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Probiotic property of lactic acid bacteria from traditional fermented condiments: *Datta* and *Awaze*

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Abstract

In this study, probiotic properties consisting of acid and bile tolerance, hydrophobicity, antibacterial activity, and antimicrobial susceptibility of lactic acid bacteria (LAB) isolated from two traditional fermented condiments (*Datta* and *Awaze*) were investigated. Of 100 isolates, 24 showed 54-97% survival rates at pH 2-3 and bile salt concentration of 0.3%-1%. Of the acid and bile tolerant isolates, only five showed marked hydrophobicity indicating tissue adherence potential . These five isolates inhibited test pathogenic bacteria to varying extents. These LAB isolates were susceptible to chloramphenicol, ampicillin, clindamycin, erythromycin, gentamycin, tetracycline and penicillin but resistant to vancomycin. All the five isolates were able to survive at levels of log 6 cfu/ml for >96 days in *Datta* stored at 4°C. These isolates belonged to LAB genera comprising *Lactococcus* (2) *Leuconostoc* (2) and *Lactobacillus* (1).

Introduction

Probiotic lactic acid bacteria (LAB) are known to inhibit the growth of food-borne pathogenic microorganisms such as *Escherichia coli, Salmonella typhi, Salmonella typhimurium* and *Staphylococcus aureus* (Tadesse *et al*., 2005; Klayraung *et al.*, 2008; Tesfaye *et al*., 2011), *Shigella flexneri* (Tadesse *et al.*, 2005), *Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumoniae, Proteus* spp., *Corynebacterium* spp., and *Streptococcus pneumoniae* (Al-Allaf *et al*., 2009).

Several studies demonstrated the inhibitory effect of LAB, isolated from various Ethiopian traditional fermented foods, condiments and alcoholic beverages against some food-borne pathogens (Bacha *et al*., 2009; Dessalegn and Ashenafi, 2010; Tesfaye *et al*., 2011) and they also evaluated the *in-vitro* (Bacha *et al.*, 2009; Dessalegn and Ashenafi, 2010) and in-vivo (Tesfaye *et al.*, 2011) probiotic properties of lactic acid bacteria. The behavior of *E. coli* O157:H7 in fermenting and cold stored Datta and Awaze was also reported by Tsegaye *et al.* (2004). However there are still few research data available on the probiotic properties of LAB from condiments. Most of the traditional fermented products of Ethiopia are consumed without further heat processing. Thus they are ideal vehicles to carry probiotic bacteria into the human gastrointestinal tract.

Datta and Awaze are popular fermented condiments in Ethiopia. They are usually consumed along with raw or roasted meat and other cereal pancakes on the basis of their desirable aroma and flavor (Idris *et al*., 2001). Datta, also known as Qotchqotcha in Oromiffa, is prepared by fermenting green or fully ripened red chilli *(Capsicum frutescens)* to which a variety of spices are added. The major substrates in Awaze are red pepper *(Capsicum annum)*, garlic *(Allium ursinum)*, and ginger *(Zingber officinale)* with various other spices added to them.

The purpose of this study was, therefore, to evaluate the *in-vitro* probiotic properties of LAB isolated from two Ethiopian fermented condiments, Datta and Awaze, with respect to their acid and bile tolerance, adherence capacity by cell surface hydrophobicity, inhibitory activity against some food-borne pathogens, and antibiotic susceptibility. The storage survivability of LAB isolates with probiotic potential would also to determined in the condiments at refrigerator temperature (4°C).

Materials and Methods

Sample collection and processing

A total of 10 samples of Datta (6) and Awaze (4) were collected from different venders and

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supermarkets in Addis Ababa. Microbiological analysis was conducted within 3-6 h of arrival at the Laboratory. Twenty five grams of each sample was mixed with 225 ml of 0.1% (w/v) sterile peptone water and serially diluted for plating.

