academicJournals

Vol. 8(13), pp. 1419-1425, 26 March, 2014
DOI: 10.5897/AJMR2014.6639
Article Number: D80ED0B46925
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation and identification of probiotic lactic acid bacteria from curd and *in vitro* evaluation of its growth inhibition activities against pathogenic bacteria

Estifanos Hawaz

Microbiology Laboratory, Department of Biology, College of Natural and Computational Science, Haramaya University, Ethiopia.

Received 16 January, 2014; Accepted 10 March, 2014

The aim of this study was to isolate and identify probiotic lactic acid bacteria from curd and evaluate *in vitro*, its growth inhibition activities against pathogenic bacteria. A total of nine strains of *Lactobacillus* were isolated from curd and identification of strains was done by biochemical and physiological tests and *Lactobacillus leichmannii, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus coagulans, Lactobacillus acidophilus, Lactobacillus lactis* and *Lactobacillus rhamnosus* strains were identified from curd. *Lactobacillus* strains survival were also assessed under conditions simulating human GI tract. Therefore, resistance to antibiotics, resistance to low pH, resistance to bile salt and bile salt hydrolysis was performed. Results showed that all tested isolates were able to grow at low pH 3.0, and at 0.3% bile concentration. *L. casei, L. delbrueckii* and *L. brevis* showed more resistance to antibiotics. According to haemolytic activity, all examined strains did not exhibit β-haemolytic activity when grown in Columbia human blood agar. Regarding the bile salt hydrolysis, *L. casei* and *L. delbruekii* showed partial bile salt hydrolysis activity and colony morphology was recorded as differentiated in comparison with the control MRS agar plates. Finally, antimicrobial activities of lactobacillus isolates were tested against five pathogenic bacteria (*Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* sp.) at pH 6.5 by disc diffusion method. All the tested isolates showed *in vitro* inhibitory zone against pathogenic bacteria. *L. casei* and *L. delbrueckii* showed maximum inhibition zone. In conclusion, the present study showed that *L. casei* and *L. delbrueckii* can be used as potential probiotic lactic acid bacteria.

Key words: *Lactobacillus,* curd, probiotics, antibiotic resistance, resistance to low pH, resistance to bile, pathogenic bacteria.

INTRODUCTION

The term probiotic, literally meaning “for life”, was first addressed by Lilly and Stillwell (1965) and was used to describe substances produced by protozoa to stimulate the growth of other organisms. Nowadays, the term refers to viable, nonpathogenic microorganisms (bacteria or yeasts) nonpathogenic microorganisms (bacteria or

E-mail: estifanos_hawaz@yahoo.com. Tel. +251932214923.

yeasts) that, when ingested, are able to reach the intestines in sufficient numbers to confer health benefits to the host (Schrezenmeir and De Vrese, 2001). Commonly used bacterial probiotics include various species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, as well as *Lactococcuslactis* and some *Enterococcus* species (Morrow et al., 2012). It is well established that probiotics confer a number of beneficial health effects to humans and animals. Intake of probiotics stimulates the growth of beneficial microorganisms and reduces the amount of pathogens, thus improving the intestinal microbial balance of the host and lowering the risk of gastro-intestinal diseases (Fuller, 1989; Cross, 2002; Chiang and Pan, 2012). Their benefits also include the alleviation of certain intolerances (such as lactose intolerance), the enhancement of nutrients bioavailability, and prevention or reduction of the prevalence of allergies in susceptible individuals (Isolauri, 2001; Chiang and Pan, 2012). Probiotics are reported to also have antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis and immuno-modulatory effects (Chiang and Pan, 2012). They relieve the symptoms of inflammatory bowel diseases, irritable bowel syndrome, colitis, alcoholic liver disease, constipation and reduce the risk for colon, liver and breast cancers (Prado et al., 2008).

