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Original Article

Isolation, Characterization and Antimicrobial Activity of Lactic Acid Bacteria from Local Milk and Milk Products

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In the present study fifteen Lactic Acid Bacteria (LAB) from milk and milk products were isolated, identified and tested for their antagonistic activity. All the samples were found to be acidic with a pH range of 6.0 to 6.8.The collected samples showed higher number of total bacterial load ranging from $3.24\acute{}10^{5}$ to $1.04\acute{}10^{8}$ cfu/ml. Out of fifteen isolates, nine isolates were found to belong to the genus *Lactobacillus* and identified as *L. casei* subsp. *pseudoplantarum, L. homohiochii, L. salivarius, L. xylosus, L.fermentum, L.leichmannii , L.heterohiochii, L.casei,* and *L.plantarum,*.The others were found to belong to the genus *Streptococcus* and identified as *S. thermophilus , S. lactis, S. uberis, S.suis, S. faecalis,* and *S. equnius*.The isolates showed antibacterial activity against four gram positive bacteria (*Bacillus cereus, B. subtilis, B.megaterium, Staphylococcus aureus*) and six gram negative bacteria (*Escherichia coli, Shigella dysenteriae, Salmonella typhi, Salmonella paratyphi, Vibrio cholerae* and *Pseudomonas aeruginosa*) by using the disc diffusion method. They also showed their antifungal activity against two fungi (*Penicillium sp.* and *Aspergillus flavus*) by modifying poisoned food technique. All of the fifteen isolates were active against one or more test pathogenic bacterial strains. Among them *L. homohiochii* (TM_3/a) showed the highest zone of inhibition (30.3mm) against *Salmonella typhi*. *Lactobacillus spp*. showed more antifungal activity than *Streptococcus spp*. and *Streptococcus uberis* (TY_4/a) showed the highest antifungal activity (50%) against *Penicillium sp.*This preliminary work shows the potential application of LAB to improve safety of traditional fermented food and milk products.

***Key words*: Lactic acid bacteria, antagonistic activity, microbial load.**

Introduction

Milk is an excellent nutrient for the microbial growth due to moderate pH (6.4-6.6), good quantity of nutrients, high water contents etc[1]. Each milk product has a characteristic flora that is responsible for its distinctive flavor and physical appearance[2]. Milk fermentation, like many traditional fermenting processes, is spontaneous and uncontrolled and could be a valuable source of autochthonous Lactic Acid Bacteria (LAB)[3].

The lactic acid bacteria (LAB) are a broad group of gram positive, catalase-negative, non-sporing rods and cocci, usually non-motile that utilize carbohydrates fermentative and form lactic acid as the major end product[4].Lactic acid bacteria exert strong antagonistic activity by producing substances (e.g. bacteriocin, lactic acid, H_2O_2) that inhibit pathogenic, non-pathogenic and spoilage organisms in fermented milk, foods and beverages[5].In addition, some strains may contribute to the preservation of fermented milk and foods by producing bacteriocins[6].Lactic acid bacteria (LAB) that possesses antibacterial or antifungal activity generally referred to as probiotic bacteria. These probiotic Lactic acid bacteria which are living and health promoting bacteria are widely distributed in the nature and are involved in various types of spontaneous food fermentation[7].LABs are used to enhance the activity of peritoneal and pulmonary macrophages, blood leukocytes, increase secretion of lysosomal enzymes, increase reactive oxygen, increase nitrogen radical production and monokines of phagocytic cells, enhance clearance of colloidal carbon *in vitro* as an indicator of the phagocytic activity[8]. Many authors described protective effects of LAB which includes boosting of the immune system, inhibition of the growth of pathogens, prevention of diarrhoea from various causes, prevention of cancer, reduction of the risk of inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins, and detoxification and protection from toxins. Members of the genera *Lactobacillus* and *Streptococcus* are the most common probiotics used in commercial fermented and non-fermented milk product today.

This study was aimed to detect and determine the antagonistic activities i.e. antibacterial and antifungal capabilities of Lactic acid bacteria (LAB) against some common microbial pathogen isolated from milk and milk products. The antimicrobial activities of LAB will affirm their use in the development of food safety for the consuming public.