From appropriate dilution, aliquots of 0.1 ml were separately spread-plated on pre-dried surfaces of MRS Agar (Oxoid) plates in duplicates. The plates were incubated anaerobically using anaerobic jar (BBL, Gas Pak Anaerobic System) at 30-32^{0}C for 48 h. After incubation, colonies that were growing on the surface of medium were counted as lactic acid bacteria (LAB). Ten colonies per sample were randomly picked from countable MRS plates and transferred in to 5 ml MRS broth (Oxoid). Then, further purification was made by repeated streaking on MRS agar plates. The pure cultures were streaked on slants of MRS Agar and kept at 4^o for further characterization.

LAB grouping

Cultures were grown overnight and wet mounted on microscopic slides. Cell shape and cell arrangement were examined under light microscope (Reichert Neovar) using oil immersion objectives (1000x). Gram characteristics of the isolates were determined by the KOH method as in Gregersen (1978). Catalase test was conducted on 24 h old pure cultures using 3% H_2O_2 (Kovacs, 1956). Homofermentative or heterofermentative nature of the isolates was assessed by production of gas from MRS broth containing 5% glucose (Nair and Surendran, 2005). Colonies identified as LAB were tentatively grouped into their respective genera as *Lactobacillus, Leuconostoc, Lactococcus, Enterococcus* and *Pediococcus* by colony characteristics, cell shape and glucose fermentation.

Determination of pH

The pH of the samples was determined by using a digital pH meter (Model: NIG 333, NAINA Solaris LTD).

Determination of titratable acidity (TA)

The titratable acidity was measured from the 1/10 dilutions of the Awaze and Datta samples following the methods of Antony and Chandra (1997). The titratable acidity (TA), expressed as gram lactic acid/100 grams of Awaze or Datta and was calculated using the following formula.

$$TA = N\ NaOH \times Vol.\ NaOH \times 0.09 \times 100$$

Vol. of sample

Determination of moisture content

To determine the moisture content of the sample, known weight of the fresh sample was kept in an oven at 80^{o}C and allowed to dry to a constant weight. Moisture content was calculated using the following formula.

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh mass - weight of dried mass} \times 100}{\text{Weight of fresh mass}}$$

Acid tolerance test

To screen for acid tolerance, 10ml MRS broth (adjusted to pH 2.5) was separately inoculated with an overnight culture of the selected LAB isolates to give a final population of 10^8-10^9cfu/ml. These were incubated at 37^{o}C for 90 minutes. An inoculated MRS broth without pH adjustment served as a control. Survival was determined by single streaking of broth culture on MRS agar plates, and the growth was observed after 24-48 h of anaerobic incubation at 37^{o}C. Isolates which grew on the agar media were considered as acid tolerant. These isolates were further cultivated in MRS broth under anaerobic condition at 37°C for 24 h. Cultures were separately inoculated in 10 ml of 0.05M sodium phosphate buffer adjusted to pH 2.0, or 3.0 to give a final population of 10^8-10^9cfu/ml. An inoculated sodium phosphate buffer without pH adjustment served as a control. Samples were incubated at 37^{o}C for 2 h. An appropriate dilution of the culture was plated onto MRS agar and incubated for 48 h for determination of the count of viable cells. The survival rate was calculated as the percentage of colonies grown on MRS agar compared to the initial cell concentration. Each experiment was conducted in triplicate.

Bile tolerance test

Screening of selected LAB isolates for bile tolerance was carried out by separately inoculating the selected isolates in MRS broth containing 0.3% of bile salt (Oxoid) to give a final population of 108-109 cfu/ml. This was followed by anaerobic incubation at 37°C for 24 h. Survival of isolates was determined by single streaking on MRS agar plates. Isolates which grew on the agar media were considered as bile tolerant. These isolates were cultivated in MRS broth under anaerobic condition at 37°C for 24 h. The cultures were separately inoculated in MRS broth containing higher concentrations of 0.5 and 1.0% (w/v) of bile salt to get a final population of 10^8-10^9 cfu/ml. MRS broth without bile salt was used as a

control. Inoculated broth tubes were incubated under anaerobic condition at 37°C for 24 h. Surviving cells were counted on MRS agar plates after incubation at 37°C for 48 h. The survival rate of each strain was expressed as the percentage of viable cells in the presence of bile salt compared to that without bile salt. The experiment was performed in triplicate and the mean values were calculated.