Lactic acid bacteria are Gram positive organisms which are safely applied in medical and veterinary functions (Holzapfel and Schillinger, 2002). Isolation and screening of lactic acid bacteria from naturally occurring processes have always been the most powerful means for obtaining useful cultures for scientific and commercial purposes and the proper selection and balance of lactic acid bacteria used for starter culture is critical for the manufacture of diary fermented food products with their desirable texture and flavor (Sanders, 2000). Mankind exploited these bacteria for thousands of years for the production of traditionally fermented dairy products because of ability to produce desirable changes in taste, flavor, texture and extending the shelf life of food products (Dave and Shah, 1996). Many studies have reported that the best matrices to deliver probiotic lactic acid bacteria are dairy fermented products such as yogurt, cheese and other fermented milk products (Salminen, 1996). Their unique presence in intestinal epithelium and human gastrointestinal tract, and their traditional use in fermented foods and dairy products without remarkable problems prove their safety (Pangallo et al., 2008). The aim of this study was to isolate and identify probiotic lactic acid bacteria from curd and evaluate *in vitro*, its growth inhibition activities against pathogenic bacteria.

MATERIALS AND METHODS

Isolation of lactic acid bacteria from curd

Lactic acid bacteria were isolated from curd obtained from different sources of dairy products. Samples were collected using sterile bottles and stored in an icebox until delivery to the laboratory for analysis. The curd samples were suspended appropriately and diluted in sterile saline and spread plated into selective medium: De Mann, Rogosa Sharpe (MRS) (Dave and Shah, 1996) and incubated at 37°C for 24 h anaerobically for isolation. The selected colonies were purified by streak plate technique. The purified bacterial strains were stored at -80°C in sterile reconstituted skim milk 12.5% (w/v) supplemented with 15% glycerol.

Physiological and biochemical characterization of lactic acid bacteria

Growth of lactic acid bacteria (LAB) isolates was examined in MRS broth at temperature 15, 37 and 45°C by incubating for 24 h anaerobically (Briggs, 1953). CO_2 and lactic acid production from glucose was tested in citrate lacking MRS broths media containing inverted Durham tubes. Salt tolerance was tested in MRS broth by incorporating at 2, 4 and 6.5% (w/v) sodium chloride, incubated at 37°C was carried out according to method described by Briggs (1953). Catalase test was performed by adding 3% of hydrogen per oxide (H_2O_2) in a test tube containing an overnight culture of lactic acid bacteria. Ammonia production in MRS broth containing 0.3% arginine and 0.2% sodium citrate instead of ammonia citrate and the production of ammonia was detected using Nessler's reagent (Briggs, 1953). Sugar fermentation test was performed using eight different sugars (mellibiose, cellobiose, lactose, sucrose, raffinose, gluconate, salicine and arabinose) as reported by Cullimore (2008). Gram positive, catalase negative and bacilli colonies were taken as lactic acid bacteria and stored in glycerol culture and kept at -80°C for further investigation.

Survival of lactic acid bacteria isolates under conditions simulating human GI tract

Major selection criteria (antibiotic resistance, resistance to low pH, tolerance against bile salt, bile salt hydrolysis and antimicrobial activity) were used for the determination of probiotic properties of lactic acid bacteria isolates (Bassyouni et al., 2012).

Antibiotic resistance

Lactic acid bacteria strains were assessed their antibiotic resistance by disc diffusion method using antibiotics discs. One milliliter of actively growing cultures was mixed with 10 ml of MRS agar and poured into a petriplates. After solidification, the antibiotic disks were placed on the solidified agar surface, and the plates were left over for 30 min at 4°C for diffusion of antibiotics and then anaerobically incubated at 37°C for 48 h. Resistance was defined according to the disc diffusion method by using antibiotic discs of streptomycin, gentamycin, chloramphenicol, ciprofloxacin, norfloxacin and tetracycline (Hi Media Laboratories Pvt. Ltd. Mumbai, India.) (Felten et al., 1999). The zone of inhibition was measured by calipers in millimeters (Lleó et al., 1998; Mir-hoseini, 2004).

