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Screening of Antimycotic Activity of Bacteriocin Producing Lactic Acid Bacteria against Food Spoilage Fungi

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Abstract

The preservative approach of foods using bacteriocinogenic lactic acid bacteria (LAB) is a novel approach. They are often called as "GRAS" organisms (Generally Regarded as Safe). The main aim of the research was to isolate bacteriocins producing LAB from various fermented and non fermented food products and to evaluate their antifungal activity. On the basis of colony characteristics of LABs 30 bacterial isolates were isolated from diverse sources and based upon the distinct morphology, phenotypic characteristics and genotypic characteristics sixteen lactic acid bacterial isolates were identified from 30 isolates. These LAB isolates were selected for screening of bacteriocins production and then antifungal activity. Only 6 lactic acid bacterial isolates had a high-quality of bacteriocin production activity against indicator pathogenic or food spoilage Bacteria. These LABs isolates was further tested for antifungal activity against food spoilage fungi. These food contaminating fungi were isolated from spoiled fruit (Papaya) and spoiled vegetables (Tomato, Brinjal, and Onion) etc. The indicator fungi were *Fusarium moniliformis* and *Penicillium notatum.* The LAB isolates and their metabolites have antifungal and antibacterial activity, as a result representing the use of lactic acid bacteria use as a potent food preservative agent.

Keywords

Phenotypic, Genotypic, Spoilage fungi, Fermented, Preservative, Contaminating, lactic acid bacteria (LAB).

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INTRODUCTION

The lactic acid bacteria are principal flora of human and animal body due to their ability to colonize and survive in an acidic environment [30, 31]. Human beings as well as animals are generally born sterile but shortly after birth, colonization start with different body parts being occupied via the fittest

microbes from the environment, hence creating a balanced ecological organism. LAB is considered as beneficial microorganism because of their capability to metabolize proteins, carbohydrates and fats present in food and help in the absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for the survival of humans and different animals (32). They have the capability to produce lactic acid either through homo or hetero fermentative pathway. Lactic acid bacteria are utilized in probiotic feeds as they can tolerate low pH and high salt concentration, enabling them to survive, grow and perform their therapeutic role inside the intestinal tract (36, 38, 33, 37, 34, 35)

Diverse kinds of food products are spoiled by moulds and yeasts cause great economic losses at international levels. In addition, moulds produces mycotoxins causes health hazards considerable general spoilage of food and feed [6]. Common spoilage organisms such as yeasts and filamentous moulds have capability to spoiled food products as fermented milk products, cheese, bread, stored crops and feed such as hay and fodder [5,14]. It is expected that about 5 and 10% of the world's food production is lost due to fungal deterioration [23]., it is estimate that in Western Europe £242 million economic loss annually due to mould spoilage of bread alone [9].

Newly reported spoilage organisms as *Penicillium* and *Aspergillus* species have been formed during storage of a wide variety of food and feed. High capacity of mycotoxins produced by *Fusarium* species are frequently established on cereal grains [14, 23]. Different *Penicillium* species examples P*. roqueforti* and *P.commune* commonly spoil cold storage hard cheese [14, 25]. Different types of Yeasts:

Candida parapsilosis,Rhodotorula mucilaginosa, Kluyveromyces marxianus and *Debaromyces hansenii* are common spoilage organisms of yoghurt and other fermented dairy products [25, 18].

Numerous techniques are used for the preventing the contamination of food and feed. Some preservation techniques like cold-storage, drying, freeze drying, modified atmosphere storage, and heat treatments are all way of food preservation through physical methods [14]. Many organic chemicals and acids are used as food preservation additives; the most active are acetic acid, lactic acid, propionic acid, sorbic acid and benzoic acid [4]. Benzoic acids and sodium benzoate are used primarily as antifungal agents [10] and both sorbic and benzoic acids have a broad range of antifungal activity [22]. There are several chemical additives also function as preservatives, even though the exact mechanisms or targets often are not known [10].

