Isolation and Characterization of *Lactobacillus* Species from Head *Cabbage (Brassica oleracea var. capitate*) and its Potential Application as a Probiotic Agent

Estifanos Hawaz*

Department of Biology, College of Natural and Computational Sciences, Haramaya University, Ethiopia

Abstract: Lactic acid bacteria (LAB) are one of the most important groups of microorganisms used in food fermentation, contribute to extended shelf life of the fermented products, and are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products. The purpose of this study was to isolate and characterize *Lactobacillus* species from head cabbage (*Brassica oleracea var. capitate*) and to evaluate their probiotic properties under conditions simulating human gastrointestinal (GI) tract. For this purpose, a total of 15 head cabbage samples were collected randomly from Haramaya district during the period from May to August, 2014. Based on cultural and biochemical characteristics, *Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus cellobiosus,* and *Lactobacillus brevis* were isolated from the cabbage heads. The probiotic effect of each isolate was evaluated for antimicrobial activity against human pathogenic bacteria, resistance to bile acids, resistance to low pH, antibiotic resistance, and haemolytic activity. Results showed that all isolates had antagonistic effects against pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia*) with different degree of inhibition zone but *L. brevis* shown the highest inhibition zone followed by *L. cellobiosus* and *L. plantarum*. According to resistance to bile acid, *L. plantarum, L. cellobiosus* and *L. brevis* retained their viability with a negligible reduction. Regarding antibiotics resistance, *L. plantarum, L. cellobiosus* and *L. brevis* were resistant strains to Streptomycin, Gentamycin, and Tetracycline antibiotics. According to a haemolytic test, all isolates did not exhibit β–haemolytic activity. The growth of *Lactobacillus* species recorded under all the pH values were viable. In conclusion, the present study indicated that *L. brevis* and *L. cellobiosus* possess potential probiotic properties but further *in-vivo* tests are required to elucidate their particular effects on human health.

Keywords: Antimicrobial susptibility; Gastrointestinal (GI) Tract; Pathogenic Bacteria; Probiotic.

1. Introduction

Lactic acid bacteria (LAB) are a broad group of Gram positive, non-spore forming, and catalase-negative, facultative anaerobic and nutritionally fastidious organism. They are widespread in soil, vegetables, meat, milk and the human body. LAB are among the most important groups of microorganisms used in food fermentation where they play an essential role and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products (Noopur *et al*., 2010; Hassanzadazar and Ehsani, 2013). One of the most important contributions of these microorganisms is the extended shelf life of the fermented products. Growth of spoilage and pathogenic bacteria in these foods is inhibited due to competition for nutrients and the presence of starter-derived inhibitors such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins (Noopur *et al*., 2010; Noordiana *et al*., 2013).

Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). 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 (Chiang and Pan, 2012), alleviatinglactose 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 have also anti-mutagenic, anti-carcinogenic, hypo-cholesterolemic, antihypertensive, anti-osteoporosis, and immune-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).

The selection of probiotic bacteria includes several criteria: safety, viability, resistance to acid and bile salts, adherence to gut epithelial tissue, ability to colonize the gastrointestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose reduction (Tkhruni *et al.,* 2013). In the past years, a lot of work has been done to isolate and characterize lactic acid bacteria from different sources but the probiotic properties of these lactic acid bacteria strains were not well determined. Therefore, the

*Corresponding Author. E-mail: estifhawaz19@gmail.com

ISSN 1992-0407

purpose of this research was to isolate and characterize *Lactobacillus* species from head cabbage (*Brassica oleracea var. capitata*) and to evaluate its probiotic properties under conditions simulating the human gastrointestinal (GI) tract.

2. Materials and Methods

2.1. Description of the Study Area

Haramaya district is one of the woreda in the Oromia Region of eastern Hararghe Zone, Ethiopia. The altitude of this district ranges from 1400 to 2340 meters above sea level. Haramaya district is located at the distance of out 500 km from Addis Ababa in the easterly direction. The Latitude and Longitude of Haramaya is 9.4062 and 42.0014 respectively.