Materials and methods

Collection of samples

Milk and milk product include curd, butter, yogurt, and cheese were collected from different places of Chittagong for isolating

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Lactic acid bacteria (LAB). The types of sample, color and pH of the collected samples were recorded carefully at the time of sample collection. After collection, samples were preserved in the refrigerator at 4^0C until analysis was conducted.

Enumeration of Bacteria

Ten-fold serial dilution and pour plate technique were followed for isolation and enumeration of bacteria. The total bacterial load of the samples was determined by using Nutrient agar (NA) media.

Isolation and Identification of Lactic acid bacteria

Different dilutions were made to isolate LAB from the collected samples. Two different selective media, namely MRS (De Man, Rogosa and Sharpe) agar media for *Lactobacillus* and YGLA (Yeast Glucose Lamco Agar) media for *Streptococcus* were used for isolation[9-10].After plating they were incubated at 37°C for 24- 48hrs aerobically. Several colonies having different morphologies were picked for identification. These isolated colonies were purified by repeated plating and were Gram stained for studying their colony and cell morphology. These isolated colonies were transferred to MRS agar and YGLA slant for further study. These isolates were examined for their catalase activity. These isolates showed negative result. These isolates produced gas in 5% glucose containing nutrient broth at 37°C for 24- 48hrs. They were observed to be catalase-negative. The isolates showed characteristics that were similar to those of Lactic acid bacteria. Further biochemical tests showed that the isolates were belonging to the genus *Lactobacillus* and *Streptococcus*.

Determination of antibacterial activity of LAB in broth culture

Test organisms (bacteria)

The test organisms were collected from the stock collection of laboratory of Dept. of Microbiology, University of Chittagong and were grown in nutrient broth at 37°C for 48hrs.The test pathogenic bacteria were *Bacillus cereus* BTCC19, *Bacillus subtilis* BTCC17, *Bacillus megaterium* BYCC18, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 25922, *Shigella dysenteriae* AE14396, *Salmonella typhi* AE14612, *Salmonella paratyphi* AE14613,*Vibrio cholerae* AE14748, *Pseudomonas aeruginosa* CRL(ICDDR,B) were used as test organisms to evaluate the antimicrobial effects of LAB isolated from milk and milk products.

Isolated LABs were screened for detecting and determining antibacterial activity against some pathogenic Gram positive and Gram negative bacteria. The antibacterial activity of the isolated LABs were determined by modifying the disc diffusion method of Ashenafi[11].Isolated LABs were grown in MRS and YGL broth media for 48 hrs at 37°C. After incubation these isolated LABs were centrifuged at 7000 rpm for 20 mins at 4°C. Then these were filtered across filter paper to remove residual cells. Filtrates were soaked in 4mm filter paper discs. The cultures of the target pathogenic bacteria were spread on solid Muller- Hinton agar media. Dried paper discs impregnated with known amount of filtered cell-free supernatant were placed on Muller-Hinton agar media uniformly seeded with the test pathogenic strains. Discs soaked with sterile media without isolated LABs were used as positive control. All the inoculated plates were kept at low temperature (4°C) for an hour to allow the maximum diffusion. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the organisms at inverted position. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving a clear distinct zone called "zone of inhibition". The antibacterial activity of the test agents (supernatants) were determined by measuring the zone of inhibition expressed in millimeter in diameter. The experiment was carried out more than once and mean of the reading was taken.

Determination of antifungal activity of LAB in broth culture

Test organisms (Fungi)

Test fungi used for observing antifungal activity were *Penicillium sp.* and *Aspergillus flavus*. Link collected from the stock culture of laboratory of Dept. of Microbiology, CU. These fungi were grown on Czapek's Dox Agar media and incubated at 25 ± 2ºC for 6 days.

The modified poisoned food technique[12] was used to screen for antifungal activity of Lactic acid bacteria. For this purpose, 0.5ml filtered cell-free supernatants were taken by sterilized pipette in a sterile Petri plate, sterilized nutrient agar media was poured in the Petri plate, mixed well and allowed to solidify. For inoculation 5mm hole was made at the center of each plate with the help of sterile cork borer. Inoculation was done at the hole of the center of each plate placing with 5mm mycelium block for each 6days old fungus by modifying poisoned food technique described by Grover and Moore[12]. The inoculated plates were incubated at 25ºC ± 2. Proper control (nutrient agar media without culture extract) was also maintained. After 5 days of incubation, the diameter of fungal redial mycelial growth was measured in mm. The percentage of inhibition of mycelial growth of the test fungi was calculated by following formula:

$$I = \frac{(C-T)}{C} \times 100$$

Here, I = Percentage of inhibition.C = Diameter of the fungal colony in control.T = Diameter of the fungal colony in treatment.

