Intl. J. Ferment. Food. 8(2): 89-96, December 2019

DOI: 10.30954/2321-712X.02.2019.5

Evaluation of Bile Salt Hydrolase Activity, Bile Salt Deconjugation Ability, Cholesterol Assimilation Ability and Antioxidative Activity of *Lactobacillus* Cultures

Dhvany Kinariwala[1] and Subrota Hati[2]*

[1]Department of Biochemistry, St. Xavier's College (Autonomous), Ahmedabad-380009, Gujarat, India
[2]Department of Dairy Microbiology, Anand Agricultural University, Anand-388110, Gujarat, India

*Corresponding author: subrota_dt@yahoo.com

Received: 09-07-2019 **Revised:** 14-10-2019 **Accepted:** 28-11-2019

ABSTRACT

Probiotic potential of three cultures of *Lactobacillus* namely *L.fermentum* (M2), *L.fermentum* (M7) and *L.paracasei* (M11) were carried out by performing various *in vitro* tests such as bile salt hydrolase activity, bile salt deconjugation ability, cholesterol assimilation ability and antioxidative activity (ABTS method). M7 and M11 showed positive bile salt hydrolase activity. M2, M7 and M11 have the ability to deconjugate bile salt and M11 culture exhibited for the highest deconjugation of bile salt (616.25µg/mL). These three cultures were able to survive in MRS broth containing 0.2% sodium thioglycolate and 0.3% sodium taurocholate (conjugated bile salt). Antioxidant activity was also evaluated among these *Lactobacillus* cultures and found in the range of 46.55% to 78.66%. But, these three cultures were very poor to assimilate the cholesterol. This study indicated that *L. paracasei* (M11)was more potent than other cultures.

Keywords: *Lactobacillus*, Bile salt hydrolase, Bile deconjugation ability, Cholesterol assimilation, Antioxidative activity

Many studies show the usefulness of lactic acid bacteria (LAB) as probiotics for human and animals (Lee and Salminen, 1995; Tannock, 1997; Brashears *et al.* 2003; Hamilton-Miller, 2003). It has been claimed that lots of commercial products or food containing LAB are healthy (Rolfe, 2000; Sakamoto *et al.* 2001; Sanders, 2003). Some LAB items have been demonstrated to be valuable as aides to keep human from gastrointestinal issue and from intense enteritis (Coconnier *et al.* 2000; Armuzzi *et al*. 2001). Probiotics have been used in various fermented foods for a long time. There are many commercial products available in the markets all over the world which contains LAB. These LAB products are available in liquid form, such as yogurt, and solid form, such as powder, granule and tablet. Most of the LAB cultures are used in combination which means product contains two or more LAB cultures. These cultures include *Lactobacillus thermophilus*, *Streptococcus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium longum*, etc (Analie and Viljoen, 2001). The genus *Lactobacillus* is the biggest of the genera remembered for lactic corrosive microorganisms with more than hundred species perceived at present. The genus *Lactobacillus* is heterogeneous containing species with considerable contrasts in their phenotypic, biochemical, physiological and genotypic attributes. The food and feed industry focus to utilize the lactobacilli for the fermentation of vegetables, silage, sourdough bread, and other milk and meat items, albeit a few species additionally found to ruin the food sources (Stiles and Holzapfel,

1997). LAB species are generally recognized as safe (GRAS) and European Food Safety Authority (EFSA) has stated that several LAB speciesare Qualified Presumption of Safety (QPS). Aside from their role in food fermentations and preservation, there is a developing intrigue for LAB regarding health benefits and other medicinal applications. Some species of LAB have the potential to improve human health by various means such as strengthening the body's natural defense to fend off undesirable microorganisms, modulating the immune system by enhancing host's immune response (Reid *et al.* 2003), binding and removal of carcinogens, production of antimutagenic compounds and improving overall intestinal and urinary-genital health and so on (Parvez *et al.* 2006). Proof from *in vitro* systems, animal models and human clinical examinations propose that LAB work as immunomodulators and can improve both explicit and vague insusceptible reactions (Ouwehand *et al.* 2002; O'Flaherty and Klaenhammer, 2010) legitimizing their utilization as wellbeing advancing enhancements or probiotics both for people and animals. Utilization of food containing probiotic microscopic organisms influence the gastrointestinal tract (GIT) as they develop and increase in GIT to applies different wellbeing advancing activities. Main purpose of this study is to check safety and efficacy of *Lactobacillus* cultures.