Cell surface hydrophobicity

The *in-vitro* microbial cell surface hydrophobicity was evaluated by adherence to a non-polar solvent according to Heravi *et al.* (2011). Cultures in stationary phase (18-24 h) were centrifuged at 5,000 rpm for 10 min, washed twice, re-suspended in PBS and their absorbance was adjusted to 0.6 - 0.64 at 600 nm (A0) (UV-7804C Ultraviolet-Visible Spectrometer). A volume of 1 ml of p-xylene (Riedel-de Haen, Germany; cat.16469) was added to 2 ml of adjusted cell suspension. After 10 min of pre-incubation at room temperature, the two-phase system was mixed on a vortex for 2 min. To allow complete phase separation of the mixture, the aqueous phase was carefully removed after 15 min and the separated aqueous phase was incubated at 37°C for 30 min and its absorbance was measured at 600 nm (A). Hydrophobicity was calculated as the percentage decrease in the optical density of the initial aqueous bacterial suspension due to cell partitioning into a hydrocarbon layer. This experiment was conducted in triplicates. The percentage of cell surface hydrophobicity (%H) of the strain adhering to p-xylene was calculated using the equation: $\%H = [(A_0 - A)/A_0) \times 100]$.

Antibacterial activity

The antimicrobial activity of the selected LAB isolates (cell free filtrate) against *Escherichia coli, Shigella boydii, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Staphylococcus aureus* was tested by the Agar well diffusion assay (Schillinger and Lucke, 1989). The test pathogens were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI). A volume of 1.5 ml overnight cultures of LAB was transferred to 2 ml capacity Eppendrof tube and centrifuged at 5,000 rpm for 10 minutes. The cell-free filtrate was removed from cell pellets carefully. The pathogenic test bacteria were incubated in Nutrient broth (Oxoid) at 37°C for 24 h. A volume of 0.1 ml of 24 h broth culture of pathogenic bacteria was spread on pre-dried surfaces of Muller Hinton Agar (Oxoid) plates. Four wells were made using sterile cork-borer on previously inoculated Muller Hinton Agar and each well was filled with 100 µl of cell-free filtrate of LAB isolates. The plates were incubated at 37°C for 24 h. Then the diameter of inhibition zone was measured by calipers in mm. The antimicrobial activity was determined by measuring the clear zone around the wells (Saranya and Hemashenpagam, 2011). This experiment was conducted in triplicates.

Antimicrobial susceptibility assay

The antimicrobial susceptibility test for the selected LAB isolates was conducted by the disc diffusion method (CLSI, 2011). Ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (10 µg), penicillin (6 µg), clindamycin (2 µg), tetracycline (30 µg), and vancomycin (30 µg) antimicrobial discs were employed. Inoculum density of LAB isolates was adjusted to McFarland 0.5 turbidity standard (equivalent to cell density of ca:108 cfu/ml). A sterile cotton swab was dipped into the adjusted suspension and swabbed over the entire surface of pre-dried Mueller-Hinton agar plate. The antimicrobial discs were dispensed onto the surface of the inoculated agar plates. The plates were incubated at 35 ± 2°C for 24 h and the diameter of the zones of complete inhibition was measured.

Survival of LAB in Datta at 4°C.

The survival of isolates showing the best probiotic properties in fermented Datta stored at refrigeration temperature (4°C) was determined according to Jayamanne and Adams (2006). Datta was purchased from Supermarkets around Arat killo and pasteurized in water bath at 80°C for 10 minutes. An overnight culture of LAB was introduced into 250 g portion of pasteurized Datta to give a final inoculums level of 10^7 cfu/g. Another flask with 250 g pasteurized Datta without inoculum served as a control. The flasks were stored in the refrigerator (4°C) and samples were taken every 4 days to determine the population of probiotic isolates. A sample of one gram of Datta was aseptically withdrawn and serially diluted using 9 ml of 0.1% (w/v) sterile peptone water. Volumes of 100 µl was spread onto duplicate MRS agar plates and counted after anaerobic incubated at 37°C for 48 h. Absence of growth from control samples on MRS plates indicated elimination during pasteurization. This experiment was conducted in duplicates.

