Biotechnol Res 2018; Vol 4(2):62-68
eISSN 2395-6763

ORIGINAL RESEARCH

Relative probiotic potential of milk bacteria from *Desi* and cross-breed cows and its compatibility with prebiotics

Jyoti CHOUDHARY*, Ramesh Chand DUBEY*
Department of Botany and Microbiology, Gurukula Kangri Vishwavidyalaya, Haridwar – 249404, India
*Corresponding Authors email: profrcdubey@gmail.com, jyotichoudharyibt@gmail.com

• *Received: 03 January 2018* • *Revised: 12 February 2018* • *Accepted: 28 February 2018* • *Published: 03 March 2018* •

ABSTRACT
Fifty two lactic acid bacteria (LAB) were isolated from milk of *Desi* and cross-breed cows of India and its functional characteristics were investigated on the basis of morphological and biochemical properties. Among them four bacterial isolates were screened on the basis of *in vitro* probiotic attributes. Isolate CP-12^{d} and CP-8^{d} which were isolated from *Desi* Indian cows showed high tolerance to low pH, bile, NaCl and certain antibiotics. These isolates exhibited the highest adhesion to hydrocarbons xylene, n-octane and n-hectane. Isolate CP-8^{d} exhibited the highest auto-aggregation rate (62%). It was the most resistant isolate against different antibiotics. All the four isolates inhibited the enteric pathogens viz., *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi* but the isolate CP-12^{d} and CP-8^{d} exhibited high antagonistic activity against all pathogens. These two bacterial isolates exhibited good probiotic properties and also grew well in presence of inulin as compared to honey.

KEY WORDS: *Lactic acid bacteria, Probiotics, Antibacterial activity, Prebiotics*

INTRODUCTION

Probiotics are the live microbial food supplements which beneficially affect the host by improving its intestinal microbial balance. There are many benefits of probiotics including improved nutrition, growth and prevention of various gastrointestinal (GI) disorders. Traditionally, physicians used various antibiotics for treating gastrointestinal disorders. However, the incidence of antibiotic-associated diarrhea (AAD) and resistance of the pathogens to antibiotics require alternative strategies for treatment. AAD results from disruption of the normal microflora of the gut by antibiotics (Wistrom *et al.,* 2001). Lactic acid bacteria (LAB) are widely used in food and pharmaceutical industry, especially for the fermentation of milks and as medicine to improve the gastrointestinal health (Battcock and Azam-Ali, 1998). Some LAB strains can be used as probiotics for human and animals (Chou and Weimer, 1999). In general, LAB used as probiotic should be resistance to host gastrointestinal conditions, adhesion to host intestinal epithelium, and the prevention of growth or invasion of pathogenic bacteria, such as *Salmonella* spp. and *Escherichia coli* in the animal intestine (Chou and Weimer, 1999; Jin *et al*., 1996). Furthermore, certain LAB strains have been reported earlier for other health benefits, such as stimulation of the immune system of the human hosts (Schiffrin *et al*. 1997). Bacterial strains to be considered as probiotic should contain some essential properties, such as origin of strain, safety, acid, bile resistance, survivability during processing, and storage with beneficial effects (Saarela *et al*., 2000; Holzapfel and Schillinger, 2002). The growing competence in

characterizing and harnessing the potential of these minute, short-lived, health promoting microorganisms has added new dimensions to the understanding of their usefulness to humans.

Probiotics produce a variety of compounds responsible for their antimicrobial activity *i.e*., exopolysaccharides, organic acids and bacteriocins, etc. (Ouwehand *et al*., 1999). An effective probiotic should be viable and able to survive during the passage in GI tract (Casey *et al*., 2004; Singh *et al*., 2011). Probiotic bacteria should adhere and colonize on gut epithelial cells (Walker and Duffy, 1998). Bacterial Adhesion to Hydrocarbons (BATH) supports the adherence capability of bacteria to gut surfaces to enhance their interaction with the host (Kumar *et al*., 2012).