Resistance to low pH

Lactic acid bacteria isolates obtained from overnight culture were harvested by centrifugation for 10 min at 5000 rpm and 4°C, washed twice with PBS buffer (pH 7.2), and adjusted to pH 2.5. Resistance were assessed in triplicates in terms of viable colony counts and enumerated on MRS agar after incubation at 37°C for 0, 1, 2 and 3 h, indicating the time spent by food in the stomach as described by Maragkoudakisa et al. (2006) and Zoumpopoulou et al. (2008). Also, growth was monitored at OD620 (Bassyouni et al., 2012).

Resistance to bile salt

The tolerance against bile was carried out based on the intestinal bile concentration, 0.3% (w/v) and the staying time of food in small intestine is 4 h (Kumar and Murugalatha, 2012). MRS medium containing 0.3% (w/v) bile concentration was inoculated with overnight culture of lactic acid bacteria. Viable colonies were counted for every hour of incubation time on MRS agar and also growth was monitored at OD620 (Bassyouni et al., 2012).

Bile salt hydrolysis

Bacterial cultures were grown overnight in MRS broth at 37°C with 5% CO_2 and streaked in triplicates on MRS agar containing 0.5% (w/v) taurodeoxycholic acid. The hydrolysis effect was indicated by different colony morphology (partial hydrolysis) from the control MRS plates, after 48 h of anaerobic incubation at 37°C.

Antimicrobial activity against bacterial pathogens

The lactic acid bacteria isolates were tested for antagonistic activity against five bacterial pathogens (*Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* sp.) (Mir-hoseini, 2004) was obtained from Haramaya University, Microbiology Laboratory. The lactic acid bacteria strains were inoculated in MRS broth for 24 h at 37°C and centrifuging the culture at 5000 rpm for 10 min at 4°C, and the bacterial cells were removed. The pH values of supernatants were neutralized to 6.5 by the addition of 0.1 N NaOH, and filtered through a sterilized cellulose acetate filter and stored at 4°C. Supernatants were directly used for antagonistic test. Pellet was diluted with sterile distilled water and used for antimicrobial activity test.

Antimicrobial assay

The antimicrobial activity of the lactic acid bacteria isolates was determined by using the disc diffusion method as described by Sandra et al. (2012). These assays were performed in triplicate. The plates were poured with 20 ml Nutrient Agar. The pathogenic strains (*Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* sp.) were spread on the surface of nutrient agar. The agar plates inoculated with test organism were incubated for 1 h before placing the extract impregnated paper discs on the plates. Five sterile papers blank disks of 6 mm diameter were placed on the surface of agar plate which was inoculated by indicator strains of 5, 10 and 15 µl of the filtered supernatant and pellets of *Lactobacilli* were applied for screening of antimicrobial activity. The plates were kept at 4°C for 30 min to permit diffusion on the assay material, and incubated at 37°C for 24 h. Discs dipped in sterile water served as control and the antibiotics Chloramphenicol, Penicillin G and Streptomycin were used as positive control. Zones of inhibition were measured by calipers in millimeters (Lleó et al., 1998; Mir-hoseini, 2004).

Haemolytic activity

Fresh overnight bacterial cultures was streaked in triplicates on Columbia agar plates, containing human blood and incubated at 30°C for 48 h. Haemolytic activities of the bacterial culture were examined for signs of β-haemolysis (clear zones around colonies), α-haemolysis (green zones around colonies) or γ-haemolysis (no clear zones around colonies) on human blood agar plates (Hargrove and Alford, 1978).

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria (LAB)

Nine *Lactobacillus* strains were isolated from curd and based on their physiological and biochemical characterristics isolates were designated as: A, B, C, D, E, F, G, H and I.