MATERIALS AND METHODS

The present study was planned to check probiotic potential of lactic acid bacterial isolates by *in vitro* tests.

Collection of LAB isolates and their maintenance

The LAB cultures used in the present study were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand (Table 1). The LAB cultures were propagated in sterilized reconstituted skim milk. The transfer was given every week during the study. Before use in the study, they were activated by propagation in MRS broth by daily transfers.

Evaluation of probiotic potential of LAB isolates by *in vitro* tests

Three potent *Lactobacillus* cultures i.e*L. fermentum* (M2), *L. fermentum* (M7) and *L. paracasei* (M11) were taken for evaluating their probiotic potential as follows.

Bile salt hydrolase activity

It was performed by plate assay method (Jayashree *et al.* 2014) which is found ubiquitously in fermented milk products. Bile salt hydrolase (BSH with some modifications. For the well assay, wells were prepared using a gel puncture on MRS agar plates with 0.3% bile salts and 0.2% sodium thioglycolate. In each well, 150 µL of supernatant of activated cultures were added and incubated at 6±2°C for 3 h. The bile acid precipitates around the colonies (opaque halo) or the formation of opaque granular white colonies with silvery shine was considered as BSH activity.

Bile deconjugation ability

Bile deconjugation ability of LAB cultures were tested by the method of Irvin *et al.* (1994) as modified by Walker and Gilliland (1993) and Ashar and Prajapati (1998). MRS broth medium containing 0.2% sodium thioglycollate to which conjugated bile salt (sodium taurocholate) was separately added at 0.3% rate, was used to test the same. Active test cultures were inoculated at the rate of 2% into 20 mL MRS broth tubes. An uninoculated broth tubes were also kept

Table 1: Gene accession number and isolation source of LAB isolates

Sl. No.	Culture Name	Source of Isolation	Selective Media	Growth Conditions	Gene bank Accession no.
1	*Lb. fermentum* (M2)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951094
2	*Lb. fermentum* (M7)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951099
3	*Lb. paracasei* (M11)	Fermented rice beverage	MRS Agar	37°C for 24h	MG027695

along with the test. The tubes were incubated at 37°C under reduced O_2 atmosphere in a gas pack jar for 24 h. An anaerobic indicator tablet was kept in gas pack jar to indicate reduced condition.

Extraction of free cholic acid

After incubation, the spent broth was adjusted to pH 7 using 1 [N] NaOH. Then volume was made to 25 mL with distilled water. The cells were removed by centrifugation at 10, 000 rpm for 15 min at 4°C. Fifteen mL of the resultant supernatant fluid was adjusted to pH 1 with 10 [N] HCL and the volume was increased to 25 mL with distilled water. Three mL of this fluid broth was taken as sample and 9 mL of ethyl acetate was added. The contents were thoroughly mixed on cyclomixer and the tubes were kept undisturbed for some time to allow phase separation. Thereafter, 3 mL of ethyl acetate layer was taken in 18 mm diameter test tubes and was evaporated to dryness in a water bath at 60°C.

Estimation of free cholic acid

One mL of 0.1[N]NaOH was added to the tubes to dissolve the residue followed by the addition of 6 mL of [16] N H_2SO_4 and 1 mL of 1% furfuraldehyde. The tubes were heated for 15 min in a water bath at 65°C followed by subsequent cooling to room temperature. Five mL of glacial acetic acid was added finally to stop the colour development. Then the tubes were measured for the absorbance at 660 nm wavelength against a reagent blank using Systronic PC based double beam Spectrophotometer, 2206. Standard curve for free bile acid (cholic acid) was prepared by taking 100, 200, 400, 600, 800 and 1000 μg of cholic acid. Cholic acid used for preparation of standard curve was dissolved in 0.1 [N] NaOH and then further diluted with distilled water as per the concentration desired per mL and following the above-mentioned procedure for estimation. The free cholic acid content in the uninoculated as well as inoculated tubes was obtained by interpolation from the standard curve. The difference of cholic acid in test and blank was calculated individually for each strain and this value was expressed as μg/mL of free cholic acid released in the medium.