Prebiotics are the 'non-digestible food' ingredients that beneficially affects host by selectively stimulating the activity of probiotic bacteria and normal microflora residing in colon (Gibson *et al*., 2004). Milk of indigenous cows (also called *Desi* cows) has more nutritional value as compared to cross-breed cows (De *et al*., 2015). Therefore, the aim of this study was to investigate the comparative probiotic potential of LAB isolated from *Desi* and cross-breed cows and effect of prebiotics on its growth.

RESULTS AND DISCUSSION

In this study, a total of fifty two isolates were isolated from milk samples collected from *Desi* cows and cross-breed cows. Among them 22 were isolated from milk of *Desi* cows, and 30 were isolated from that of cross-breed cows. The isolates were identified as LAB that appeared white, creamish-yellow, Gram-positive and non-endospore forming. Probiotic bacteria have also been isolated earlier by Garabal *et al*. (2008) but we report the comparative probiotic potential of *Desi* and cross-breed cows milk first time. Milk is considered the most accessible and the best supplement for children and adults also. The nutritive value of milk also depends on the microbial composition and the benefits imparted by these microbes (Cromie *et al*., 1991; Brouillaud *et al*., 1997).

On the basis of the primary screening, two representative isolates from each referred source (CP-4^{c} and CP-26^{c} from cross-breed, while CP-12^{d} and CP-8^{d} from *Desi* breed) were selected for further study. Primary screening for *in vitro* probiotic attributes revealed that the survival rate of bacterial isolates from *Desi* cow milk was high at low pH and in different concentration of bile as compared to that from cross-breed cow. Bacterial isolates from *Desi* breed were also able to tolerate the high salt concentration as compared to cross-breed cows.

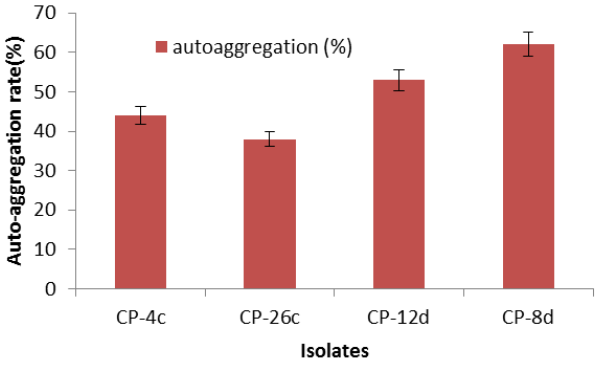

Figure 1: Auto-aggregation rate of bacterial isolates of *Desi* and cross-breed cow's milk.

The main *in vitro* selection criteria for any probiotic bacteria are acid and bile-resistance which indicate their ability to survive in GI tract (Pennacchia *et al*., 2004; Garabal *et al*., 2008). Before the entry in GI tract, these probiotic bacteria transit through the stomach where pH varied from 1.5 to 2.0. All the selected isolates were able to resist the low pH. The survival rate of isolate CP-12^{d} and CP-8^{d} was higher than that of CP-4^{c} and CP-26^{c} in acidic condition (Table 1).

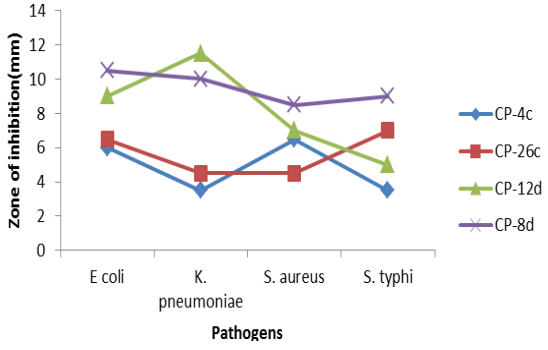

Figure 2: Antibacterial activity of selected of *Desi* and cross-breed cow's milk bacterial isolates.