Streptomyces natalensis produces an antibiotic agent Natamycin which is very effective against yeasts and moulds and often used as a preservative on surfaces of hard cheese [10]. In addition, isolates of *P. roqueforti* have been found to be resistant to benzoate [22].

The mould *Penicillium discolor* has recently acquire a higher capability of resistance to natamycin used in food processing [22, 14, 7].

Most of the yeast like Debaromyces hansenii, Candida versatilis and Torulaspora delbrueckii, have also shown resistance to chemical sanitizers and cleaning compounds in dairy industries [28].

The combination of antibiotic resistance and general chemical resistance of spoilage organisms, together with an increased general awareness among consumers calls for alternative measures to preservation of food products [4]. Therefore, the application of biopreservation for control of one organism by another, has received much attention in recent years [26].

Lactic acid bacteria are found in many nutrient-rich environments and occur naturally in various food products such as dairy and meat products, and vegetables [9]. LAB are of special interest as biopreservation organisms since they have a long history of use in food and are 'generally regarded as safe' organisms. They have, by tradition, been established as a natural, consumer and environment-friendly way of preserving food and feed. Their preserving effect mainly relates to the production of organic acids, i.e. lactic and acetic acid [16], but bacteriocins, produced by some strains, are also of importance [12]. The long tradition of using lactic acid bacteria in food processing in combination with trecent information of probiotic [19, 1] makes them a promising alternative.

The majority of the large numbers of reports on the antimicrobial activity of LAB have focused on antibacterial effects [12], while reports on antifungal effects are few. Lavermicocca et.al., reported production of the antifungal compounds phenyllactic acid and 4-hydroxyphenyllactic acid by a sourdough Lactobacillus plantarum strain. In addition, bacteriocin-like substances and other low molecular mass compounds produced by LAB have been reported as [23, 24]. Recently discovered Lactobacillus coryniformis strain Si3 can produce a proteinaceous antifungal compound [18, 20].

Research dealing with fungal inhibition by lactic acid bacteria and the compounds produced by these bacteria is still novel. While the number of

publications regarding the antibacterial activity of LAB is large, our knowledge of the antifungal activities of these bacteria is still limited. This is the new ways to extend shelf life, suppressing fungal growth and produce safe, preservative-free food. This need stimulates the search for lactic acid bacteria with these abilities.

2. MATERIALS AND METHODS:

2.1 Isolation and identification of bacterial strains

The LAB isolated from dairy sources such as milk of (cow, buffalo, goat), cheese, shrikhand and nondairy sources such as fermented Loki, soaked Soybean, Garlic fermented, fermented green gram, sugarcane juice, soaked cereal Rice, and Dosa batter etc. On the basis of colony characterstics 30 bacteria was isolated from a variety of sources. Such bacterial Isolates were further identified by Biotyping and genotypically (Bergey's Manual of Systematic Bacteriology, 1989). Bio-typing includes morphological identification and various biochemical test followed by isolates was send for genotypic identification using 16s rDNA sequencing. **2.2 Bacteriocin Production Assay**

The bacteriocin production assay was performed by Agar well diffusion method was used to detect antibacterial activity of LAB isolates [21, 2, 27]. The bacteriocins collection is the first step of bacteriocins production assay. In first step, LAB isolates were grown in 20 ml of MRS Broth of pH 5.6 for 48 h at 35ºC in orbital shaker incubator. After incubation suspended cell were removed by centrifugation at 15000 x g for 20 min at 4^0C and filtered through a 0.45 µm pore-size filter (Millipore, USA). Each cell-free supernatant containing bacteriocin was precipitated by addition of 40% ammonium sulphate and stored at 4^0C for 24 hrs, next day addition of 20% ammonium sulfate in same tube for additional precipitate formation. The precipitate of cude Bacteriocin was pelleted by centrifugation at 15000 xg for 20 min. at 4^0C. The precipitated crude bacteriocin was dissolved in 40µl of 0.5 M potassium phosphate buffer (solution must be in concentrated form) and stored the solution at 4^0C for further testing antimicrobial activity. Quantity of bacterial cell produced in medium is directly proportion to amount of bacteriocins produced by LAB isolates, therefore calculates the dry weigh of bacterial cell mass and bacteriocins.