Cholesterol assimilation ability

The procedure of Gilliland and Walker (1990) adopted by Ashar and Prajapati (1998) was followed for the study of cholesterol assimilation activity by the culture. Fifty μg/mL of cholesterol was aseptically added into 10 mL of MRS broth base containing 0.2% sodium thioglycollate and 0.3% sodium taurocholate. To this broth media tubes, 24 h active test strain of LAB was inoculated at the rate of 2%. The tubes were incubated anaerobically under reduced O_2 conditions in a gas pack jar at 37°C up to 24 h. Thereafter, the content of the tubes was centrifuged at 10,000 rpm for 10 min at 4°C (Eppendorf Centrifuge, US). Supernatant broth obtained thus was treated as sample and 0.5 mL of the same was transferred into a clean test tube.

Extraction

To the above sample, 3 mL of 95% ethanol followed by 2 mL of 50% KOH were added to the tubes and the contents were mixed thoroughly on a cyclomixer. Thereafter the tubes were heated for 10 min in a water bath maintained at 60°C and cooled subsequently. Further, 5 mL of hexane was added, and the tubes were mixed thoroughly. Then, 3 mL of distilled water was added, and the mixing was repeated. To permit phase separation, the tubes were allowed to stand for 15 min at room temperature. Thereafter, 2.5 mL hexane layer was transferred into clean test tubes. The hexane was evaporated from the tubes by heating them at 60°C using hot water bath for an overnight period.

Estimation

The method of Rudel and Morris (1973) using O-phthalaldehyde (OPA) was followed. In this method, 4 mL of OPA reagent (50 mg OPA per liter of glacial acetic acid) was added in above dried extracts and the tubes were allowed to stand at room temperature for 10 min. Then, 2 mL of concentrated sulphuric acid was added slowly from the side of the test tube and the contents mixed thoroughly on cyclomixer. The tubes were allowed to stand at room temperature for further 10 min. Then, the

test absorbance was read against blank at 550 nm wavelength on Systronic PC based double beam Spectrophotometer, 2206. Results were recorded in terms of percentage reduction in cholesterol in the test supernatant broth as compared to that in the uninoculated blank supernatant broth.

$$\% \text{ cholesterol removal from media} = \frac{c_0 - c_1}{c_0} \times 100$$

Where,

C_0:OD_{550} of MRS broth supernatant containing no culture

C_1:OD_{550} of MRS broth supernatant containing culture

Antioxidative activity

Free radical scavenging activity was determined by the ABTS method followed by Hati *et al.* (2018) with slight modifications.

The ABTS working solution was prepared by mixing 88 µL of 140 mM potassium persulphate with 5 mL of 7 mM ABTS stock solution and incubating overnight in dark bottles for generation of radicals. An aliquot of 5 mL of this solution was added to 150 mL PBS to adjust the absorbance at 734 nm to 0.7 ± 0.02. Active culture supernatant was collected by centrifuging at 10,000 rpm for 10 min at 4°C (Eppendorf Centrifuge, US). 200 µL of cell supernatant was added to 2.0 mL ABTS in PBS solution and absorbance was measured at 734 nm. As a blank double distilled water was used. ABTS activity was calculated as follow:

$$\text{Antioxidant (\%)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Statistical methods

All the data were subjected to statistical analysis using one factor and two factor Completely Randomized Design (CRD) as per the requirement. The significance was tested on basis of comparison between calculated value and Table F-value. Standard error of mean value, co-efficient of variance (C.V.) and critical difference (C.D.) were determined. The values for microbial counts were log transformed before analysis.

RESULTS AND DISCUSSION

Evaluation of *Lactobacillus* cultures for probiotic potential

Probiotic potential of three *Lactobacillus* cultures (M2, M7 and M11) were carried out by performing a battery of *in vitro* tests i.e. bile salt hydrolase activity, bile deconjugation ability, cholesterol assimilation ability and antioxidative activity.

Evaluation of *Lactobacillus* cultures for bile salt hydrolase activity (BSH)

Lactobacillus cultures M2, M7 and M11 were evaluated for BSH activity following the method of Jayashree *et al.* (2014). It was observed that M7 showed positive activity up to 3 h of incubation followed by M11 whether M2 did not show any significant bile hydrolyzation (Fig. 1).