Physiological and biochemical identification

According to biochemical characterization, all tested isolates were grown at 37, 45°C where as isolate B, D, F, and I were grown at 15°C. All tested isolates did not produce ammonia from arginine and did not produce gas from glucose. All isolates tolerated 2, 4 and 6.5% of NaCl concentration and was catalase negative. The recognition of lactobacillus strains was made mainly on the results of carbohydrate fermentation tests. According to carbohydrate fermentation, isolate A ferment cellobiose, lactose, sucrose and salicine. Isolate B ferment sugar lactose, sucrose, gluconate and salicine. Isolate C ferment only sucrose. Isolate D ferment sugars mellibiose, gluconate and arabinose. Isolate E ferment carbohydrates mellibiose, sucrose, raffinose and gluconate. Isolate F ferment cellobiose, lactose, sucrose and salicine. Isolate G ferment carbohydrates cellobiose, lactose, sucrose, salicine. Isolate H ferment lactose, sucrose, salicine, and isolate I ferment mellibiose, cellobiose, gluconate, salicine and arabinose carbohydrates. Based on the biochemical characterrization, isolate A is *Lactobacillus leichmannii*, isolate B: *Lactobacillus casei*, isolate C: *Lactobacillus delbrueckii*, isolate D: *Lactobacillus brevis*, isolate E: *Lactobacillus fermentum*, isolate F: *Lactobacillus coagulans*, isolate G: *Lactobacillus acidophilus*, isolate H: *Lactobacillus lactis* and isolate I: *Lactobacillus rhamnosus*. This in agreement with Karimi et al. (2012) and Liu et al. (2012) who isolated *L. acidophilus* from cheese and found that *L.I actis* and *L. casei* were considered as the predominated species in fermented dairy products (Tables 1 and 2).

Survival of lactic acid bacteria isolates under conditions simulating human GI tract

In order to use lactic acid bacteria as a probiotic, the bacteria must be able to survive the acidic conditions in the stomach and resist bile acids concentration (Holzapfel et al., 1998; Klaenhammer and Kullen, 1999) and possess antagonistic effect against pathogenic bacteria (Mir-hoseini, 2004).

Resistance to low pH

Being resistant to low pH is one of the major selection criteria for probiotic strains (Çakır, 2003). To reach

Table 1. Morphological and cultural characteristics of *Lactobacillus* isolates.

Laboratory isolate	Cell morphology	Spore forming	Capsule	Motility	Growth at			Co_2 production	Ammonia production	Catalase test
					15^0C	37^0C	45^0C			
A	SR	-	-	-	-	+	+	-	-	-
B	SR	-	-	-	+	+	+	-	-	-
C	SR	-	-	-	-	+	+	-	-	-
D	SR	-	-	-	+	+	+	-	-	-
E	SR	-	-	-	-	+	+	-	-	-
F	SR	-	-	-	+	+	+	-	-	-
G	SR	-	-	-	-	+	+	-	-	-
H	SR	-	-	-	-	+	+	-	-	-
I	SR	-	-	-	+	-	-	-	-	-

[SR]Straight rods, [v]variable, [-]negative, and [+]positive.

Table 2. Carbohydrate fermentation of lactic acid bacteria.

Laboratory isolate	Mellibiose	Cellobiose	Lactose	Sucrose	Raffinose	Gluconate	Salicine	Arabinose	Strain
A	-	+	+	+	-	-	+	-	*L. leichmannii*
B	-	-	+	+	-	+	+	-	*L. casei*
C	-	-	-	+	-	-	-	-	*L. delbrueckii*
D	+	-	V	v	V	+	-	+	*L. brevis*
E	+	-	+	+	+	+	-	V	*L. fermentum*
F	-	+	+	+	-	-	-	+	*L. coagulans*
G	V	+	+	+	V	-	+	-	*L. acidophilus*
H	-	-	+	+	-	-	+	-	*L. lactis*
I	+	+	V	-	-	+	+	+	*L. rhamnosus*

[+] Positive, [-]negative, and [v]variable.