2.2.1 Antimicrobial activity Assay:

The indicator pathogen strains were employed in this experiment: *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pnuemoniae* and *Bacillus mycoides.* These bacterial isolates were inoculated in Nutrient Broth and incubate at 35ºC for 24 h.

The indicator bacteria swab over the surface nutrient agar media using an L shape spreader, rest for 15 min, prepare well using 6 mm size borer and then fill the wells with 40µl bacteriocin containing a solution. The plates were placed at 4ºC overnight to allow the diffusion of this solution in the agar and then incubated at 35ºC for 48 h. Subsequently, the diameter of the clear zone around each well was recorded in millimetres with a Vernier calliper.

2.3 Screening of bacteria for antifungal activity

The screened bacteriocin-producing LAB isolates were further screened for antifungal activity using a dual culture overlay assay [18]. In this method LAB isolates were inoculated into 10 ml of MRS Broth and incubate at 35°C for 48 h under anaerobic condition. Visualize the broth for optimum growth. After incubation, 4 cm lines was drawn on selective MRS agar media using inoculation loop over the surface of MRS agar plates and allowed to grow at 30^0C for 48 h under anaerobic jars. After necessary time of incubation bacterial growth was observed along the streak. The indicator food spoilage fungi were used for the detection of antifungal activity of LAB isolates. The above plates were then overlaid with 10 ml of potato dextrose soft agar (1% agar type I) containing 10^4 fungal spores per ml.

The indicator fungi were Fusarium pallisodorium and Penicillium notatum. After 72h-120h of aerobic incubation at 300C, the zone of inhibition was measured. The inhibition was graded by relating the inhibited growth area per inoculation streak to the total area of the Petridish. The inhibition area was also related to the variation in length of the bacterial streak.

3. RESULT

3.1 Isolation and identification of bacterial strains

The bacteria isolated from dairy and non-dairy sources such as milk (cow, buffalo, goat), cheese, shrikhand fermented Loki, soaked soybean, fermented garlic, fermented green gram, sugarcane juice, soaked cereal and Rice and Dosa batter etc. MRS agar media and CATC agar media are selective media for the isolation of different members of lactic acid bacteria. Approximately 30 bacterial isolates were isolated on the basis of its colony morphology of lactic acid bacteria. These isolates were further identified by biotyping as well as genotyping and then match with Bergey's manual key. On the basis of morphological and biochemical identification, 16 of the 30 bacteria were identified as lactic acid bacteria. These isolates were further selected for

detection of bacteriocin production activity by Agar well diffusion method was used to detect antibacterial activity [2, 21, 27].

3.2 **Bacteriocin Production Assay**

Number of bacterial cell produced in medium is directly proportion to amount of bacteriocins produced by LAB isolates, thus calculates the dry weigh of bacterial cell mass and bacteriocins. Dry weight of both cell and bacteriocins has been done in triplicate. The bacteriocin production assay was performed by Agar well diffusion method was used to detect antibacterial activity [2, 21, 27], against *Bacillus mycoides, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Proteus vulgaris.* After measuring a zone of inhibition around the well there is 6 lactic acid bacterial isolates have shown maximum activity unit. These LAB isolates was designated as S1 (soy), B1 (5), B2 (9), G1 (Gr1), SJ1 (13) and SCR 1(15). These bacterial isolates further tested for antifungal activity by Dual overlay method [18].

Table 3.2.1: The table showing a Dry weight of cellular mass and dry weight of bacteriocins.

