhammer (Klaenhammer, 1993) [14].

Earlier (E1-shafei *et al.*, 2000) [9] screened one hundred lactic acid bacterial strains isolated from traditional fermented foods (yoghurt, milk, sour cream, sour dough, cheese) for bacteriocin production. The most striking is that this bacteriocin was limited by extreme broad antimicrobial spectrum which is similar to the report for some bacteriocin of LAB with narrow spectrum for example lactococcin A (Halo *et al.*, 1991) [12] and lactacin B (Barefoot and Kleanhammer, 1983). The results of our study showed, that strains of lactic acid bacteria isolated from dairy products, which were tolerant against acidic conditions and antimicrobial effects and could be used widely in production of industrial products and native probiotic strains, contributing to enhance health in the society.

Table 1: Screening of LAB for antibacterial activity against *Escherichia coli*

S. No	Lactic acid bacteria	Diameter of inhibition zone	
		Disc diffusion method (mm)	Agar well diffusion (mm)
1	*Lactobacillus fermentum*	10.8	10.3
2	*Lactobacillus delbrueckii sub sp. bulgaricus*	14.6	15.2
3	*Lactobacillus acidophilus*	14.8	14.5
4	*Lactobacillus plantarum*	7.4	7.1
5	*Leuconostoc*	15.1	15
6	*Streptococcus thermophilus*	7.5	7.3
7	*Pediococcus acidilactici*	-	-
8	*Pediococcus pentosaceus*	10.2	10.4
9	*Lactococcus lactis*	-	-

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 2: Screening of LAB for antibacterial activity against *Salmonella Typhi*

S. No	Lactic acid bacteria	Diameter of inhibition zone	
		Disc diffusion method (mm)	Agar well diffusion (mm)
1	*Lactobacillus fermentum*	10.5	10.2
2	*Lactobacillus delbrueckii* sub sp. *bulgaricus*	15.1	14
3	*Lactobacillus acidophilus*	14.6	14.7
4	*Lactobacillus plantarum*	7.3	6.8
5	*Leuconostoc*	14.5	14.6
6	*Streptococcus thermophilus*	6.9	6.6
7	*Pediococcus acidilactici*	-	-
8	*Pediococcus pentosaceus*	10.3	10.1
9	*Lactococcus lactis*	-	-

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 3: Screening of LAB for antibacterial activity against *Shigella*

S. No	Lactic acid bacteria	Diameter of inhibition zone	
		Disc diffusion method (mm)	Agar well diffusion (mm)
1	*Lactobacillus fermentum*	10.3	10
2	*Lactobacillus delbrueckii* sub sp. *bulgaricus*	14.4	14.7
3	*Lactobacillus acidophilus*	14	14.2
4	*Lactobacillus plantarum*	6.5	6.2
5	*Leuconostoc*	14.1	14.5
6	*Streptococcus thermophilus*	6.3	6.1
7	*Pediococcus acidilactici*	-	-
8	*Pediococcus pentosaceus*	10.1	10
9	*Lactococcus lactis*	-	-

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 4: Screening of LAB for antibacterial activity against *Staphylococcus aureus*

S. No	Lactic acid bacteria	Diameter of inhibition zone	
		Disc diffusion method (mm)	Agar well diffusion (mm)
1	*Lactobacillus fermentum*	11.6	12.1
2	*Lactobacillus delbrueckii* sub sp. *bulgaricus*	15.3	15.5
3	*Lactobacillus acidophilus*	14.8	15.1
4	*Lactobacillus plantarum*	8.2	7.3
5	*Leuconostoc*	15	14.9
6	*Streptococcus thermophilus*	8	7.8
7	*Pediococcus acidilactici*	-	-
8	*Pediococcus pentosaceus*	11.1	11.4
9	*Lactococcus lactis*	-	-

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 5: Screening of LAB for antibacterial activity against *Listeria monocytogenes*