the small intestine they have to pass through stressful conditions of the stomach (Çakir et al, 2003). Although in the stomach, pH can be as low as 1.0, in most *in vitro* assays pH 3.0 has been preferred. Due to the fact that a significant decrease in the viability of strains is often observed at pH 2.0 and below (Prasad et al., 1998), for selection of the strains resistant to low pH, PBS pH was adjusted to 3.0. The time taken during the digestion in the stomach is 3 h. So, all the isolates were detected whether they were resistant to pH 3.0 during 3 h or not. All the tested isolates were able to survive at pH 3.0 during three hours of incubation. *L. casei* and *L. delbrueckii* were more resistant to low pH than other strains. This correlated with the previous report by Argyri et al. (2013) were nine strains of *Lactobacillus* showed very high resistance to low pH (*L. plantarum L. pentosus*, *L. paracasei* subsp.*paracasei*) with final populations exceeding 8 log cfu/ml, whereas 12 strains showed high resistance to low pH (*L. plantarum*, *L. pentosus*) which along with the reference strains *L. casei* and *L. rhamnosus* GG showed final populations within 6-8 log cfu/ml and according to Bassyouni et al. (2012), eight of eleven tested lactobacillus isolates were resistance to pH 3.0 during three hours. All isolates that survive in pH 3.0 were taken to the next step for further investigation (Table 3).

Table 3. Survival in pH 3.0 - OD620 value.

Laboratory isolate	OD at 620nm at different time interval (hour)			
	0	1	2	3
B	1.1	1.1	1	1.2
C	0.99	1	0.98	1
D	0.71	0.7	0.7	0.7
H	0.56	0.58	0.59	0.58
I	0.4	0.38	0.35	0.3

Resistance to bile salt and bile salt hydrolysis

The strains, resistant to low pH, were screened for their ability to tolerate the bile salt. Although the bile concentration of the human gastro intestinal tract varies, the mean intestinal bile concentration is believed to be 0.3% (w/v) and the staying time is suggested to be 4 h (Prasad

et al., 1998). Strains were detected in 0.3% during 4 h of stay. Results showed that all isolates after four of exposure retaining their viability with negligible reduction in viable counts to 0.3% (w/v) bile concentration and among all of tested isolates, *L. casei* and *L. delbruekii* were more tolerant than others. This in agreement with Jensen et al. (2012) report that *Lactobacillus* species tolerate gastric juice well with no reduction in viability. Similarly observation by Abriouel et al. (2012), showed all lactic acid bacteria isolated from fermented olive were able to grow and survive at 0.3% (w/v) bile salt. The survival at bile salt condition is one of the main criteria for *in vitro* selection of potentially probiotic bacteria and critical points for the microbes. Regarding the bile salt hydrolysis, *L. casei* and *L. delbruekii* show partial bile salt hydrolysis activity and colony morphology was recorded as differentiated in comparison with the control MRS agar plates (Table 4).

Table 4. Tolerance against 0.3% bile – OD620 values.

Laboratory isolate	OD at 620nm at different time interval (hour)				
	0	1	2	3	4
B	0.055	0.042	0.049	0.049	0.07
C	0.038	0.036	0.037	0.04	0.042
D	0.031	0.03	0.029	0.031	0.032
H	0.02	0.023	0.03	0.028	0.031
I	0.03	0.028	0.025	0.02	0.018

Haemolytic activity

Non-haemolytic activity and antibiotic resistance strains are considered as a safety prerequisite for the selection of a probiotic strain. Results showed that all examined strains did not exhibited β-haemolytic activity when grown in Columbia human blood agar. Most of the strains (7 strains) were γ-haemolytic (non-haemolysis), while two strains exhibited α-haemolysis. These were *L. coagulans, L. rhamnosus.* Isolate *L. casei, L. delbruekii* and *L. lactis* showed γ-haemolysis (Maragkoudakisa et al., 2006).