2.2. Treatments and Experimental Design

The treatments consisted of examination comprised isolation of *Lactobacillus* species, physiological and biochemical characterization, and probiotic feasibility tests. The experiment was designed as a complete randomized design and replicate three times.

2.3. Sample Collection

A total of 15 samples of head cabbage were collected randomly during the period from May to August, 2014. The cabbage samples were collected from Haramaya University's student cafeteria (HUSC), Haramaya town market (HaTM) and also from Bate town market (BaTM). The cabbage samples were taken in accordance with the instructions given in Health Protection Agency (HPA, 2004) and the Food and Drug Administration (FDA, 2003). The cabbage samples were packaged into sterile plastic containers, transported to Microbiology Laboratory, Haramaya University and experiment was done immediately to prevent deterioration.

2.4. Isolation of *Lactobacillus* Species

Osmotic pressure stabilization was done by taking one gram of each sample and homogenizing it in nine ml of a sterile salt solution (0.85% NaCl) using the vortex. Then, sequential decimal dilutions of the homogenate were obtained. One ml of each aliquot dilution was spread plated on MRS agar for the isolation of *Lactobacillus* species and incubated anaerobically for 48 h at 37°C. The colonies were randomly picked from plates and purified by successive streaking on MRS agar media before being subjected to characterization. Gram-positive, catalase-negative and rod and/or bacilli isolates were considered as *Lactobacillus* as described by (Harrigan and McCance, 1990). The cultures were stored and maintained at -20°C on MRS agar slants supplemented with 10% (v/v) glycerol for further studies.

2.5. Physiological and Biochemical Characterization of *Lactobacillus* Isolates

Growth of *Lactobacillus* isolates was examined in MRS broth at the temperatures of 15, 37 and 45°C for 24 h anaerobically according to (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 2, 4 and 6.5 % (w/v) sodium chloride and incubating it at 37°C according to the method described by (Briggs, 1953). A catalase test was performed by adding 3% of hydrogen per oxide (H_2O_2) in a test tube containing an overnight culture of *Lactobacillus* species. Ammonia production in MRS broth (containing 0.3% arginine and 0.2% sodium citrate instead of ammonia citrate) was detected using Nessler's reagent according to method described by (Briggs, 1953). The isolates were further characterized by their carbohydrate fermentation pattern using different sugars (lactose, raffinose, sucrose, salicine, cellobiose, gluconate, arabinose, and mellibiose). Gram positive, catalase negative, and bacilli colonies were taken as *Lactobacillus* species and stored in a glycerol culture at -20°C for further investigation.

2.6. Probiotic Feasibility Test of *Lactobacillus* Isolates

All *Lactobacillus* isolates were examined for their probiotic properties under conditions simulating human gastrointestinal (GI) tract (resistance to low pH and resistance to bile acids), antibiotic resistance, haemolytic activity and antimicrobial activity against human pathogenic bacteria. Resistances to low pH and bile acids were determined according to reduction in cell viability (log cfu/ml) comparing with the reference microbial count. Accordingly, 2.80 log cfu/ml was used as a reference to all *Lactobacillus* isolates.

2.6.1. Antibiotic Resistance

All *Lactobacillus* isolates were evaluated for their antibiotic resistance by the disk diffusion method using three different antibiotics (streptomycin, tetracycline and gentamycin). Six milliliters of overnight grown cultures were inoculated in to de Man Ragosa and Sharpe (MRS) broth and mixed thoroughly using vortex and spread plated over MRS agar in triplicates. After solidification of the media, the antibiotic disks were kept on the solidified agar surface and the plates were left over for 10 minute at 4°C for diffusion of antibiotics and then anaerobically incubated at 37°C for 48 h. Zone of inhibition was measured using calipers in millimeter (mm) according to the method described by (Todorov and Dicks, 2004).

2.6.2. Resistance to Low pH

Lactobacillus isolates were assessed for their resistance to pH 2.0, 3.0, and 4.0 incubated anaerobically at 37°C for 3 h. All overnight grown *Lactobacillus* cultures were harvested by centrifugation (5000 rpm for 10 min at 4°C). Pellets were washed once in phosphate-saline buffer (PBS at pH 7.0). Then cell pellets were suspended with different pH ranges and incubated at 37°C. Enumeration of viable colonies on MRS agar was carried out using a colony counter at 3 h gap.