Sr. No.	Designation	Dry Weight of cells(g) for 150ml			Average dry weight of cells	Dry Bacteriocin Weight			Average dry Weight of Bacteriocin
		1	2	3		1	2	3	
1	LK1	80	79	79.9	79.63	30	29.9	30	29.96
2	LK2	79.2	80	79.2	79.46	29.5	29.6	29.6	29.56
3	S1	80	80.3	80.3	80.2	32.5	32.8	32.8	32.7
4	B1	78	79	78	78.33	32.8	32.9	32.9	32.86
5	B2	77.4	78	77.5	77.633	31	31.2	31.5	31.23
6	G1	78	78	78.6	78.2	29.5	29.3	29.3	29.36
7	GG1	79	80	79	79.33	29.6	29.6	29.8	29.66
8	GG2	79	78	78	78.33	29	28.9	29	28.96
9	Cw1	80.4	81	80.4	80.6	30.5	30.8	30.8	30.7
10	CW4	76.8	77.1	77.1	77	25.6	24.4	24.4	24.8
11	SJ1	78.1	78.6	78.6	78.43	28.6	28.8	28.6	28.66
12	BR1	74	74	74.7	74.23	28.2	28.6	28.6	28.46
13	BR2	75.3	75	75.4	75.23	28	28.1	28.1	28.06
14	SCR1	78.9	78.5	78.5	78.63	27.8	27.8	27.9	27.83
15	DD1	76.5	76.9	76.5	76.63	23.9	23.6	23.6	23.73
16	DD2	79.5	79.6	79.6	79.56	27.5	27.6	27.5	27.53

Figure 3.2.2: The Graph showing relationship between bacterial cell mass and bacteriocins production.

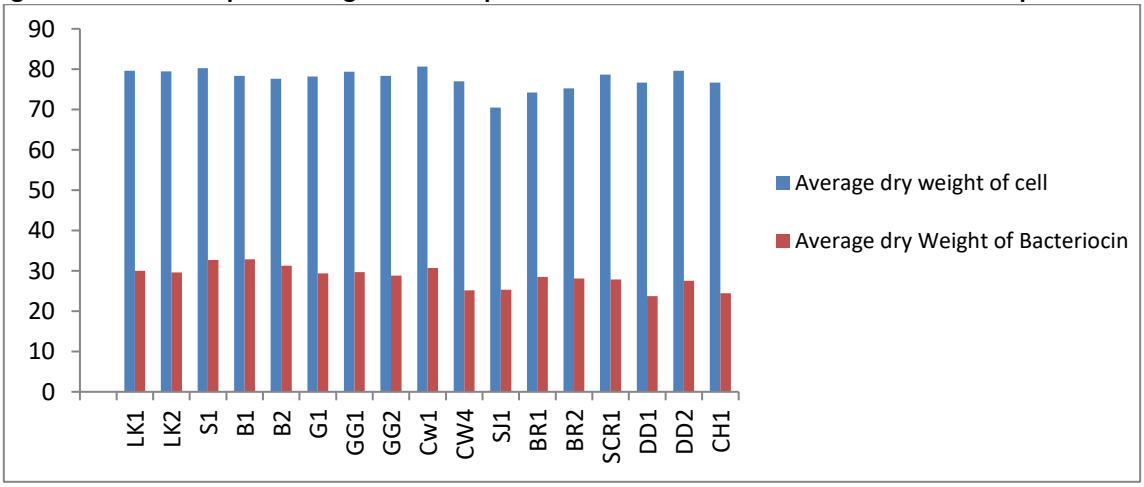


Table 3.2.3: Showing antibacterial activity against S*taphylococcus aureus*

Sr. No.	Isolates	Borer size (A)	zone of inhibition (mm) (B)	Difference between (B-A)	Activity Unit/ml
1	LK1	6mm	8	2	40
2	LK2	6mm	9	3	60
3	S1(soy)	6mm	12	6	120
4	B1 (5)	6mm	10	4	80
5	B2(9)	6mm	12	6	120
6	G1(Gr1)	6mm	10	2	40
7	GG1	6mm	10	2	40
8	Cw1	6mm	9	3	60
9	CW4	6mm	9	3	60
10	SJ1(13)	6mm	10	4	80
11	BR1	6mm	10	4	80
12	BR2	6mm	11	5	100
13	SCR1(15)	6mm	11	5	100
14	DD2	6mm	10	4	80