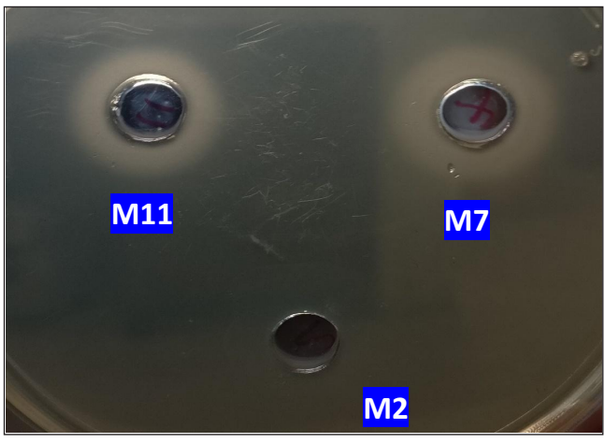

Fig. 1: BSH activity of *Lactobacillus* cultures

Certain probiotic strains of lactic acid bacteria have capacity to enzymatically deconjugate bile acids through bile salt hydrolase catalysis. Partial absorbance of the deconjugated bile acids are done through the intestinal cell wall while remaining deconjugated bile acid will be discharged out. The utilization of cholesterol to blend new bile would prompt a diminished convergence of cholesterol in blood because cholesterol is the precursor of bile acids (Lye *et al.* 2009). Tanaka *et al.* (2000) had observed that almost all bifidobacterium species and strains have

bile salt hydrolase activity, while this action must be found in chosen species of lactobacilli. Argyri *et al.* (2013) had reported that *Lactobacillus plantarum* and *Lactobacillus pentosus* (strains isolated from fermented olives)exhibit partial bile salt hydrolase activity. Commercial probiotic strains and culture collection strains were studied for screening of BSH activity by using TLC method and plate assay. Among all the 59 *Lactobacillus* strains, 15 strains were shown to be BSH positive by TLC method and 8 strains of them by plate assay. Most of the BSH positive strains were isolated from feces (Sedláčková *et al.* 2015).

Evaluation of *Lactobacillus* cultures for bile salt deconjugation ability

Bile salt deconjugation was determined by the amount of cholic acid released. Free cholic acid released from sodium taurocholate by cultures is presented in Table 2. The values were calculated from the standard curve prepared using various concentration of cholic acid.

Table 2: Amount of free cholic acid released by *Lactobacillus* cultures

Lactobacillus cultures	Free cholic acid (μg/mL)	Free cholic acid (μg/mL)
Control	532.875	—
M2	922.875	390
M7	679.125	146.25
M11	1125.125	616.25

M11 exhibited highest bile deconjugation ability (616.25 μg/mL cholic acid from sodium taurocholate) followed by M2 (390 μg/mL cholic acid) and M7 (146.25 μg/mL cholic acid). Results showed that control has also some capability to deconjugate bile salts.

Begley *et al.* (2008) had said that deconjugated bile salts are just somewhat reabsorbed in the intestinal lumen, which brings about discharge of free bile salts into feces; accordingly, cholesterol is utilized for de-novo synthesis of bile acids, in this manner diminishing serum cholesterol level. Free bile salts likewise wastefully solubilize cholesterol (lipids) in digestion tracts along these lines further reducing cholesterol absorption. Sontakke (1992) had performed bile deconjugation ability test for different strains of lactobacilli and he had observed that only *Lactobacillus acidophilus* (CH) was able to deconjugate sodium taurocholate and sodium glycocholate. Ashar and Prajapati (1998) had checked bile deconjugation ability of different strains of acidophilus namely *Lb. acidophilus* H3, *Lb. acidophilus* C2, *Lb. acidophilus* V3 and *Lb. acidophilus* I4. Among them H3 released maximum amount (443 μg/ ml) of cholic acid from sodium taurocholate, followed by C2 (422 μg/ ml), V3 (389 μg/ ml) and I4 (332μg/ ml) after 24 h of growth at 37°C. In our findings, M2 (*Lb. fermentum*)has capacity to deconjugate bile acids about 390 μg/mL which value is almost nearest with V3 (389 μg/mL).