Data analysis

IBM SPSS (version 20, SPSS Inc, Chicago, IL, USA, 2012) was used to analyze mean, standard deviation and standard error of the mean. Independent T Test was performed for comparison of the mean at ($p<0.05$) using the same program.

Results

Bacterial isolation

The pH values of the condiments samples were within pH 3.6 and 5.0, with an average pH of 4.1 for Datta samples and pH 4.3 for Awaze samples. The average titratable acidity values of Datta and Awaze samples were 0.1% and 0.2%, respectively. Datta anf Awaze had average moisture content of 20.1% and 30.9%, respectively. The average LAB count of Awaze was 1.0 x 10^8 cfu/ml and that of Datta was 8.4 x10^7 cfu/ml.

Of the 100 isolates selected from the two types of fermented condiments and purified, 62 were identified as LAB. The LAB consisted of 55 cocci and seven rods. Glucose fermentation experiments showed that, among the cocci, 46 were homofermentative and, thus, belonged to the *Lactococcus* spp, and nine were heterofermentative and belonged to *Leuconostoc* spp. The rods were grouped under *Lactobacillus* and consisted of both homofermentative and heterofermentative isolates. Awaze yielded only *Leuconostoc* isolates whereas all the three types of LAB were isolated from Datta.

Acid tolerance

The acid tolerance test showed that 87% of the isolates survived at pH 3 and 81% at pH 2 to varying degrees for two hours. Eight isolates showed over 90% survival rate at pH 3 whereas the population of three isolates decreased markedly with time of exposure. Four resistant isolates at pH 3 were sensitive when exposed to pH 2. At pH 2, only 13 isolates (54%) showed resistance to the reduced pH for two hours. Eleven isolates (46%) did not show marked difference in their tolerance to pH 2 and pH 3.

Bile tolerance

Variations were observed among isolated in their tolerance to bile salt. Over 60% of the isolates showed a survival rate of more than 85% when exposed to 0.5% bile salt for 24 hrs. Eight isolates were the most resistant showing 90-97% survival rate. The isolates also showed different levels of tolerance to 1% bile salt concentration although 16 of them did not show much difference in their tolerance to the two bile concentrations.

Cell surface hydrophobicity

A total of 24 acid and bile tolerant isolates were selected and evaluated for *in-vitro* determination of microbial adhesion to p-xylene. The isolates showed hydrophobicity varying from 1.5% to 99.2% (Table 1). Eleven isolates showed higher than 75% hydrophobicity. The highest hydrophobicity observed was 99.2%.

Table 1. Mean values of hydrophobicity of tested isolates

Isolates	Genus	Hydrophobicity (%)
LD6	*Lactococcus*	7.8 ± 1.6
LD8 *	*Lactococcus*	91.9 ± 0.3
LD10 *	*Lactococcus*	18.9 ± 4.2
LD11 *	*Lactococcus*	81.1 ± 8.0
LD24 *	*Lactococcus*	17.5 ± 2.5
LD33	*Lactococcus*	5.6 ± 5.9
LD35 *	*Lactococcus*	31.3 ± 4.8
LD41 *	*Lactococcus*	35.2 ± 0.1
LD43	*Lactococcus*	3.9 ± 1.8
LD45 *	*Lactobacillus*	31.6 ± 2.5
LD46	*Lactobacillus*	7.7 ± 4.7
LD47 *	*Lactobacillus*	83.7 ± 2.0
LD55	*Lactococcus*	2.4 ± 1.5
LD56	*Lactococcus*	8.1 ± 1.6
LD59	*Lactococcus*	6.5 ± 3.2
LD60	*Lactococcus*	1.5 ± 0.6
LD52 *	*Leuconostoc*	94.3 ± 2.5
LD25 *	*Leuconostoc*	99.2 ± 0.8
LD14 *	*Leuconostoc*	96.3 ± 0.6
LD18 *	*Leuconostoc*	75.9 ± 4.8

*Isolates selected for antimicrobial testing

In general, eight isolates showed desirable probiotic properties in terms of overall resistance to the tested pH values and bile salt concentrations and, at the same time, a higher degree of hydrophobicity. Most of the potential probiotic isolates belonged to the genus *Leuconostoc* and *Lactobacillus*. Although *Lactococcus* spp were the most dominant isolates from Datta and Awaze samples, they was represented by a few potential probiotic isolates.