Table 3.2.4: Showing antibacterial activity against *Proteus vulgaris*

Sr. No.	Isolates	Borer size (A)	zone of inhibition in(mm) (B)	Difference between (B-A)	Activity Unit/ml
1	LK1	6mm	9	3	60
2	LK2	6mm	9	3	60
3	S1(soy)	6mm	10	4	80
4	B1 (5)	6mm	11	5	100
5	B2(9)	6mm	11	5	100
6	G1(Gr1)	6mm	10	4	80
7	GG1	6mm	10	4	80
8	Cw1	6mm	10	4	80
9	CW4	6mm	9	3	60
10	SJ1(13)	6mm	10	4	80
11	BR1	6mm	11	5	100
12	BR2	6mm	10	4	80
13	SCR1(15)	6mm	13	7	140
14	DD2	6mm	9	3	60

Table 3.2.5: Showing antibacterial activity against *Bacillus mycoides*

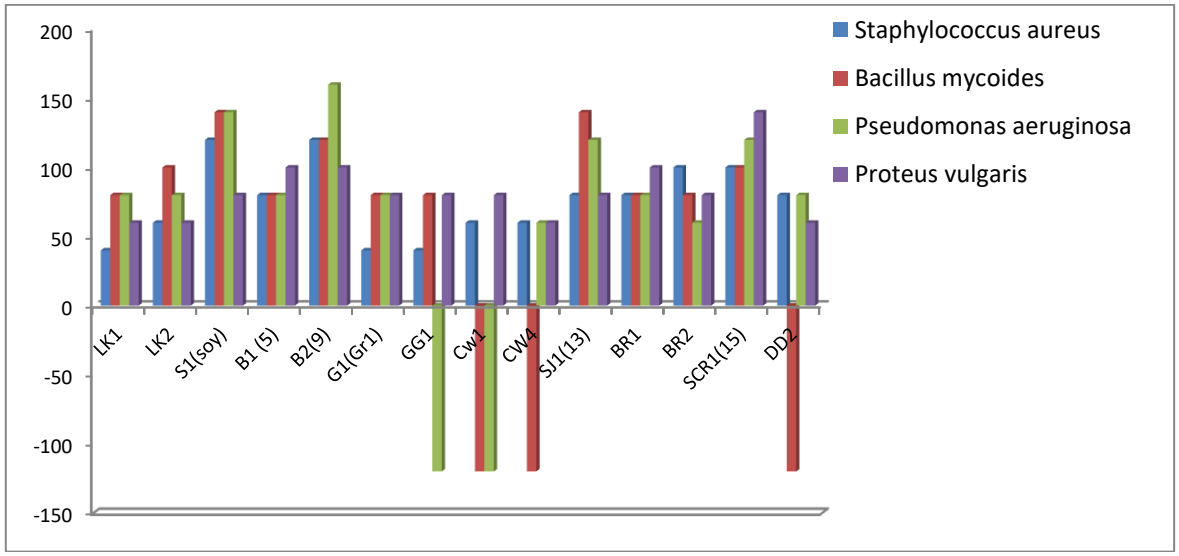
Sr. No.	Isolates	Borer size (A)	zone of Inhibition (B)	Difference between (B-A)	Activity Unit/ ml
1	LK1	6mm	10	4	80
2	LK2	6mm	11	5	100
3	S1(soy)	6mm	13	7	140
4	B1 (5)	6mm	10	4	80
5	B2(9)	6mm	12	6	120
6	G1(Gr1)	6mm	10	4	80
7	GG1	6mm	10	4	80
8	Cw1	6mm	0	-6	-120
9	CW4	6mm	0	-6	-120
10	SJ1(13)	6mm	13	7	140