Evaluation of *Lactobacillus* cultures for cholesterol assimilation ability

Cholesterol assimilation ability of *Lactobacillus* cultures after 24 h anaerobic incubation in MRS broth was determined. The results of cholesterol assimilation by cultures of lactobacilli after 24 h incubation under reduced O_2 tension maintained in gas pack jar are presented in Fig. 2 and it was found that these cultures [M2 (3.15%), M7 (1.05%) and M11 (5.11%)] have very low ability to assimilate cholesterol.

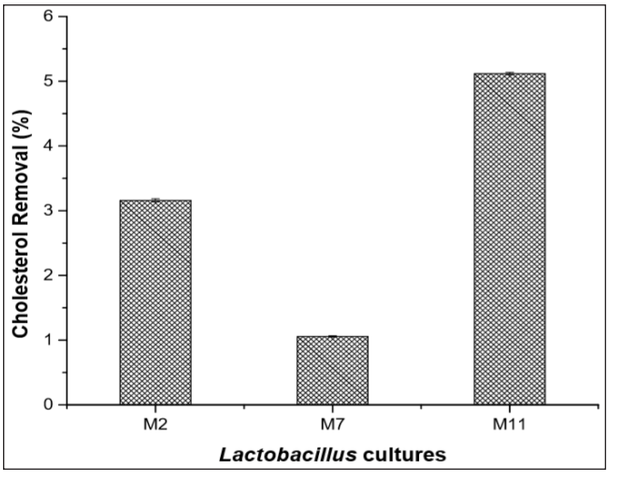

Fig. 2: Cholesterol assimilation ability of *Lactobacillus* cultures

Several theories of how probiotics remove host cholesterol have been put forward. They are mainly: (1) Assimilation (2) Coprecipitation (3) Adsorption and incorporation4) Reduction of the cholesterol absorption by host (Li 2012). Ashar and Prajapati (1998) had performed cholesterol assimilation ability test for four strains of *Lactobacillus acidophilus* (V3, I4, H3 and C2). They observed that strains have ability to reduce cholesterol ranged from 3.2 to 25.3 percent within 48 h by *in vitro* study.

Evaluation of *Lactobacillus* cultures for antioxidative activity

The antioxidant activity was measured in terms of free radical scavenging activity (RSA) using ABTS assay method and results were expressed in terms of percentage (%) of inhibition. Absorbance was measured at 734 nm for 6 min 20 sec at every 10 sec. The results obtained for 3 *Lactobacillus* cultures by ABTS (2, 2'-Azinobis (3-ethylene benzothiazoline) 6-Sulphonicacid) assay is and the trend of inhibition rate of selected *Lactobacillus* cultures has shown in Fig. 3.

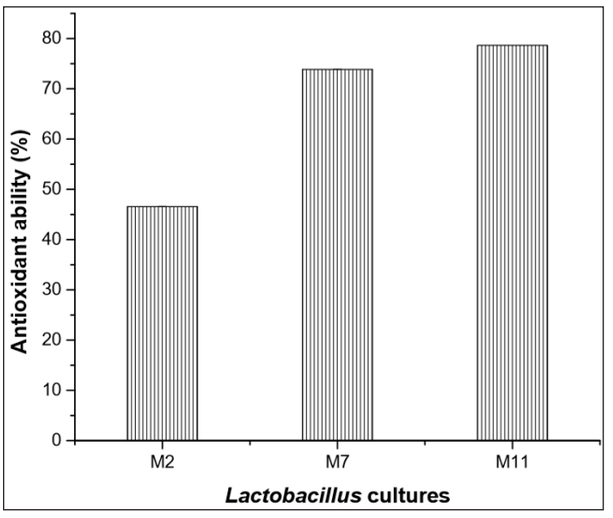

Fig. 3: Antioxidative activity of *Lactobacillus* cultures

M11 culture had higher antioxidant activities (78.66%) but according to statistical analysis, there is no significant difference between M7 and M11. Only 46.55% inhibition of oxidation process was observed by M2 culture.