Antibacterial activity

All *Lactococcus*, and *Lactobacillus* isolates with potential probiotic properties inhibited all test organisms, except *S. aureus*. *Leuconostoc* isolates were able to inhibit only *P. aeruginosa* (Table 2).

Antibiotic susceptibility assay

A total of 11 isolates were tested for their antibiotic susceptibility to eight drugs (Table 3). All isolates were susceptible to chloramphenicol, clindamycin, erythromycin, tetracycline, gentamycin and ampicillin. Susceptibility to penicillin and vancomycin was seen in ten and five isolates, respectively (data not given).

Determination of survival of isolates in Datta stored at 4°C

The effect of refrigeration storage on the survival of five selected LAB isolates was determined. The five isolates were selected based on their overall probiotic properties (Table 3). The count of the isolates decreased only by less than 2 log units during the 96 days of storage (Figure 1).

Table 2. Antimicrobial activity (mean inhibition diameter) of isolates against the test organisms

Isolates	Genus	Inhibition zone (mm) of test pathogens[a]				
		E. coli ATTC 25922	*S. boydii* ATTC 9289	*S. typhimurium* ATTC 13311	*P. aeruginosa* ATTC 27852	*S. aureus* ATTC 25923
LD8	*Lactococcus*	9.0 ± 1.0	13.7 ± 0.6	11.3 ± 0.6	14.3 ± 0.6	-
LD10	*Lactococcus*	10.3 ± 0.6	13.3 ± 0.6	10.3 ± 0.6	16.3 ± 1.5	-
LD14	*Leuconostoc*	-	-	-	14.3 ± 0.6	-
LD18	*Leuconostoc*	-	-	-	11.0 ± 1.0	-
LD24	*Lactococcus*	11.7 ± 0.6	12.0 ± 0.6	12.0 ± 1.0	14.0 ± 1.0	-
LD25	*Leuconostoc*	-	-	-	9.7 ± 0.6	-
LD35	*Lactococcus*	12.3 ± 0.6	14.0 ± 1.0	14.0 ± 1.0	14.3 ± 0.6	-
LD41	*Lactococcus*	11.3 ± 0.6	13.7 ± 0.6	13.7 ± 0.6	13.7 ± 0.6	-
LD45	*Lactobacillus*	10.3 ± 0.6	12.3 ± 0.6	10.7 ± 0.6	14.3 ± 0.6	-
LD47	*Lactobacillus*	11.3 ± 0.6	14.3 ± 1.2	13.0 ± 1.0	16.7 ± 0.6	-
LD52	*Lactococcus*	10.3 ± 0.6	13.7 ± 0.6	11.3 ± 0.6	11.3 ± 0.6	-

Discussion

The pH and titratable acidity values of Datta samples in this study were in agreement with the observation of Idris *et al.* (2001), who recorded pH values of 3.7-5.0 and titratable acidity values of 0.1-0.3%. No significant difference ($p > 0.05$) was observed in counts of lactic acid bacteria between the Datta or Awaze samples in our study. Similar to the finding of Idris et al. (2001), Awaze fermentation in our study was initiated by LAB levels of log 6 cfu/g and, as observed by Idris *et al*. (2001), it reached the maximum counts of log 9 cfu/g. The counts remained greater than log 8 cfu/g during the period of fermentation. The LAB counts during the fermentation of Awaze and Datta in our study were within the ranges of the counts observed by Dessalegn and Ashenafi (2010).