2.6.3. Resistance Against Bile

Resistance to bile acids was carried out using all overnight grown *Lactobacillus* cultures and spread plated on MRS agar containing 0.3% (w/v) bile concentration in triplicates and incubated anaerobically at 37°C for 4 h according to the method described by (Kumar, 2012). Enumeration of viable colony on MRS agar was carried out for every hour using a colony counter.

2.6.4. Haemolytic Activity

Lactobacillus isolates were screened for haemolytic activity on Colombia human blood agar plates incubated anaerobically at 37°C for 48 h. Haemolytic activity of the cultures were evaluated for signs of β-haemolysis, γ-haemolysis and α-haemolysis on Colombia human blood agar plates (Nour-Eddine, 2006).

2.6.5. Antibacterial Activity of *Lactobacillus* Isolates

Antimicrobial effects of *Lactobacillus* isolates were determined by the agar diffusion method as described by (Todorov and Dicks, 2004). The *Lactobacillus* isolates were evaluated for antimicrobial activities against human pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)* obtained from Haramaya University, Microbiology Laboratory. The tested *Lactobacillus* species were inoculated in to MRS broth and incubated at 37°C for 48 h anaerobically. Cell free supernatant and cell mass were screened for antibacterial activities against the pathogenic bacteria. A cell-free supernatant was obtained by centrifugation of the liquid culture at 8000 rpm for 20 minutes at 4°C. Wells with a diameter of six milliliters were loaded with 15µl of the cell free supernatant. The wells were prepared in the Mueller Hinton agar previously seeded with the test isolates. The plates were then incubated at 37°C for 24 h after which the diameter of inhibition zones was measured using a caliper in millimeter (mm).

3. Data Analysis

Analysis of variance was done in accordance with the complete randomized design. Significant differences among the means were separated using the least significant difference (LSD) test at 5% level of significance.

4. Results and Discussion

4.1. Isolation of *Lactobacillus* Species

A total of 65 lactic acid bacteria colonies were isolated from the MRS agar medium; 22 of the isolates shared characteristics of *Lactobacillus* species which are gram positive, catalase negative, non-motile, non-sporulating, anaerobic, and bacilli (Table 1). According to biochemical characterization, eleven isolates were *Lactobacillus cellobiosus,* eight isolates were *Lactobacillus plantarum*, and three isolates each were *Lactobacillus salivarius, Lactobacillus fermentum* and *Lactobacillus brevis*. All isolates were well identified based on their sugar fermentation profile. *Lactobacillus cellobiosus* and *Lactobacillus plantarum* was the dominant bacteria isolated in all the head cabbage samples. Similar reports by (Hernández *et al.,* 2005) indicated isolation of *L. plantarum* from cucumber fermentation. Similarly, (Daeschel *et al*., 1990), isolated *L. plantarum* from Italian ewe cheeses, and (Hassanzadazar and Ehsani, 2013) reported that the dominant isolated bacillus genus from Koopeh Cheese was *Lactobacillus plantarum* (58% of the lactobacilli population).

4.2. Physiological and Biochemical Characterizations of *Lactobacillus* Species

All *Lactobacillus* isolates were able to grow at the temperatures of 37 and 45°C (Table 1). Similar reports by (Iman *et al.* 2014) indicated that two isolates *L. plantarum* K3 and *L. plantarum* SH4 and *L. fermentum* C12 were able to grow at 45°C, while 12 isolates were not able to grow at the same temperature in isolated Syrian fermented foods. All isolates were able to grow in NaCl at the concentrations of 2 to 4% (Table 1). The growth of two *L. plantarum* isolates isolated by (Pal *et al,* 2004) from cabbage showed very weak growth in the presence of 4% NaCl concentration. All tested isolates did not produce ammonia from arginine. This is comparable with the results reported by (Estifanos Hawaz, 2014) that all isolates of lactic acid bacteria isolated from cow milk curd of dairy products did not produce ammonia. In this study, *L. plantarum* and *L. fermentum* isolates produced CO_2 gas from glucose, and all isolates are catalase negative (Table 1).