Results and Discussion

In this study, the milk samples were collected from different places of Chittagong. The types of sample, color, pH and total bacterial load of collected samples were recorded and shown in Table-1.All the samples were found to be acidic with a pH range of 6.0 to 6.8. The bacterial count in samples ranged between 3.24×10^5 to 1.04×10^8 cfu/ml.

Thirty LAB isolates were primarily selected and among them fifteen LAB isolates were finally selected for identification. On the basis of their morphological, cultural and biochemical characteristics, all the isolates were found to be closely related to *Lactobacillus casei* subsp. *pseudoplantarum* (TM_2/a_1) , *L.homohiochii* (TM_3/a) , *L.salivarius* (TM-$_3$/b), *L. xylosus* (TM_3/c), *L. fermentum* (TM_4/d), *L. leichmannii* (TM_6/a), *L. heterohiochii* (TM_6/b), *L. casei* (TM-$_6$/c), *L. plantarum* (TM-$_6$/d), *Streptococcus thermophilus* (TY_2/f), *S. Lactis* (TY_3/b), *S. uberis* (TY_4/a), *S. suis* (TY_5/b), *S. faecalis* (TY_6/e) and *S. equnius* (TM-$_6$/d), while compared with the standard description given in Bergey's Manual of Determinative Bacteriology, 8th edition[13].(Table-2, 3 ,4)

These isolates were screened for their efficiency of antibacterial and antifungal activity.

Determination of antibacterial activity

Fifteen LABs, isolated from fermented milk sample, were screened for their antagonistic activity against some Gram positive and Gram negative pathogenic bacteria. The inhibitory effects of cell-free supernatants were evaluated *in vitro* and shown in Table.5-8.

From this study it is found that among the LABs isolates, *Lactobacillus homohiochii* (TM_3/a), *L. leichmannii* (TM_6/a), *L. heterohiochii* (TM_6/b), *L. casei* (TM_6/c), *Streptococcus thermophilus* (TY_2/f), *S. equnius*(TY_6/f) showed antibacterial activity against all test pathogenic bacteria. Among them *L. homohiochii* (TM_3/a) showed the highest zone of inhibition (30.3mm) against *Salmonella typhi* . *L. plantarum* (TM_6/d), *S. uberis* (TY_4/a), *S. suis* (TY_5/b) showed antibacterial activity against nine test pathogenic bacteria. *S. lactis* (TY_3/b), *S.*

Table 1: *The types of sample, color, pH and total bacterial load of the collected sample*

No.of sample	Types of sample	Color of sample	pH	Total bacterial count (cfu/ml)
1	Yogurt	Whitish	6.1	$1.04´10^8$
2	Yogurt	Cream	6.0	$8.8´10^7$
3	Buffalo dahi	Whitish	6.8	$8.9´10^7$
4	Cow milk	Whitish	6.0	$5.8´10^6$
5	local goat milk	Whitish	6.3	$7.8´10^6$
6	Curd from cow milk	Whitish	6.6	$1.2´10^7$
7	Arong butter	Cream	6.3	$1.39´10^6$
8	Yogurt	Whitish	6.2	$3.06´10^6$
9	Milkvita butter	Cream	6.8	$3.24´10^5$
10	Slice cheese	Cream	6.0	$3.0´10^6$
11	Local cheese	Whitish	6.2	$2.4´10^6$
12	Cow milk	Whitish	6.6	$5.8´10^6$