Antimicrobial activity

The cell free supernatants and cell masses of different lactobacillus strains were tested for antimicrobial activity against five pathogenic bacterial strains (*Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* sp.) by using disc diffusion method. The production of antimicrobial compounds such as organicacids, short chain fatty acids and bacteriocins is one of the functional properties used to characterize probiotics (Fuller, 1989). The capacity to produce different antimicrobial compounds may be one of the critical characteristics for effective competitive exclusion of pathogen survival in the intestine and expression of a probiotic effect for the host (Salminen et al., 1998). In this study, the antimicrobial activities of supernatants and cell masses were conducted at three different concentrations of 5, 10 and 15 µl/discs were inoculated and compared with those of positive control such as tetracycline, penicillin G and streptomycin and distilled water as negative control. The data obtained from the disc diffusion method indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. The inhibitory effect was increased with the increase of the extract concentration from 5 to 15 µl. The diameters of inhibition zones were measured and compared. Result showed that *L. casei* and *L. delbrueckii* showed maximum inhibition zone against bacterial pathogens among all the *Lactobacillus* isolates. This in agreement with Bassyouni et al. (2012) that *Lactobacillus* strains tested against *Salmonella thyphimurium, E. coli,* and *Staphylococcus* spp. and all of tested isolates have antibacterial effect against the pathogenic bacteria (Tables 5 and 6).

Resistance to antibiotics

Resistance was defined according to the standard disc diffusion method by using antibiotic discs of Streptomycin, Gentamycin, Chloramphenicol, Ciprofloxacin, Norfloxacin and Tetracycline (Hi Media Laboratories Pvt. Ltd. Mumbai, India.). The zone of inhibition was measured by calipers in millimeters (Lleó et al., 1998; Mir-hoseini, 2004). Results showed that *L. casei*, *L. delbruekii* and *L. brevis* were resistant to all antibiotics and *L. fermentum, L. lactis* and *L. rhamnosus* were sensitive to all antibiotics except Streptomycin. *L. leichmannii, L. acidophilus* and *L. coagulans* were sensitive to all antibiotics. *L. casei*, *L. delbruekii, L. lactis, L. rhamnosus* and *L. brevis* were taken to the next step for further investigation (Table 7).

Conclusions

The *Lactobacillus* cultures were isolated and identified according to their physiological and biochemical characteristics. The used tests were: lactic acid and CO_2 production test, ammonia production test, catalase test, growth at 15, 37 and 45°C, salt tolerance test and sugar fermentation test. Results showed that the strains *L. leichmannii, L. casei, L. delbrueckii, L. brevis, L. fermentum, L. coagulans, L. acidophilus, L. lactis* and *L. rhamnosus* were isolated from curd. *Lactobacillus* strains survival was also assessed under conditions simulating human GI tract which were performed followed by antibiotic resistance, resistance to bile salt, bile salt hydrolysis and resistance to low pH. Results showed that all isolates were able to grow at low pH 3.0, at 0.3% bile concentration. *L. casei, L. delbrueckii* and *L. brevis* were found to be resistant to five antibiotics, that is, Tetracycline, Streptomycin, Gentamycin, Ciprofloxacin and Norfloxacin. According to haemolytic activity all

Table 5. Antimicrobial activity of lactic acid bacteria cell mass.

Laboratory isolate	Volume of cell mass (µl)	Diameter of inhibition zone (mm)				
		E. coli	*Pseudomonas*	*Staphylococcus*	*Klebsiella*	*Bacillus*
B	5	–	11	10	9	8
	10	10	13	12	12	9
	15	10	20	14	15	12
C	5	8	–	8	–	–
	10	10	7	11	–	–
	15	11	10	14	–	10
D	5	–	–	–	8	–
	10	10	–	–	10	–
	15	10	–	–	10	12
H	5	–	–	–	–	–
	10	8	–	–	–	–
	15	11	–	–	–	–
I	5	8	–	–	–	–
	10	10	–	–	–	–
	15	13	10	9	–	–

Table 6. Antimicrobial activity of lactic acid bacterial supernatant.