4.3. Probiotic Feasibility Test of *Lactobacillus* Species

4.3.1. Resistance to Low pH

All selected *Lactobacillus* isolates were examined for pH resistance (2.0, 3.0, and 4.0). Results showed that the counts of *Lactobacillus* species recorded under all the pH values were viable. *L. plantarum, L. cellobiosus,* and *L. salivarius* were able to survive at pH ranging from 2 to 4 with significant reduction in viability but *L.brevis* retained its viability with negligible reduction. *L. fermentum* species was able to survive only at pH 4 (Table 2).

4.3.2. Antibiotic Resistance

According to the antibiotic resistance test, *Lactobacillus plantarum, Lactobacillus cellobiosus* and *Lactobacillus brevis* were potent antibiotic resistant strains (Table 3). *Lactobacillus salivarius* and *Lactobacillus fermentum* were not resistant to Gentamycin and Tetracycline, respectively. This is in agreement with (Halami *et al.,* 2000) reported that *Lactobacillus* species are resistant to β-lactam, cephalosporin, aminoglycosides, quinolone, imidazole, nitrofurantoin and fluoroquinolines. Belletti *et al.* (2009) also reported that *Lactobacilli* are generally resistant to aminoglycosides.

Table 1. Cultural, physiological and biochemical characteristics of the *Lactobacillus* isolates.

	Lactobacillus isolates				
Tests	*L. brevis*	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*
Growth at					
15°C	-	-	-	-	+
37°C	+	+	+	+	+
45°C	+	+	+	+	+
Growth at pH					
2.0	+	+	-	+	+
3.0	+	+	-	+	+
4.0	+	+	+	+	+
Production of					
CO_2	-	-	+	+	-
Ammonia	-	-	-	-	-
Lactic acid	+	+	+	+	+
Growth at NaCl					
2%	+	+	+	+	+
3%	+	+	+	+	+
4%	+	+	+	+	+
Catalase test	-	-	-	-	-
Cell shape	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Motility test	-	-	-	-	-
Aerobicity	f.a	f.a	f.a	f.a	f.a
Spore forming	-	-	-	-	-
Capsule formation	-	-	-	-	-
Fermentation of					
Lactose	+	+	+	-	+
Raffinose	-	-	+	-	+
Sucrose	+	-	+	v	+
Salicine	+	+	-	-	-
Cellobiose	-	-	-	-	-
Gluconate	-	-	+	+	-
Arabinose	-	-	v	-	-
Mellibiose	-	-	+	-	v

Note: *+ = positive, - = negative , v = variable, and f.a = facultative anaerobic*

Table 2. Resistance to low pH of *Lactobacillus* isolates (log cfu/ml).

	Means ± SD Resistance to low pH (log cfu/ml)				
pH range	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*	*L. brevis*
2.0	1.15 ± 0.11[a]	N	1.12 ±0.00[a]	1.00 ±0.00[a]	2.63 ±0.00[a]
3.0	1.22 ± 0.12[a]	N	1.22 ±0.50[a]	1.20 ±0.22[a]	2.75 ±0.21[a]
4.0	1.30 ± 0.10[a]	1.34±0.00[a]	1.28 ±0.00[a]	1.22 ±0.03[a]	2.80 ±0.01[a]
Average	1.22 ±0.11	1.34±0.00	1.21±0.16	1.14±0.08	2.73±0.07

Note*: a = Means bearing similar superscripts in the same column differ insignificantly (p>0.05); N = negative*

Table 3. Antibiotic resistance of *Lactobacillus* isolates.