Table 2. *Colony morphology of the selected isolates*

No.ofIsolates	Cultural characters					Slant Characters
	Form	Color	Elevation	Margin	Surface	
TM_2/a_1	Circular	Cream	Raised	Entire	Smooth	Echinulate
TM_3/a	Circular	Whitish	Convex	Entire	Smooth	Echinulate
TM_3/b	Circular	Whitish	Convex	Entire	Rough	Echinulate
TM_3 /c	Circular	Whitish	Convex	Entire	Smooth	Filiform
TM_4/d	Filamentous	Whitish	Flat	Filamentous	Rough	Umbonate
TM_6/a	Circular	Whitish	Round	Entire	Smooth	Echinulate
TM_6/b	Circular	Whitish	Flat	Entire	Smooth	Filiform
TM_6/c	Circular	Whitish	Flat	Entire	Smooth	Echinulate
TM_6/d	Circular	Whitish	Flat	Entire	Smooth	Echinulate
TY_2/f	Circular	Cream	Convex	Entire	Smooth	Filiform
TY_3/b	Punctiform	Transparent	Convex	Entire	Smooth	Beaded
TY_4/a	Circular	Whitish	Raised	Entire	Smooth	Spreading
TY_5/b	Circular	Cream	Convex	Entire	Smooth	Echinulate
TY_6/e	Circular	Whitish	Round	Entire	Smooth	Beaded
TY_6/f	Circular	Whitish	Raised	Entire	Smooth	Echinulate

Table 3. *Microscopic features and staining characteristics of the selected isolates.*

No.ofIsolates	Vegetative cells Form	Staining Arrangement	Gram	Spore
TM_2/a_1	Short rod	Single and in pair	Positive	Non-spore former
TM_3/a	Rod	Single and in pair	Positive	Non-spore former
TM_3/b	Rod	Single, pair & in chain	Positive	Non-spore former
TM_3/c	Rod	Single, pair & in long chain	Positive	Non-spore former
TM_4/d	Short rod	Single, pair & in chain	Positive	Non-spore former
TM_6/a	Short rod	Single & in chain	Positive	Non-spore former
TM_6/b	Short rod	Single & in pair	Positive	Non-spore former
TM_6/c	Short rod	Single, pair & short chain	Positive	Non-spore former
TM_6/d	Short rod	Single & in pair	Positive	Non-spore former
TY_2/f	Spherical or ovoid	Single, pair & in short chain	Positive	Non-spore former
TY_3/b	Cocci	Single & in pair	Positive	Non-spore former
TY_4/a	Spherical or ovoid	Single	Positive	Non-spore former
TY_5/b	Cocci	Single & in pair	Positive	Non-spore former
TY_6/e	Cocci	Single	Positive	Non-spore former
TY_6/f	Spherical or ovoid	Single, pair and short chain	Positive	Non-spore former

Table 4. *Biochemical characteristics of the selected isolates*

No .of Isolates	Catalase Test	Motility Test	Starch Hydrolysis	Casein hydrolysis	H_2S production	Indole test	Methyl red test	Voges-Proskauer	Citrate utilization	Sugar Fermentation Test									
										Glucose	Fructose	Sucrose	Maltose	Lactose	Galactose	Mannitol	Innulin	Raffinose	Xylose
TM_2/a_1	-	-	-	+	+	-	-	-	+	A	A	A	A	A	A	A	A	-	-
TM_3/a	-	-	-	+	+	-	-	-	+	A	A	-	A	-	-	A	-	-	-
TM_3/b	-	-	-	-	+	-	-	-	+	A	A	A	A	A	A	A	A	A	-
TM_3/c	-	-	-	-	+	-	-	-	+	A	A	A	A	A	Al	A	A	-	A
TM_4/d	-	-	+	+	+	-	-	-	+	A	A	A	A	A	A	A	A	A	A
TM_6/a	-	-	-	+	-	-	-	-	+	A	A	A	-	Al	-	-	A	A	-
TM_6/b	-	-	-	-	+	-	-	-	+	A	-	-	-	-	-	A	A	-	-
TM_6/c	-	-	-	-	+	-	-	-	+	A	A	A	-	Al	-	-	-	-	-
TM_6/d	-	-	-	+	+	-	-	-	+	A	A	A	-	Al	A	A	-	A	A
TY_2/f	-	-	+	-	-	-	-	-	+	A	A	A	-	A	A	Al	-	Al	-
TY_3/b	-	-	-	-	+	-	-	-	+	A	A	A	-	A	A	A	A	A	-
TY_4/a	-	-	+	-	-	-	-	+	+	A	A	A	A	A	A	A	A	A	A
TY_5/b	-	-	+	-	+	-	-	-	+	Al	Al	A	A	A	A	Al	Al	A	Al
TY_6/e	-	-	-	-	+	-	-	-	+	A	A	A	A	A	A	A	-	-	A
TY_6/f	-	-	+	-	+	-	-	-	+	A	A	A	A	Al	A	A	A	A	-