The average bile concentration in GI tract remains around 0.3% (Gupta and Tiwari, 2014). Therefore, these probiotic bacteria must tolerate such bile concentration to survive in intestine and maintain its microflora. Isolate CP-12^{d} and CP-

Table 1: Growth rate of lactic acid bacterial isolates at low pH

Isolates	Acid tolerance (viable counts, log cfu/ml)					
	pH 2.0		pH 3.0		pH 6.5	
	0h	4h	0h	4h	0h	4h
CP-4[c]	6.7 ± 0.2	3.4 ± 0.2	7.5 ± 0.4	4.1 ± 0.6	8.0 ± 0.3	8.9 ± 0.2
CP-26[c]	6.2 ± 0.5	3.9 ± 0.2	7.4 ± 0.3	4.5 ± 0.3	8.1 ± 0.1	9.2 ± 0.2
CP-12[d]	8.7 ± 0.4	6.7 ± 0.5	9.2 ± 0.7	7.5 ± 0.2	9.0 ± 0.8	10.1± 0.0
CP-8[d]	8.3 ± 0.3	6.1 ± 0.4	8.8 ± 0.5	7.1 ± 0.1	9.5 ± 0.1	10.7± 0.1

Values are mean of triplicate ± standard error

Table 2: Effect of bile on lactic acid bacterial isolates

Isolates	Bile tolerance (viable counts, log cfu/ml)			
	0.50 %		1.00 %	
	0h	8h	0h	8h
CP-4[c]	7.9 ± 0.5	5.9 ± 0.3	7.7 ± 0.2	4.7 ± 0.2
CP-26[c]	7.4 ± 0.4	5.6 ± 0.2	7.2 ± 0.1	5.1 ± 0.3
CP-12[d]	9.6 ± 0.3	8.9 ± 0.2	9.7 ± 0.2	7.6 ± 0.1
CP-8[d]	10.3 ± 0.1	9.9 ± 0.5	10.1 ± 0.4	8.5 ± 0.2

Values are mean of triplicate ± standard error

Table 3: Hydrophobicity (%) of lactic acid bacterial isolates

Isolates	Hydrophobicity (%)		
	Xylene	n-Octane	n-Hectane
CP-4[c]	13.5 ± 0.3	22.5 ± 0.7	29.0 ± 0.4
CP-26[c]	26.2 ± 0.5	19.2 ± 0.4	37.1 ± 0.3
CP-12[d]	38.3 ± 0.2	36.2 ± 0.1	56.2 ± 0.1
CP-8[d]	54.6 ± 0. 4	47.7 ± 0.6	45.5 ± 0.5

Values are mean of triplicate ± standard error

Table 4: Antibiogram of lactic acid bacterial isolates

Antibiotics	Conc. (mg)	CP-4[c]	CP-26[c]	CP-12[d]	CP-8[d]
Tetracyclin	25	R	R	R	R
Streptomycin	10	S	R	R	R
Chlormaphenicol	25	S	S	R	R
Erythromycin	5	R	S	R	R
Penicillin	1	R	S	R	R
Novobiocin	5	S	S	S	R
Meticillin	10	S	S	R	R
Fusidic acid	10	R	R	S	R

R- Resistance; S-Sensitive

8[d] grew well in presence of bile, though CP-8[d] displayed the highest (9.9 and 8.5 log cfu/ml) survival rate at 0.5% and 1.0% bile till 8h, respectively (Table 2). But the survival rate of bacterial isolates from cross-breed cows was not satisfactory.