	Lactobacillus isolates				
Antibiotics	*L. salivarius*	*L. fermentum*	*L. plantarum*	*L. cellobiosus*	*L. brevis*
Streptomycin	R	R	R	R	R
Gentamycin	N	R	R	R	R
Tetracycline	R	N	R	R	R

Note*: R = resistance; N = negative*

4.3.3. Resistance to Bile Acids

All *Lactobacillus* isolates were screened for bile salt tolerance. *Lactobacillus salivarius* and *Lactobacillus fermentum* were survived with significant reduction in viable count but *L. plantarum, L. cellobiosus* and *L. brevis* were retaining their viability with negligible reduction after 4 h of exposure to 0.3 % (w/v) bile acid (Table 4). This is in agreement with (Jensen *et al.,* 2012) who reported that *Lactobacillus* species tolerate gastric juice well with no reduction in viability. Similarly (Vitali *et al.,* 2012) determined the probiotic potential of large number of lactic acid bacteria isolated from fruit and

vegetables were survived in gastric and intestinal conditions.

Table 4. Resistance to bile acids 0.3 % (w/v) of *Lactobacillus* isolates (log cfu/ml).

	Means ± *SD* Resistance to bile acids 0.3 % (w/v)				
Time	*L. salivarius*	*L. fermentum*	*L.plantarum*	*L. cellobiosus*	*L. brevis*
0 h	1.42±0.11[a]	1.50±0.00[a]	2.51±0.00[b]	2.53±0.00[b]	2.80±0.00[b]
1 h	1.00±0.00[a]	1.25±0.03[a]	2.41±0.00[b]	2.51±0.02[b]	2.63±0.00[b]
2 h	0.78±0.02[a]	1.01±0.01[a]	2.40±0.00[b]	2.50±0.00[b]	2.50±0.03[b]
3 h	0.35±0.00[a]	0.98±0.02[a]	2.35±0.00[b]	2.45±0.04[b]	2.48±0.00[b]
Average	0.89±0.03	1.19±0.02	2.42±0.00	2.50±0.12	2.60±0.08

***Note**: a, b = Means bearing similar superscripts in the same column differ insignificantly (p > 0.05); N = negative*

4.3.4. Haemolytic Test

All isolates were tested for hemolytic activity and gave negative results. These results agree with the findings of (Sandra *et al.* 2012) who reported that none of the fifteen putative probiotics was found to be β-hemolytic. This is also comparable with (Estifanos Hawaz, 2014) who reported that all examined *Lactobacillus* strains isolated from cow milk curd did not exhibit β-hemolytic activity.

4.3.5. Antibacterial Activity of *Lactobacillus* Species

The *Lactobacillus* species cell free supernatants (CFS) were tested for their antimicrobial activities against human pathogenic microorganisms (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)*. Results showed (Table 5) that all the CFSs were found to produce inhibition zone against pathogenic bacteria but *L. brevis* produced a wider inhibition zone against the tested microorganism followed by *L. cellobiosus*, *L. plantarum* and the lowest antimicrobial effect was recorded for *L. salivarius* and *L. fermentum* isolates. This may be due to production of short-chain organic acids (lactic, acetic, propionic), bacteriocins (nisin, acidolina, acidofilina, lacatcyna, lacocydyna, reutryna, laktoline, entrocine) and hydrogen peroxide. Bacteriocins have a high antibacterial activity against *Escherichia coli, Salmonella, Staphylococcus aureus, Clostridium perfringers,* and *Campylobacter* (Patil and Ajay, 2010; Karimi *et al.,* 2012). A similar result on antagonistic activity of LAB was reported by (Hernandez *et al.* 2005) in that, the CFS of the selected *Lactobacillus* isolates from vegetables inhibited the growth of *K. pneumoniae, S. aureus*, and *E. coli.* Vitali *et al.* (2012) isolated *lactobacillus* strains from olives which had antimicrobial effect against *S. aureus, E. facalis,* and *Salmonella enteric.* Messaoudi (2012) also reported that *Lactobacillus* strains isolated from chicken showed inhibition against *L. monocytogenes, S. aureus* and *Salmonella.*

Table 5. Antimicrobial activity of *Lactobacillus* isolates (CFS).