Note: '+' = positive, '-' = negative, A= Acid, Al=Alkali

Table 5. *Diameter of zone of inhibition (mm) produced by Lactobacillus isolates on the test Gram positive bacteria.*

No. of isolates	Test organism			
	Bacillus cereus	*Bacillus subtilis*	*Bacillus megaterium*	*Staphylococcus aureus*
TM_2/a_1	-	-	-	-
TM_3/a	8.5	22	7	16.5
TM_3/b	9.5	15.6	10	16
TM_3/c	9.2	-	-	9.5
TM_4/d	-	-	-	9
TM_6/a	6	10.1	11	10.5
TM_6/b	8	10	8.5	12.5
TM_6/c	13.5	15	7.5	15
TM_6/d	10	8	11	16

Table 6. *Diameter of zone of inhibition (mm) produced by Lactobacillus isolates on the test Gram negative bacteria*

No. of isolates	Test organism					
	E. coli	*Shigella dysenteriae*	*Salmonela typhi*	*Salmonella paratyphi*	*Vibrio cholerae*	*Pseudomonas aeruginosa*
TM_2/a_1	-	-	-	9	6	10.5
TM_3/a	16	12.5	29.1	30.3	13	14.5
TM_3/b	12.5	12.5	25.8	23.6	-	12.2
TM_3/c	-	9	8.5	11	11	8
TM_4/d	16	8	10	8	10	10.5
TM_6/a	25	8.5	11	12.2	10.5	12.5
TM_6/b	8.5	9.5	7.5	11	12	14.5
TM_6/c	11.5	11	13.2	11.1	9	14
TM_6/d	-	8.5	11.5	13	6.5	11

Table 7. *Diameter of zone of inhibition (mm) produced by Streptococcus isolates on the test Gram positive bacteria as assessed by disc diffusion method.*

No. of isolates	Test organism			
	Bacillus cereus	*Bacillus subtilis*	*Bacillus megaterium*	*Staphylococcus aureus*
TY_2/f	8.5	7.9	13.5	11.5
TY_3/b	10	-	-	14.5
TY_4/a	6	10	-	12
TY_5/b	8	10	7	12.5
TY_6/e	12	-	-	12
TY_6/f	13.5	8.2	7	12.4

Table 8. *Diameter of zone of inhibition (mm) produced by Streptococcus isolates on the test Gram negative bacteria*

No. of isolates	Test organism					
	E. coli	*Shigella dysenteriae*	*Salmonela typhi*	*Salmonella paratyphi*	*Vibrio cholerae*	*Pseudomonas aeruginosa*
TY_2/f	7.5	9	10	13.5	9	10
TY_3/b	10	11.5	13.5	10.5	8	14
TY_4/a	10	11.2	14	10.8	10.5	10
TY_5/b	7	9.5	8.5	11	-	11
TY_6/e	9	8.5	10	12	8	15
TY_6/f	9	9	10.5	11	11.5	13.5

faecalis (TY_6/e) showed antibacterial activity against eight test pathogenic bacteria. *L. xylosus* (TM_3/c), *L. fermentum* (TM_4/d) showed antibacterial activity against seven test pathogenic bacteria. *L. casei* subsp. *pseudoplantarum* (TM_2/a_1) and *L.salivarius* (TM_3/b) showed antibacterial activity against three and one test pathogenic bacteria respectively. Among streptococcal isolates *S. faecalis* (TY_6/e) showed the highest antibacterial activity (15mm) against *Pseudomonas aeruginosa*. The result showed that all of the fifteen isolates were active against one or more tested organisms.