11	BR1	6mm	10	4	80
12	BR2	6mm	10	4	80
13	SCR1(15)	6mm	11	5	100
14	DD2	6mm	0	-6	-120

Table 3.2.6: Showing antibacterial activity against *Pseudomonas aeruginosa*

Sr. No.	isolates	Borer size (A)	zone of Inhibition (B)	Difference between (B-A)	Activity unit/ml
1	LK1	6mm	10	4	80
2	LK2	6mm	10	4	80
3	S1(soy)	6mm	13	7	140
4	B1 (5)	6mm	10	4	80
5	B2(9)	6mm	14	8	160
6	G1(Gr1)	6mm	10	4	80
7	GG1	6mm	0	-6	-120
8	Cw1	6mm	0	-6	-120
9	CW4	6mm	9	3	60
10	SJ1(13)	6mm	12	6	120
11	BR1	6mm	10	4	80
12	BR2	6mm	9	3	60
13	SCR1(15)	6mm	12	6	120
14	DD2	6mm	10	4	80

Figure 3.2.7: Showing results of antibacterial activity (activity unit) of bacteriocins against different indicator bacteria.



3.3 Screening of bacteria for antifungal activity

Dual overlay method

Varying degrees of inhibition were detected against the indicator fungi in the overlay method. Most of the isolates showed good inhibitory activity against of the fungi in the dual-culture agar overlay methods.

The results of antifungal activity of LAB isolate shows in the form of Positive and negative.

(+++) indicates lack of fungal groth inside the plate, (++) indicates fungal growth was found but not along the streak. (+) indicates fungal growth not along the streak, (-) indicates fungal growth along the streak.

Figure 3.3 Indicate Antifungal Activity activity against food spoilage fungi *Penicillium notatum* and *Fusarium pallisodorium* after 3 day of incubation.

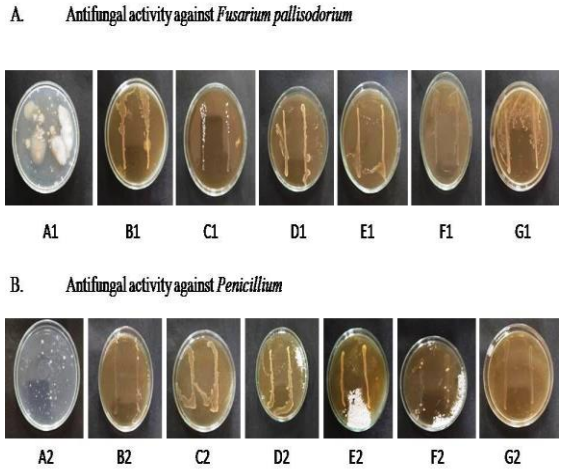


Table 3.3.1 and 3.3.2: Showing antifungal activity of LAB isolates against food spoilage fungi.

Fusarium pallisodorium		
Sr. No.	Isolates	Result
A1	Control	-----
B1	S1(soy)	+++
C1	B1 (5)	+++
D1	B2(9)	+++
E1	G1(Gr1)	+++
F1	SJ1(13)	+++
G1	SCR1(15)	+++

Pencillium notatum		
Sr. No.	Isolates	Result
A2	Control	
B2	S1(soy)	(+++)
C2	B1 (5)	(++)
D2	B2(9)	(+)
E2	G1(Gr1)	(++)
F2	SJ1(13)	(+++)
G2	SCR1(15)	(+++)

+++ indicates lack of fungal groth inside the plate.
++ indicates fungal growth was found but not along the streak.
+ indicates fungal growth not along the streak.
- indicates fungal growth along the streak.

4. DISCUSSION:

In bacteriocins production assay dry weight of cellular mass and dry weight of Bacteriocin was calculated. 14 maximum quantities of bacteriocins producing bacteria were selected for further studies. After bacteriocins production assay results was analyzed, 6 bacterial isolates showing maximum activity unit against the food pathogenic bacteria. These superior bacterial isolates also showing antifungal activity against food contaminating fungi by Dual agar overlay method. The table 2.3.1 and 2.3.2 showing results of antifungal activity. All 6 bacterial isolates have maximum antifungal activity against *Fusarium pallisodorium.* Isolates S1, SJ1, SCR1 have maximum antifungal activity and isolates B1 and G1 showing a lesser amount of antifungal activity. Isolate B2 have least amount antifungal activity.