During Datta fermentation, homofermentative LAB initiated and dominated the fermentation for the first two days and the heterofermentative LAB took over thereafter. This observation was similar to the findings of Idris *et al.* (2001) and Dessalegn and Ashenafi (2010). On the other hand, our Awaze isolates were initiated and dominated by heterofermentative LAB, which was in agreement with the observation of Idris *et al.* (2001) but different from that of Dessalegn and Ashenafi (2010). The absence of *Pediococcus* and the dominant appearance of Lactococcus in the fermenting samples could be due to the chemical nature and composition of the raw materials which contained different spices and a considerable amount of salt.

Most of our LAB isolates showed higher survival rate (>75%) at pH 3.0 but failed to a lowered pH value of 2.0. Nevertheless, considerable number of our isolates also showed strong survival at pH 2.

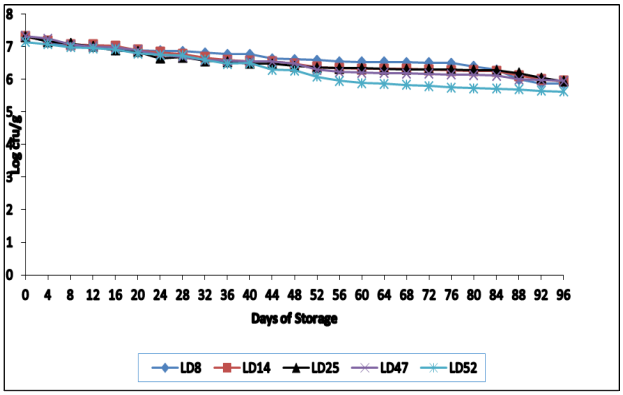

Figure1. Survival of LAB isolates in Datta at refrigerator temperature (4°C)

Mishra and Prasad (2005) also observed that LAB showed higher survival rate at a raised pH (3.0) than at pH 2. In another study on fermented pork, fish, tea leaves and garlic, raising the pH to 3.0 resulted in a higher survival rate (>75%) of most LAB isolates (Klayraung *et al.*, 2008). It is worth noting that of the LAB isolates which tolerated pH 2, 98% were obtained from Datta samples. These could possibly be due to fact that LAB stress response to low moisture content, and consequently low aw, in Datta could also result in tolerance to lower pH. This property is important to warrant passage of ingested probiotic LAB through the highly acidic environment of the stomach.

Another crucial property of probiotic LAB is tolerance to bile in the small intestine. The physiological concentration of bile salts in the small intestine is considered to be within the range of 0.2% to 2.0% (Gunn, 2000). In our study, >50% of isolates survived well in 0.5% bile salt concentration similar to results reported by Klayraung *et al.* (2008). Similar to the observation of the same workers (Klayraung *et al.*, 2008), a gradual decrease of viable cells was

Table 3. A summary of properties of potentially probiotic isolates

probiotic property	Tested against	Potentially probiotic LAB Isolates				
		LD8	LD14	LD25	LD47	LD52
	pH 2	70	59	72	83	71
	pH 3	71	88	88	92	90
	0.5%	86	95	95	78	97
	1.0%	70	93	93	77	73
Hydrophobicity (%H)	% Adherence	92	96	99	84	94
Antibacterial activity (mm of inhibition)	*E. coli*	9.0±1.0	-	-	11.3±0.6	10.3±06
	S. boydii	13.7±0.6	-	-	14.3±1.2	13.7±0.6
	S. typhimurium	11.3±0.6	-	-	13.0±1.0	11.3±0.6
	P.aeurginosa	14.3±0.6	14.3±0.6	9.7±0.6	16.7±0.6	11.3±0.6
	S. aureus	-	-	-	-	-
Antibiotics Susceptibility	Amp (10 µg)	S	S	S	S	S
	Chl (30 µg)	S	S	S	S	S
	Cli (2 µg)	S	S	S	S	S
	Ery (15 µg)	S	S	S	S	S
	Gen (10 µg)	S	S	S	S	S
	Tet (30 µg)	S	S	S	S	S
	Pen (6 µg)	S	S	S	S	S
	Van (30 µg)	S	S	S	S	R
Survival at 4°C	Time (days)	96	96	96	96	64

Amp, Ampicillin; Chl, Chloramphenicol; Cli, Clindamycin; Ery, Erythromycin; Gen, Gentamycin; Tet, Tetracycline; Pen, Penicillin; Van, Vancomycin.
S, Susceptible; R, Resistant.

observed when the concentration of bile salt was increased up to 1.0%. Our isolates, however,showed stronger bile tolerance at 1.0% bile concentration than those in Papamanoli *et al*. (2003) and Klayraung *et al.* (2008).