All lactic acid bacteria generally show antibacterial activity against many pathogenic bacteria. These antagonistic activities of LAB were observed by many scientists. Based on the differences in inhibition zones produced by LAB cultures and the controls, *Lactobacillus* isolates were the most inhibitory to the test strains followed by *Streptococcus, pediococcus* and *Leuconostoc* isolates was reported by Ashenafi *et al.*[11]. Hütt[14] found antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uro pathogens. Srikanjana and Siriporn[15] isolated strains of *Lactobacillus fermentum* from miang and investigated their antibacterial and antioxidant activities. They found that *L. fermentum* FTL2311 and *L. fermentum* FTL10BR showed antibacterial activity against several pathogenic bacteria: *Listeria monocytogenes*, *Salmonella typhi*, *Shigella sonnei* and *Staphylococcus aureus* subsp. *aureus*. *Streptococcus thermophilus*T2 strain showed the wide inhibitory spectrum against the Gram positive bacteria[16]. Our results were in concurrence with them.

Determination of antifungal activity

In the present study, the inhibitory effect of the culture filtrates of all LAB isolates against *Penicillium sp.* and *Aspergillus flavus* were studied and shown in Table 9-10.

The result showed that *Streptococcus uberis* (TY_4/a) was the most efficient LAB which showed 10% and 15.78% fungal inhibition against *Penicillium sp*.ad *Aspergillus flavus* respectively. *L. leichmannii* (TM_6/a), *L. casei* subsp. *pseudoplantarum* (TM_2/a_1), *L. xylosus* (TM_3/c), *L. plantarum*

Table 9. *Percentage of inhibition (%) produced by Lactobacillus isolates on the test fungi*

No. of isolates	*Penicillium sp.*		*Aspergillus flavus*	
	Zone of fungal inhibition (%)	Percentage of growth (mm)T $I = \frac{(C-T)}{C} \times 100$	Zone of fungal growth (mm) T	Percentage of inhibition (%) $I = \frac{(C-T)}{C} \times 100$
TM_2/a_1	1.2	40	-	-
TM_3/a	1.5	25	-	-
TM_3/b	1.4	30	-	-
TM_3 /c	1.25	37.5	-	-
TM_4/d	-	-	-	-
TM_6/a	1.15	42.5	-	-
TM_6/b	-	-	-	-
TM_6/c	1.3	35	-	-
TM_6/d	1.25	37.5	-	-
Control(C)	2		1.9	

Table 10. *Percentage of inhibition (%) produced by Streptococcus isolates on the test fungi*

No. of isolates	*Penicillium sp.*		*Aspergillus flavus*	
	Zone of fungal inhibition (%)	Percentage of growth (mm)T $I = \frac{(C-T)}{C} \times 100$	Zone of fungal growth (mm) T	Percentage of inhibition (%) $I = \frac{(C-T)}{C} \times 100$
TY_2/f	-	-	-	-
TY_3/b	-	-	-	-
TY_4/a	1	50	1.6	15.79
TY_5/b	-	-	1.5	21.05
TY_6/e	-	-	-	-
TY_6/f	-	-	-	-
Control(C)	2		1.9	

(TM_6/d), *L. casei* (TM_6/c), *L. salivarius* (TM_3/b) and *L. homohiochii* (TM_3/a) showed 42.5%, 40%, 37.5%, 37.5%, 35%, 30% and 25% fungal inhibition against *Penicillium sp.* respectively. *S. suis* (TY_5/b) and *S. uberis* (TY_4/a) showed 21.05% and 15.78% fungal inhibition against *A. flavus* respectively. This result corroborates with the findings of Karunaratne *et al.* [17] and Gourama and Bullerman[18] who concluded that the lactic acid bacterial suspensions were active against more than one *Aspergillus* spp. Indeed, Vanne *et al.*[19] showed that the growth of toxigenic storage fungi could be restricted by LAB *in vitro*. Onilude *et al.* [20] showed the antifungal effects of *L. plantarum, L. fermentum, L.brevis* and a *Lactococcus* spp. on aflatoxigenic fungal isolates. Our results showed similarity to their findings.

Conclusion

From the above study we can conclude that, the antimicrobial activity of LABs isolated from the different milk products may be due to the production of acetic and lactic acids that lowered the pH of the medium. *Lactobacillus* isolates resulted in the higher diameter of inhibition than *Streptococcus* isolates. Among the test pathogens *S. paratyphi* and *Pseudomonas aeruginosa* were most sensitive to LAB. Besides *Lactobacillus* isolates showed higher fungal inhibititory effects against *Penicillium sp*. than *Streptococcus* isolates. Between test fungi, *Penicillium sp*. was more sensitive to LAB than *Aspergillus flavus*.This preliminarily works showed the potential application of autochthonous lactic acid bacteria to improve safety of traditional fermented food. Further study of the genetics of Lactic Acid Bacteria (LAB) may be an interesting topic in food science.