5. CONCLUSION

In this study isolates from different dairy and non-dairy sources have been identified as a member of *Lactobacillaceae* family and characterized as cultures with promising antifungal activity. This is the new ways to extend shelf life, suppressing fungal growth and produce safe, preservative-free food. This will need to stimulate the search for lactic acid bacteria with these abilities. The combination of natural origin and strong inhibitory activity of the LAB isolates is a prerequisite for their possible application as starters and/or bioprotective antifungal agent.

CONFLICT OF INTEREST

No conflict of interest.

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REFERENCES

1. Corsetti, M. Gobebetti, J. Rossi, P. Damiani, Antimould activity of sourdough lactic acid bacteria: Identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. Applied Microbiology and Biotechnology 50 (1998) 253-256.
2. B.C. Viljoen, The interaction between yeasts and bacteria in dairy environments, International Journal of Food Microbiology 69 (2001) 37-44.
3. S. Brul, P. Coote, Preservative agents in foods and mode of action and microbial resistance mechanisms, International Journal of Food Microbiology 50 (1999) 1-17.
4. M.P. Davidson, Chemical preservatives and natural antimicrobial compounds. In Food microbiology: Fundamentals and frontiers. Edited by MP, Doyle, and LR. Beuchat, T.J. Montville, ASM press. Washington, USA. (2001) 593-627.
5. P.M. Davidson, L.S. Post, A.L. Branen, A.R. McCurdy, Naturally occurring and miscellaneous food antimicrobials. In antimicrobials in foods. Edited by PM. Davidson, AL. Branen. Marcel Dekker Inc. New York, USA. (1983) 385-392.
6. F.J. Carr, D. Chill, N. Maida, The lactic acid bacteria: a literature survey. Critical Reviews in Microbiology 28 (2002) 281-370.
7. E.L. Anderssen, D.B. Diep, I.F. Nes, V.G.H. Eijsink, J. Nissen-Meyer, Antagonistic activity of *Lactobacillus plantarum* C11: Two new two-peptide bacteriocins, plantaricins EF and JK, and the induction factor plantaricin A, Applied and Environmental Microbiology 64 (1998) 2269-2272.
8. H. Andersson, N.G. Asp, A. Bruce, S. Roos, T. Wadström, AE.Wold, Health effects of probiotics and prebiotics. A literature review on human studies. Scandinavian Journal of Nutrition 45 (2001) 58-75.
9. T.Mattila-Sandholm, J. Mättö, M.Saarela, Lactic acid bacteria with health claims interactions and interference with gastrointestinal flora. International Dairy Journal 9 (1999) 25-35.
10. S.E. Lindgren, W.J. Dobrogosz, Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiology Reviews 87(1990) 149-164.
11. N. Van Hai, R. Fotedar, N. Buller, Selection of probiotics by various inhibition test methods for use in the culture of western king prawns, *Penaeus latisulcatus* (Kishinouye). Aquaculture 272 (2007) 231-239.
12. V. Mishra, D.N. Prasad, Application of in vitro methods for selection of *Lactobacillus casei strains* as potential probiotics. International Journal of Food Microbiology 103 (2005) 109-115.
13. J.L. Balcazar, I. de Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell, JL. Muzquiz, The role of probiotics in aquaculture. Veterinary Microbiology 114 (2006) 173-186.
14. RS. Breed, E.G.D. Murray, NR. Smith, Bergey's Manual of Determinative Bacteriology, 7th ed., 1957, the Williams and Wilkins Co., Baltimore.
15. L.B. Bullerman, Incidence and control of mycotoxin producing molds in domestic and imported cheeses. Annales de la nutrition et de l'alimentation 31(1977) 435-46.
16. M.L. Cabo, A.F. Braber, P. Koenraad, apparent antifungal activity of several lactic acid bacteria against Penicillium discolors is due to acetic acid in the medium. J. Food Protect 2002; 65: 1309-1316.
17. H.M. Dodd and M.J. Gasson, Bacteriocins of lactic acid bacteria. In: Genetics and Biotechnology of Lactic Acid Bacteria (Eds. M.J. Gasson, and W.M. De Vos) Blackie Academic and Professional, London) 1994 (211-251).
18. O. Filtenborg, JC. Frisvad, U. Thrane, Moulds in food spoilage. Int J Food Microbiol 33(1996) 85-102.
19. P. Lavermicocca, F. Valerio, A. Evidente, S. Lazzaroni, A. Corsetti, M. Gobetti, Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl Environ Microbio 66 (2000) 4084-4090.
20. SE. Lindgren, WJ. Dobrogosz, Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. 87(1990) 149-164.
21. J. Magnusson, J. Schnurer, Lactobacillus coryniformis subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Appl. Environ. Microbiol 67 (2001) 1-5.
22. W. Messens, L. De Vuyst, Inhibitory substances produced by *Lactobacilli* isolated from sourdoughs - a review. Int. J. Food Microbiol 72 (2002) 31-43.
23. PV. Nielsen, E. de Boer, Food preservatives against fungi. In Introduction to food and airborne fungi. Edited by R.A. Samson, E.S. Hoekstra, J.C. Frisvad & O. Filtenborg. Centraalbureau voor