Laboratory isolate	Different volume of supernatant mass (µl)	Diameter of inhibition zone(mm)				
		E. coli	*Pseudomonas*	*Staphylococcus*	*Klebsiella*	*Bacillus*
B	5	-	–	9	8	–
	10	10	8	11	9	10
	15	12	10	12	10	10
C	5	–	10	–	7	8
	10	8	12	8	10	10
	15	9	15	9	14	13
D	5	–	–	7	–	–
	10	10	–	8	–	–
	15	11	–	10	–	–
H	5	–	7	7	–	–
	10	8	10	9	10	8
	15	11	12	1	15	9
I	5	–	–	–	–	–
	10	–	–	–	–	–
	15	–	–	–	–	–

examined strains did not exhibited β-haemolytic activity when grown in Columbia human blood agar. Most of the strains (7 strains) were γ-haemolytic (non-haemolysis), while two strains exhibited α-haemolysis. These were *L. coagulans, L. rhamnosus.* Isolate *L. casei, L. delbruekii* and *L. lactis* showed γ–haemolysis. Regarding the bile salt hydrolysis, *L. casei* and *L. Delbruekii* show partial bile salt hydrolysis activity and colony morphology was recorded as differentiated in comparison with the control MRS agar plates. Finally, antimicrobial activities of *Lactobacillus* isolates were tested against five pathogenic bacteria (*Staphylococcus* spp., *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., and *E. coli* spp.) at pH 6.5 by disc diffusion method. All the tested isolates showed *in vitro* inhibitory zone against pathogenic bacteria at pH 6.5. Among the *Lactobacillus* strains, *L. casei,* and *L. delbrueckii* showed maximum inhibition zone. In conclusion, the present study showed that from all the isolated *Lactobacillus* strains, *L. casei* and *L. delbrueckii* can be used as potential probiotic lactic acid bacteria.

Table 7. Drug resistance of *Lactobacillus* strains.

Laboratory isolate	Streptomycin	Tetracycline	Gentamycin	Norfloxacin	Ciprofloxacin
A	12	25	20	26	25
B	R	R	R	R	R
C	R	R	R	R	R
D	R	R	R	R	R
E	R	25	R	10	10
F	14	40	25	30	35
G	10	40	20	35	35
H	R	12	15	17	R
I	R	25	16	10	15

[R]Resistant.

ACKNOWLEDGEMENT

The authors acknowledge Department of Biology, College of Natural and Computational Science, Haramaya University, Ethiopia for giving permission to use microbiology laboratory.

REFERENCES

Abriouel H, Benomar N, Cobo A, Caballero N, Fernández Fuentes MÁ, Pérez-Pulido R, Gálvez A (2012). Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. Food Microbiol. 32(2):308-316.

Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJ, Panagou EZ, Tassou CC (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests. Food Microbiol. 33:282-291.

Bassyouni RH, Abdel-all WS, Fadl MG, Abdel-all S, kamel Z (2012). Characterization of Lactic Acid Bacteria Isolated from Dairy Products in Egypt as a Probiotic. Life Sci. J. 9:2924-2930.

Briggs M (1953). The Classification of Lactobacilli by means of Physiological Tests. J. Gen. Microbial. 9: 234-2444.

Çakır İ (2003). Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of Ph.D.

Chiang P (2012). Beneficial effects of *Lactobacillus paracasei subsp. Paracasei NTU* 101 and its fermented products. Appl. Microbiol. Biotechnol. 93:903-916.

Cross ML (2002). Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS Immunol. Med. Microbiol. 34(4):245-253

Cullimore R (2008). Practical atlas for bacterial identification. chapter gram positive rods. p. 91.

Dave RI, Shah NP (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus* and*Bifidobacterium spp*. J. Dairy Sci. 79:1529-1536.

Felten A, Barreau C, Bizet C, Lagrange PH, Philippon A (1999). Lactobacillus species identification, H_2O_2 production, and antibiotic resistance and correlation with human clinical status. J. Clin. Microbiol. 37(3):729-733.

Fuller R (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66 (5):365-378.

Holzapfel WH, Schillinger U (2002). Introduction to pre- and probiotics. Food Res. Int. 35:109-116.