LAB, after successful passage over the hurdles presented by the stomach and the small intestine, have to establish themselves through adhesion to epithelial cells of the large intestine where they can manifest their probiotic properties. We used hydrophobicity to indicate adhesion properties in this study as the positive relationships between hydrophobicity and adhesion were reported by different researchers (Rosenberg *et al*., 1983 and Klayraung *et al*., 2008). Eight of our LAB isolates showed more than 75% hydrophobicity and this agreed with the results of Heravi *et al*. (2011).

The probiotic activity of our screened LAB isolates was assessed by their antimicrobial activity on known foodborne pathogens. The results revealed that all isolates had varying levels of inhibition towards the test pathogens. According to Schillinger and Luck (1989), diameters of >0.5 mm of clear zone around the colonies of the producer strain were scored positive for inhibition. Thirteen of our isolates inhibited four test pathogens at various extent. Similarly, Klayraung *et al.* (2008) and Saranya and Hemashenpagam (2011) showed inhibition of similar pathogens by LAB at varying degrees.

The antibiotic susceptibility pattern of lactic acid bacteria is important because bacteria used as probiotics may serve as host for antibiotic resistant genes which can horizontally transfer to the pathogenic bacteria. All our isolates were susceptible to the various antibiotics tested in this study. This observation was in agreement with that of Liu *et al.* (2009) where their isolates were susceptibility to most of the drugs used in our study. Most of our isolates were resistant to vancomycin, as reported by Dessalegn and Ashenafi (2010) but susceptible to penicillin as in Danielson and Wind (2003). This same susceptibility may be of a disadvantage, however, if the host takes orally administered antibiotics which may eventually eliminate established probiotic LAB.

To have any effect, probiotic bacteria must remain viable until they reach the large intestine (Jayamanne and Adams, 2006). This would require survival in the food vehicle until consumption (Kailasapathy and Rybka, 1997). Food vehicles must, thus, contain at least log 6 cfu/g of probiotic bacteria at consumption (Samona and Robinson, 1994). Our potentially probiotic LAB isolates were able to survive in fermented Datta for three months under refrigeration temperature at a level of log 6 cfu/g. This indicated that the functionality of our potentially probiotic LAB would be maintained if the product had to be stored for a considerable time under refrigeration conditions. Similarly, Jayamanne and Adams (2006) kept probiotic bacteria for five weeks in skimmed milk, and Adhitama *et al*. (2012) for four weeks in probiotic yoghurt at a level >log 6 cfu/ml at refrigeration temperature. The survival of our LAB

isolated for a much longer time in fermented Datta qualifies the product to be an appropriate vehicle for probiotic bacteria.

References

Adhitama, G., Maheswari, R. and Wulandari, Z. 2012. Microbiological quality of probiotic yoghurt jelly drink during storage in refrigerator. Proceeding of the 2nd International Seminar on Animal Industry, p. 631. Jakarta.

Al-Allaf, M., Al-Rawi, A. and Al-Mola, A. 2009. Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria. Iraqi Journal of Veterinary Science 23: 115-117.

Antony, U. and Chandra, T. 1997. Microbial population and biochemical changes in fermenting finger millet *(Eleusine coracana)*. World Journal of Microbiology and Biotechnology 13: 533-537.

Bacha, K., Mehari, T. and Ashenafi, M. 2009. *In vitro* probiotic potential of some Lactic Acid Bacteria (LAB) isolated from Wakalim, a traditional Ethiopian fermented beef sausage. Ethiopian Journal of Health Sciences 19: 21-27.