References

1. Dubey RC and Maheshwari DK. 2002. *A Text book of Microbiology*, 3rd edn, pp 408-427. S. Chand & Company Ltd. NewDelhi, India.
2. Foster EM, Nelson F E, Speck ML, Doetsch RN and Olson JC. 1958. *Dairy Microbiology,* pp 1-7. MacMillan & Co Ltd. London.
3. El Soda M, Ahmed N, Omran N, Osman G and Morsi A. 2003. Isolation, identification and selection of lactic acid bacteria cultures for cheesemaking.*Emir. J. Agric. Sci.***15**(2): 51-71.
4. Aguirre M and Collins MD. 1993. Lactic acid bacteria and human clinical infection. *J. Appl. Bacteriol*. **75**: 95-107.
5. Gilliland SE and Speck ML. 1975. Inhibition of psychotropic bacteria by lactobacilli and pediococci in non-fermented refrigerated foods. *Journal of Food Science.***40**: 903.
6. Brink ten B, Minekns M, Vander Vossen JMBM, Leer RJ. and HuisVeld JJ. 1994. Antimicrobial activity of *Lactobacillus.*--- *J. Appl. Bacteriol.***77**: 140-148.
7. Holzapfel WH, Geisen R and Schillinger U.1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Intern. J. Food Microbiol*. **24**(3):343–362.
8. Herich R and Levkut M. 2002. Lactic acid bacteria, probiotics and immune system**.** *Vet. Med. – Czech.* **47** (6): *169–180*
9. De Man JD, Rogosa M, Michell JA and Sharpe ME. 1960. A Medium for the Cultivation of Lactobacilli. *J. Appl. Bact.* **23**: 130-135.
10. Naylar J and Sharpe ME. 1958. Lactobacilli in chedder cheese.1.The use of selective media for isolation and of serological typing for Identification. *J.Dairy Res*. **25**:42.
11. Ashenafi M, Tadesse G and Ephraim E. 2005.Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita,traditional Ethiopian fermented beverages on some food borne pathogens and effect of growth medium on the inhibitory activity.*Internt. J. Food Safety*. **5**:12-20.
12. Grover RK and Moore JD. 1962. Assessment of antifungal property in vitro by poison-food technique.*J. Phytopathology*. **52**: 876.
13. Buchanan RE and Gibbons NE. 1974. *Bergey's Manual of Determinative Bacteriology*, 8th edn. The Williams and Wilkins Company, Baltimore.
14. Hütt P, Shchepetova J, Lõivukene K, Kullisaar T and Mikelsaar M. 2006. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J. ApplMicrobiol.***100**(6):1324-32.
15. Srikanjana K and Siriporn O. 2009. Antibacterial and Antioxidant Activities of Acid and Bile Resistant Strains of *Lactobacillus fermentum* Isolated from Miang. *Braz. J. Microbiol.* **40**(4): 757-766.
16. Abdelkader M, Nour-Eddine C, Abdelkader DB, Naima NA and Jean PH. 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. *J. Environ. And Public Health*. Article ID 678495, pp 1-8.
17. Karunaratne A, Wezenberg E and Bullerman LB. 1990. Inhibition of mould growth and aflatoxin production by *Lactobacillus* spp. *J. Food Protection.* **53:** 230–236.
18. Gourama H and Bullerman LB. 1995. Antimycotic and antiaflatoxigenic effects of LAB: A review. *J. Food Protection.* **57**: 1275-1280.
19. Vanne L, Kleemola T and Haikara A. 2000. Screening of the antifungal effects of Lactic Acid bacteria against toxigenic *Penicillium* and *Aspergillus* strains in attributes of lactic acid bacteria.British Mycological Society. Int. Symp. Bioactive fungal metabolites- impact and explotation, pp 100. Wales, UK.
20. Onilude AA, Fagade O E, Bello M M and Fadahunsi IF. 2005. Inhibition of aflatoxin-producing aspergilli by lactic acid bacteria isolates from indigenously fermented cereal gruels. *Afr. J. Biotechnol*. **4 (**12**)**: 1404-1408.