Holzapfel WH, Haberer P, Snel J, Schillinger V, Huisin't Veld J (1998). Overview of gutflora and robiotics. Int. J. Food Microbiol. 41:85-101.

Isolauri E (2001). Probiotics in the prevention and treatment of allergic disease. Pediatr. Allergy Immunol. 12(s14):56-59.

Karimi R, Mortazavian AM, Amiri-Rigi A (2012). Selective enumeration of probiotic microorganisms in cheese. Food Microbiol. 29:1-9.

Klaenhammer TR, Kullen MJ (1999). Selection and design of probiotics. Int. J. Food Microbiol. 50(1-2):45-57.

Kumar AM, Murugalatha N (2012). Isolation of *Lactobacillus plantarum* from cow milk and screening for the presence of sugar alcohol producing gene. J.f Microbiol. Antimicrob. 4(1):16-22.

Lilly DM, Stillwell RH (1965). Probiotics: growth-promoting factors produced by microorganisms. Science 147(3659):747-748.

Liu W, Bao Q, Jirimutu, Qing M, Siriguleng, Chen X, Sun T, Li M, Zhang J, Yu J, Bilige M, Sun T, Zhang H (2012). Isolation and identification of lactic acid bacteria from Tarag in Eastern Inner Mongolia of China by 16S rRNA sequences and DGGE analysis. Microbiol. Res. 167:110-115.

Lleó MM, Tafi MC, Canepari P (1998). Non culturable Enterococcus faecalis cells are metabolically active and capable of resuming active growth. Syst. Appl. Microbiol. 21(3):333-339.

Maragkoudakisa PA, Zoumpopouloua G, Miarisa C, Kalantzopoulosa G, Potb B, Tsakalidou E (2006). Probiotic potential of *Lactobacillus strains* isolated from dairy products. Int. Dairy J. 16:189-199.

Mir-hoseini (2004). Study of effect of nisin and producer bacteria of nisin on *Listeria monocytogenes*and *Bacillus cereus*. Isfahan: University of Isfahan. 33:234-342

Morrow LE, Gogineni V, Malesker MA (2012). Probiotics in the intensive care unit. Nutr. Clin. Prac. 27 (2):235-241.

Pangallo D, Drahovska H, Harichova J, Karelova E, Chovanova´ K, Ferianc P, Turn˘a J, Timko J (2008). Assessment of environmental enterococci: bacterial antagonism, pathogenic capacity and antibiotic resistance. Antonie Van Leeuwenhoek 94:555-562.

Prado FC, Parada JL, Pandey Ashok, Soccol CR (2008). Trends in non-dairy probiotic beverages. Food Res. Int. 41 (2):111-123.

Prasad J, Gill H, Smart J, Gopal PK (1998). Selection and characterisation of *Lactobacillus* and *Bifidobacterium*strains for use as probiotics. Int. Dairy J. 8(12):993-1002.

Salminen S (1996). Uniqueness of probiotic strains. IDF Nutr. News Lett. 5:16-18.

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fondén R, Saxelin M, Collins K, Mogensen G, Birkeland SE, Mattila-Sandholm T (1998). Demonstration of safety of probiotics - a review. Int. J. Food Microbiol. 44(1-2):93-106.

Sanders ME (2000). Considerations for use of probiotic bacteria to modulate human health. J. Nutr. 130:384-390.

Sandra TS, Janine Barlow, Adele C , Glenn R, Gibson, Ian R (2012). *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids J. Anaerobe 18:530-538.

Schrezenmeir J, de Vrese M (2001). Probiotics, prebiotics, and synbiotics-approaching a definition[1–3]. Am. J. Clin. Nutr. 73(2):361S-364S.

Zoumpopoulou G, Foligne B, Christodoulou K, Grangette C, Pot B, Tsakalidou E (2008). *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential*in vitro* and protects against TNBS-induced colitis and Salmonella infection in murine models. Int. J. Food Microbiol. 121:18-26.