	Means ± *SD* zone of inhibition zone (mm)			
Lactobacillus isolates	*P. aeruginosa*	*S. aureus*	*E. coli*	*K. pneumonia*
L. salivarius	12±0.01[a]	9±0.00[a]	10±0.02[a]	12±0.00[a]
L. fermentum	13±0.00[a]	10±0.03[a]	12±0.00[a]	12±0.01[a]
L. plantarum	14±0.10[a]	15±0.04[a]	14±0.01[a]	15±0.00[a]
L. cellobiosus	22±0.00[b]	20±0.01[b]	21±0.00[b]	22±0.01[b]
L. brevis	25±0.02[b]	24±0.00[b]	25±0.00[b]	23±0.01[b]

***Note:** a, b = Means bearing different superscripts in the same column differ significantly (p < 0.05)*

5. Conclusion

Cabbage is one of the potential sources of lactic acid bacteria due to its nutritional composition. In the present findings; *L. salivarius, L. brevis, L. plantarum, L. cellobiosus,* and *L. fermentum* were isolated from cabbage (*Brassica oleracea var. capitate*). Probiotic properties of all isolates were investigated under conditions simulating human gastrointestinal (resistance to low pH and resistance to bile acids), antibiotic resistance, haemolytic activity and antimicrobial activity against pathogenic bacteria. Results showed that, all *Lactobacillus* strains shown antagonistic effect against all human pathogenic bacteria (*P. aeruginosa, S. aureus, E. coli,* and *K. pneumonia)* with different degree of inhibitory effect but *L.brevis* shown high inhibition zone followed by *L. cellobiosus* and *L. plantarum.* Regarding to resistance to bile acid, *L. plantarum, L. cellobiosus* and *L. brevis* were retaining their viability with negligible reduction and did not exhibited β–haemolytic activity. The growth of *Lactobacillus* species recorded under all the pH values were viable. In conclusion, the current *in-vitro* study indicated that, *L. brevis* and *L. cellobiosus* are potential probiotic candidate but further *in-vivo* test is required for its specific outcome on human health.

6. Acknowledgements

The author would like to acknowledge Haramaya University Research Affairs Office for funding this research work.

7. References

Abriouel, H., Benomar, N., Cobo, A., Caballero, N., Fuentes, M. A., Pérez-Pulido, R. and Gálvez, A. 2012. Characterization of lactic acid bacteria from

naturally-fermented Manzanilla Aloreña green table olives. *Food Microbiology,* 32 : 308-316

Belletti, N., Benedetta-bottari, M. G., Tabaneli, G. and Gardini, A. F. 2009. Antibiotic Resistance of *Lactobacilli* Isolated from Two Italian Hard Cheeses. *Journal of Food Protection,* 72 : 2162-2169.

Briggs, M. 1953. The classification of Lactobacilli by means of physiological tests. *J. Gen. Microbial.*, 9 : 234-244

Bukola, C., Adebayo, T., and Abiodun, A. 2008. Screening of Lactic Acid Bacteria Strains Isolated from Some Nigerian Fermented Foods for EPS Production. *World Applied Sciences Journal,* 4 : 741-747.

Chiang, S. S., Pan, T. M. 2012. Beneficial effects of Lactobacillus paracasei subsp. paracasei NTU 101 and its fermented products. *Appl. Microbiol. Biotechnol.*, 93: 903-916.

Daeschel, M. A., McKenney, M. C. and Mc Donald, L. C. 1990. Bacteriocidal activity of *Lactobacillus plantarum* C-11. *Food Microbiology,* 7 : 91-98.

Estifanos Hawaz, 2014. Isolation and identification of probiotic lactic acid bacteria from curd and *in-vitro* evaluation of its growth inhibition activities against pathogenic microorganisms. *African Journal of Microbiology,* 8 : 1419-425.

FDA. 2003. Food sampling and preparation of sample homogenate. Bacteriological analytical manual on-line, 8th Edition. Food and Drug Administration (FDA), USA.

Halami, P. M., Chandrashekar, A., Nand, K., 2000. *Lactobacillus farciminis* MD, a newer strain with potential for bacteriocin and antibiotic assay. Lett. *Appl. Microbiol.*, 30 : 197-202.