According to the free radical theory of aging (Muller *et al.* 2007), the interruption of the sensitive balance between generation of reactive oxygen species (ROS) and antioxidant scavenging systems with increasing age could lead to a shift to an oxidative cellular milieu, and eventually lead to serious health problems such as diabetes and Alzheimer's disease. Therefore, it is necessary to protect the human body from free radicals and retard the progress of many chronic diseases (Bellino, 2006; Getoff, 2007). Some LAB own antioxidant activity and can reduce the risk of accumulation of ROS during ingestion of food (Kaizu *et al.* 1993).

Nearly alllactic acid bacteria have system to survive with oxygen radicals. LAB was noticed to corrupt the superoxide anion and hydrogen peroxide *in vitro* (Kullisaar *et al.* 2002). The antioxidant activity of probiotic cultures viz. *S. thermophilus* MTCC 5460 and *Lb. helveticus* MTCC 5463, was determined by ABTS method. It was observed that antioxidative activity of *Lb. helveticus* 5463, *S. thermophilus* MTCC 5460 and combination of both the cultures was 0.47%, 3.01% and 5.46% respectively (Shah, 2011).

CONCLUSION

Based on the above results, for bile salt hydrolase activity, M7 showed maximum zone around the well (in agar plate) than M11. These three *Lactobacillus* cultures M2, M7 and M11 were able to survive in presence of bile and showed their ability to hydrolase (except M2) and deconjugate. But they are poor to assimilate cholesterol. M7 and M11 culture showed maximum antioxidant activities (78.66%). Further, health claims for particular *Lactobacillus* cultures need to be validated on conducting clinical trials on human subjects.

ACKNOWLEDGEMENTS

The authors kindly acknowledge the funding support received from the Department of Biotechnology, New Delhi, Government of India (Agri/2015/02) under the Twining project-2015.

REFERENCES

Argyri, A.A., Zoumpopoulou, G., Karatzas, K.A.G., Tsakalidou, E., Nychas, G.J.E., Panagou, E.Z. and Tassou, C.C. 2013. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiology*, **33**(2): 282-291.

Armuzzi, A., Cremonini, F., Bartolozzi, F., Canducci, F., Candelli, M., Ojetti, V. and Gasbarrini, G. 2001. The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy. *Alimentary Pharmacology and Therapeutics*, **15**(2): 163-169.

Ashar, M.N. and Prajapati, J.B. 1998. Bile tolerance, bile deconjugation and cholesterol reducing properties of dietary lactobacilli. *Indian Journal of Microbiology*, **38**(3): 145-148.

Begley, T.P., Chatterjee, A., Hanes, J.W., Hazra, A. and Ealick, S.E. 2008. Cofactor biosynthesis-still yielding fascinating new biological chemistry. *Current Opinion in Chemical Biology*, **12**(2): 118-125.

Bellino, F.L. 2006. Advances in endocrinology of aging research, 2005–2006. *Experimental Gerontology*, **41**(12): 1228-1233.

Brashears, M.M., Jaroni, D. and Trimble, J. 2003. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157: H7 in cattle. *Journal of Food Protection*, **66**(3): 355-363.

Coconnier, M.H., Liévin, V., Lorrot, M. and Servin, A.L. 2000. Antagonistic activity of *Lactobacillus* acidophilus LB against intracellular Salmonella enterica serovar Typhimurium infecting human enterocyte-like Caco-2/TC-7 cells. *Applied and Environmental Microbiology*, **66**(3): 1152-1157.

Getoff, N. 2007. Anti-aging and aging factors in life. The role of free radicals. *Radiation Physics and Chemistry*, **76**(10): 1577-1586.

Gilliland, S.E. and Walker, D.K. 1990. Factors to consider when selecting a culture of *Lactobacillus* acidophilus as a dietary adjunct to produce a hypocholesterolemic effect in humans. *Journal of Dairy Science*, **73**(4): 905-911.

Hamilton-Miller, J.M.T. 2003. The role of probiotics in the treatment and prevention of Helicobacter pylori infection. *International Journal of Antimicrobial Agents*, **22**(4): 360-366.

Hati, S., Patel, N. and Mandal, S. 2018. Comparative growth behaviour and biofunctionality of lactic acid bacteria during fermentation of soy milk and bovine milk. *Probiotics and Antimicrobial Proteins*, **10**(2): 277-283.

Irvin, J.L., Johnston, C.G. and Kopala, J. 1944. A photometric method for the determination of cholates in bile and blood. *Journal of Biological Chemistry*, **153**(2): 439-457.