S. No	Lactic acid bacteria	Diameter of inhibition zone	
		Disc diffusion method (mm)	Agar well diffusion (mm)
1	*Lactobacillus fermentum*	11.2	10.7
2	*Lactobacillus delbrueckii* sub sp. *bulgaricus*	15.2	14.8
3	*Lactobacillus acidophilus*	14.7	15
4	*Lactobacillus plantarum*	7.4	7.6
5	*Leuconostoc*	14.6	14.7
6	*Streptococcus thermophilus*	7.9	7.6
7	*Pediococcus acidilactici*	-	-
8	*Pediococcus pentosaceus*	10.8	10.6
9	*Lactococcus lactis*	-	-

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Conclusion

Preventive health care can be effectively enhanced by the intake of foods that discourage pathogens that cause infection. The culture filtrates from lactic acid bacteria isolated from dairy products exhibited antibacterial activity against five indicator test strains. The bacteriocin producers are recommended to food processing industries to be employed in biopreservation of food to enhance extension of shelf life of food products, bacteriocins are not only effective, but are also safe for risk of the use of chemical preservatives and additives, as they could pose health risk generally.

References

1. Abd El Gawad, IA, Abd El Fatah AM, Al Rubbayyi KA. Identification and characterization of dominant lactic acid bacteria isolated from traditional Rayeb milk in Egypt. Journal of American science. 2010; 6(10):728-735.
2. Adams MR, Moss MO. Food Microbiology. Royal Society of Chemistry, Gulford, 1995; 252-284.
3. Axelsson L. Lactic acid bacteria: classification, physiology and functional aspects. 2nd ed Marcel dekker Inc, New York, 1998, 1-72.
4. Bare foot SF, Klaenhammer TK. Detections and activity of lactacin B on bacteriocin produced by Lactobacillus acidophilus. Applied and Environmental Microbiology 1983; 45:1808-1815.
5. Bauer AW, Kirty WMM, sheris JC. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1996; 45:493-496.
6. Cassia Regina Nespolo, Adriano Brandelli. Production of bacteriocin-like substances by lactic acid bacteria isolated from regional ovine cheese. Braz J Microbiol. 2010; 41:1009-1018.
7. Cintas LM, Casaus MP, Herranz C, Nes IF, Hernández PE. Review: Bacteriocins of Lactic Acid Bacteria. Food Sci Tech In, 2001; 7:281-305.
8. Daeschel MA. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol, 1989; 43:164-166.
9. El-shafei HA, Abd-el-sabour JI, Ibrahim M, Mostafa YA. Isolation, screening and characterization of bacteriocin producing lactic acid bacteria isolated from traditional fermented food. Microbiology Review 2000; 154(4):321-331.
10. Fawole MO, Oso BA. Laboratory Manual of Microbiology. Spectrum Book Limited, Ibadan, Nigeria, 1998, 16-35.
11. Farkye N. Cheese technology. International Journal of Dairy Technology. 2004; 57:91-97.
12. Halo H, Nilssen O, Nes IF. Lactococcin A, a new bacteiocin from Lactococcus lactis sub sp. cremoris: Isolation and characterization of the protein and its gene. Journal of Bacteriology. 1991; 173:3887.
13. Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiol. Review, 1991; 59:171-200.
14. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Reviews 1993; 12:39-86.
15. Mannu K, Riu G, Communian R, Frozzi MC, Scintu MC. A preliminary study of lactic acid bacteria in whey starter culture and industrial Pecorino Sardo ewes' milk cheese: PCR identification and evaluation during ripening. International Dairy Journal. 2002; 12:17-26.
16. Matilla Sadholm T, Matho J, Saarela M. Lactic acid bacteria with health claim- interactions and interference with gastro intestinal flora. International Dairy Journal. 1999; 9:25-35.
17. Oyeleke SB, Manga BS. Essentials of Laboratory Practical in Microbiology (first edition). Tobest Publishers, Minna, Nigeria, 2008, 28-62.
18. Rhodehamel EJ. FDA's concerns with sous vide processing. Food Technol 1992; 46(12):73-76.
19. Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of gram positive bacteria, Bacteriol. Review 1976; 40:722-756.