Harrigan, W. and Mc Cance, M. 1990. Laboratory Methods in Food and Dairy Microbiology. 8th Edition. Academic Press. London. UK.

Hassanzadazar, H. and Ehsani, A. 2013. Phenotypic Characterization of Lactic Acid Bacteria Isolated from Traditional Koopeh Cheese. *Global Veterinaria,* 10 : 148-152.

Hernández, D., Cardell, E., and Zárate, V. 2005. Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum*. *J Appl. Microbiol. TF 711*., 99 (1) : 77-84.

HPA. 2004. Preparation of samples and decimal dilutions. National Standard Method D1. Issue No. 2. Health Protection Agency (HPA), UK.

Iman A., Gorrah S. A. and Al-Amir, L. 2014. Isolation and Characterization of Bacteriocin Producing Lactic Acid Bacteria from some Syrian fermented foods. *Journal of Chem. Tech. Research*, 6 (4) : 2507-2520.

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

Jensen, H., Grimmer, S., Naterstad, K. and Axelsson, L. 2012. In vitro testing of commercial and potential probiotic lactic acid bacteria International. *Journal of Food Microbiology* 153 : 216-222.

Karimi, R., Mortazavian, A. M. and Amiri-Rigi, A. 2012. Selective enumeration of probiotic microorganisms in cheese. *Food Microbiology,* 29 : 1-9

Kumar, M. 2012. Isolation of Lactobacillus plantarum from cow milk and screenin for the presence of sugar alcohol producing gene. *Journal of Microbiology and Antimicrobials*, 4 (1) : 16-22.

Martin, R., Langa, S., Reviriego, C., Jimenez, E., Marin, L.M. Olivares, M., Boza, J., Jimenez, J., Fernandez, L., Xaus J. and Rodriguez, J. M. 2004. Tthe commensal microflora of human milk: New perspectives for food bacteriotherapy and probiotics. *Trends in Food Science and Technology,* 15 : 121-127.

Messaoudi, S., Kergourlay, G., Dalgalarrondo, M., Choiset, Y. and Mir-Hoseini, M. 2012. Study of effect of nisin and producer bacteria of nisin on *Listeria monocytogenes* and *Bacillus cereus* [Isfahan: University of Isfahan].

Nour-Eddine K. M. K. 2006. In-vitropre-selection criteria for probiotic Lacto bacillus. *International Journal of Probiotics and Prebiotics*, 1 : 27-32.

Pal, V., Jamuna, M., and Jeevaratnam, K. 2004. Isolation and Characterization of Bacteriocin Producing Lactic Acid Bacteria from A South Indian Special Dosa (Appam) Batter. *Journal of Culture Collections,* 4 : 53-60.

Patil, M. M., and Ajay, P. T. 2010. Isolation and characterization of lactic acid bacteria from curd and cucumber. *Indian Journal of Science*, 9 : 166-172.

Plantarum strains of fermented olives origin. *International Journal of Probiotics and Prebiotics*, 1 : 27-32.

Prado, F. C., Parada, J. L., Pandey, A., Soccol, C. R. 2008. Trends in non-dairy probiotic beverages. *Food Res. Int.*, 41 (2) : 111-123.

Sandra, T. S., Barlow, J., Costabile, A., Glenn, Gibson, R. and Rowland I. 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.

Tkhruni, F. N., Karapetyan, K. J., Danova, S. T., Dimov, S. G., Karimpour, F. A. 2013. Probiotic properties ofendemic strains of lactic acidbacteria. *Journal Bioscience Biotechnology,* 2 (2) : 109-115.

Todorov, S. D., Van Reenen, C. A., and Dicks, L. M. T, 2004. Optimization of bacteriocin production by *Lactobacillus plantarum* ST13BR, a strain isolated from barley beer. *J. Gen. Appl. Microbiol.*, 50 : 149-157.

Vitali, B., Minervini, G., Rizzello, C. G., Spisni, E., Simone M, S., Brigidi P., Gobbettib, M. and Di Cagno, R. 2012. Novel probiotic candidates for humans isolated from raw fruits and vegetables. *Food Microbiology,* 31 : 116-125.