Schimmelcultures. Utrecht, the Netherlands (2000) 357-363.
24. ML. Niku-paavola, A. Laitila, T. Mattila-Sandholm, A. Haikara, New types of antimicrobial compounds produced by *Lactobacillus plantarum.* J. Appl. Microbiol 86 (1999) 29-35.
25. DJ. Okkers, LMT. Dicks, M. Silvester, JJ. Joubert and HJ. Odendaal, Characterization of pentocin TV35b, a bacteriocin-like peptide isolated from *Lactobacillus pentosus* with a fungistatic effect on *Candida albicans*. J. Appl. Microbiol. 87(1999) 726-734.
26. JI. Pitt, AD. Hocking, Fungi and Food Spoilage, Chapman and Hall, New York (1999).
27. ME. Stiles, Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70 (1996) 331-345.
28. N. Van Hai, R. Fotedar, N. Buller Selection of probiotics by various inhibition test methods for use in the culture of western king prawns, *Penaeus latisulcatus* (Kishinouye). *Aquaculture* 272 (2007) 231-239.
29. BC. Viljoen, The interaction between yeasts and bacteria in dairy environments. *International Journal of Food Microbiology* 69 (2001) 37-44.
30. E. Isolauri, Probiotics in human disease. *Am J Clin Nutr.* 73 (2001) 11425-65.
31. O. Olanrewaju, Antagonistic effect of *Lactobacillus* isolates form kunnu and cow milk on selected pathogenic microorganisms, Internet J Food Safety 9 (2007) 63-66.
32. JC. Piard, M. Desmazeaud, inhibiting factors produced by lactic acid bacteria: Oxygen metabolites and catabolism en-products, Lait 71(1991) 525-541.
33. Usman, Hosono, Binding of cholesterol to the cells and peptidoglycan of *Lactobacillus gasseri*, Milchwissenchaft 54 (1999) 495- 498.
34. Pelinescu, E. Sasarman, MC. Chifiriuc, I. Stoica, AM. Nohita, I. Avram F. Serbancea, TV. Dimov, Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach, Romanian Biotechnological Letters 14 (2009) 4225-4233.
35. R. Kumar, S. Pandey, P. Kapoor, S. Awasthi and T. Bhatnagar Isolation and characterization of endemic strains of *Lactobacillus sp.* and evaluation of their probiotic activity, Int J Curr Microbiol App Sci. 3 (2014) 907-916.
36. CC. Allain, LS. Poon, CSG. Chan, W. Richmond, PC. Fu. Enzymatic determination of total serum cholesterol, Clinical Chemistry 20 (1974) 470–475.
37. IAP Dora, RG. Glenn, Cholesterol assimilation by lactic acid bacteria and *Bifidobacteria* isolated from the human gut, Applied Environmental Microbiology 68 (2002) 4689-4693.
38. SE. Gilliland, DK. Walker, Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypolcholesterolemic effect in humans, J Dairy Sci. 73(1989) 905-911.