Jayashree, S., Pooja, S., Pushpanathan, M., Rajendhran, J. and Gunasekaran, P. 2014. Identification and characterization of bile salt hydrolase genes from the genome of *Lactobacillus* fermentum MTCC 8711. *Applied Biochemistry and Biotechnology*, **174**(2): 855-866.

Kaizu, H., Sasaki, M., Nakajima, H. and Suzuki, Y. 1993. Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *Journal of Dairy Science*, **76**(9): 2493-2499.

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C. and Kilk, A. 2002. Two antioxidative lactobacilli strains as promising probiotics. *International Journal of Food Microbiology*, **72**(3): 215-224.

Lee, Y. K. and Salminen, S. 1995. The coming of age of probiotics. *Trends in Food Science and Technology*, **6**(7): 241-245.

Li, G. 2012. Intestinal probiotics: interactions with bile salts and reduction of cholesterol. *Procedia Environmental Sciences*, **12**: 1180-1186.

Lourens-Hattingh, A. and Viljoen, B.C. 2001. Yogurt as probiotic carrier food. *International Dairy Journal*, **11**(1-2): 1-17.

Lye, H.S., Kuan, C.Y., Ewe, J.A., Fung, W.Y. and Liong, M.T. 2009. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *International Journal of Molecular Sciences*, **10**(9): 3755-3775.

Muller, F.L., Lustgarten, M.S., Jang, Y., Richardson, A. and Van Remmen, H. 2007. Trends in oxidative aging theories. *Free Radical Biology and Medicine*, **43**(4): 477-503.

O'Flaherty, S. and Klaenhammer, T.R. 2010. The role and potential of probiotic bacteria in the gut, and the communication between gut microflora and gut/host. *International Dairy Journal*, **20**(4): 262-268.

Ouwehand, A.C., Salminen, S. and Isolauri, E. 2002. Probiotics: an overview of beneficial effects. In *Lactic Acid Bacteria: Genetics, Metabolism and Applications, pp.* 279-289.

Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, **100**(6): 1171-

Reid, G., Jass, J., Sebulsky, M.T. and McCormick, J.K. 2003. Potential uses of probiotics in clinical practice. *Clinical Microbiology Reviews*, **16**(4): 658-672.

Rolfe, R.D. 2000. The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition*, **130**(2): 396S-402S.

Rudel, L.L. and Morris, M.D. 1973. Determination of cholesterol using o-phthalaldehyde. *Journal of Lipid Research*, **14**(3): 364-366.

Sakamoto, I., Igarashi, M., Kimura, K., Takagi, A., Miwa, T. and Koga, Y. 2001. Suppressive effect of *Lactobacillus* gasseri OLL 2716 (LG21) on Helicobacter pylori infection

in humans. *Journal of Antimicrobial Chemotherapy*, **47**(5): 709-710.

Sanders, M.E. 2003. Probiotics: considerations for human health. *Nutrition Reviews*, **61**(3): 91-99.

Shah, C.H. 2011. Antioxidative activity of selected symbiotic fermented dairy products. M. Sc. Thesis submitted to Anand Agricultural University, Anand, Gujarat, India.

Sontakke, A.T. 1992. Beneficial activities of selected human strains of Lactobacilli in milk for its use as dietary adjunct. PhD Thesis submitted to Gujarat Agricultural University, Anand.

Stiles, M.E. and Holzapfel, W.H. 1997. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, **36**(1): 1-29.

Tanaka, H., Hashiba, H., Kok, J. and Mierau, I. 2000. Bile salt hydrolase of Bifidobacterium longum—biochemical and genetic characterization. *Applied and Environmental Microbiology*, **66**(6): 2502-2512.

Tannock, G.W. 1997. Probiotic properties of lactic-acid bacteria: plenty of scope for fundamental R and D. *Trends in Biotechnology*, **15**(7): 270-274.

Sedláčková, P., Horáčková, Š., Shi, T., Kosova, M. and Plocková, M. 2015. Two different methods for screening of bile salt hydrolase activity in *Lactobacillus* strains. *Czech Journal of Food Sciences*, **33**(1): 13-18.

Walker, D.K. and Gilliland, S.E. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. *Journal of Dairy Science*, **76**(4): 956-961.