CLSI 2009. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standards (10th edn.). CLSI (Clinical and Laboratory Standards Institute). M02-A10, Vol. 26: No. 1.

Danielson, M. and Wind, A. 2003. Susceptibility of Lactobacillus species to antimicrobial agents. Journal of Applied Bacteriology 73: 31-36.

Dessalegn, A. and Ashenafi, M. 2010. Evaluation of the Probiotic Properties and Antibiotic Resistance of Lactic Acid Bacteria Isolated from Awaze, Qotchqotch and Teff dough, traditional Ethiopian fermented foods. Internet Journal of Food Safety 12: 187-191.

Gunn, J. 2000. Mechanisms of bacterial resistance and response to bile. Microbes and Infection 2: 907-913.

Heravi, R., Kermanshahi, H., Sankian, M., Nassiri, M., Moussavi, A., Nasiraii, L. and Varasteh, A. 2011. Screening of *Lactobacilli* bacteria isolated from gastrointestinal tract of broiler chickens for their use as probiotic.African Journal of Microbiological Research 5: 1858-1868.

Idris, A., Mehari, T. and Ashenafi, M. 2001. Some microbiological and biochemical studies on the fermentation of ''Awaze'' and ''Datta'', traditional Ethiopian condiments. International Journal of Food Science and Nutrition 52: 5-14.

Jayamanne, V. and Adams, M. 2006. Determination of survival, identity and stress resistance of probiotic bifidobacteria in bio-yoghurts. Letters in Applied Microbiology 42: 89-194.

Kailasapathy, K. and Rybka, S. 1997. *Lactobacillus acidophilus* and *Bifidobacterium* spp. their therapeutic potential and survival in yoghurt. Australian Journal of Dairy Technology 52: 28-35.

Klayraung, S., Viernstein, H., Sirithunyalug, J. and Okonogi, S. 2008. Probiotic properties of lactobacilli isolated from thai traditional food. Scientia Pharmaceutica 76: 485-503.

Liu, C., Zhang, Z., Dong, K., Yuan, J. and Guo, X. 2009. Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomedical and Environmental Sciences 22: 401-412.

Mishra, V. and Prasad, D. 2005. Application of *in vitro* methods for selection of *Lactobacillus casei* strains as potential probiotics. International Journal of Food Microbiology 103: 109-115.

Nair, P. and Surendran, P. 2005. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. Journal of Culture Collections 4: 48-52.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E. and Kotzekidou, P. 2003. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat Science 65: 859-867.

Rosenberg, M., Judes, H. and Weiss, E. 1983. Cell surface hydrophobicity of dental plaque microorganisms in situ. Infection and Immunity 42: 831-834.

Samona, A. and Robinson, R. 1994. Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. Journal of the Society of Dairy Technologist 47: 58-60.

Saranya, S. and Hemashenpagam, N. 2011. Antagonistic activity and antibiotic sensitivity of Lactic acid bacteria from fermented dairy products. Advances in Applied Science Research 2: 528-534.

Schillinger, U. and Lucke, F. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Applied and Environmental Microbiology 55: 1901-1906.

Tadesse, G., Ephraim, E. and Ashenafi, M. 2005. Assesment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shameta, traditional Ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. Internet Journal of Food Safety 5: 13-20.

Gregersen, T. 1978. Rapid methods for distinction of Gram-negative and Gram-positive bacteria. European Journal of Applied Microbiology and Biotechnology 5: 123-127.

Tesfaye, A., Mehari, T. and Ashenafi, M. 2011. Evaluation of the in vitro and *in vivo* probiotic qualities of Lactic Acid Bacteria (LAB) recovered from locally fermented products. . International Journal of Probiotics and Prebiotics 6: 1-8.

Tsgaye, M., Ephraim, E. and Ashenafi, M. 2004. Behaviour of *Escherichia coli* O157:H7 during the fermentation of Datta and Awaze, traditional Ethiopian fermented condiments, and during product storage at ambient and refrigeration temperatures. Food Microbiology 21: 743-751.