BATH test explains the adhesion properties of bacterial isolates and hydrophilic and hydrophobic nature of the cell surface of bacteria (Lee *et al*., 2008). High adherence to xylene represents the hydrophobic nature of the bacterial cell surface. All the isolates followed the different range of adhesion with different hydrocarbons (Table 3). Isolate CP-12[d] and CP-8[d] showed high percentage of hydrophobicity with each hydrocarbon used in this study. CP-12[d] and CP-8[d] have showed maximum (56% and 54%) percentage of hydrophobicity with n- hectane and xylene, respectively. Aggregation capability of bacterial isolates directly relates to their colonization potential in GI tract (Casena *et al*., 2001). Auto-aggregation rate of the isolates ranged between 35 and 65% (Fig. 1). Isolate CP-8[d] aggregated rapidly as compared to the other isolates followed by CP-12[d]. Thus LABs isolated from *Desi* cows showed higher adhesion and auto-aggregation rate as compared to LABs from cross-breed isolates. Isolates CP-4[c] and CP-26[c] were found sensitive to most of the antibiotics such as strepto- mycin, chlormaphenicol, novobiocin, erythromycin, penicillin and meticillin. CP-12[d] was sensitive to only novobiocin and fusidic acid, while CP-8[d] was resistant to all the antibiotics used in this study (Table 4). Therefore, LABs isolated from *Desi* cows were more resistant to antibiotics used during this study as compared to other isolates. Antibiotic susceptibility proves the safety of bacterial strain as probiotic (Herreros *et al*., 2005); hence CP-12[d] and CP-8[d] are safer to consume as probiotic than CP-4[c] and CP-26[c].

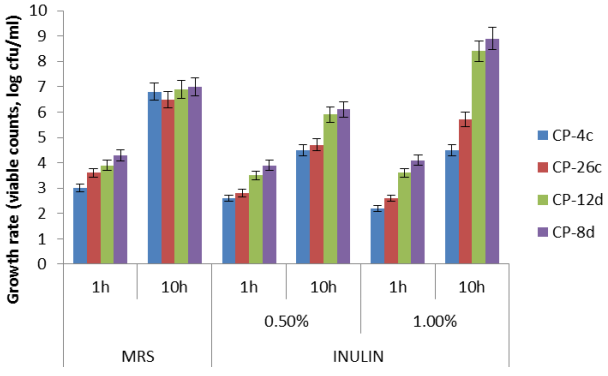

Figure 3: Effect of inulin on the growth of bacterial isolates of *Desi* and cross-breed cow's milk.

All the LABs exhibited antibacterial activity against the selected enteric pathogens. Similar research work has also been performed by Kos *et al*. (2008) to examine the inhibitory effect of some probiotic strains against food-borne pathogens. In this study, CP-8[d] has shown strong

antagonistic activity against each pathogen causing 10.5, 10, 8.5 and 9 mm zone of inhibition against *E. coli, K. pneumonia, S. aureus* and *S. typhi,* respectively (Fig 2). Antibacterial activity of LABs also increases their potential as food preservative for food industries (Gong *et al*., 2010). Both the isolates from *Desi* cow breed inhibited each pathogen efficiently which make them more suitable to treat various gastrointestinal disorders as compared to CP-4^{c} and CP-26^{c}.

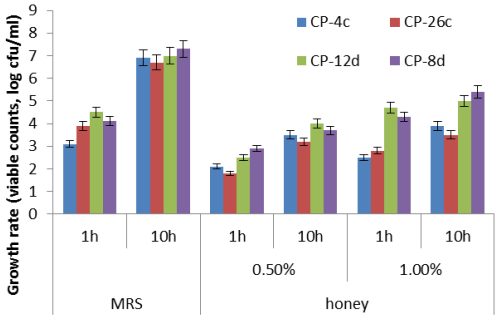

Figure 4: Effect of honey on the growth of bacterial isolates of *Desi* and cross-breed cow's milk

Inulin and honey modulates the growth of LABs by improving the quality and sensory characteristics of dairy products.

They also enhance the physical properties such as firmness and viscosity of the probiotic product (Kristo *et al*., 2003; Donkor *et al*., 2007; Oliveira *et al*., 2009). During this study, all the isolates grew well in presence of inulin as compared to honey (Fig 3 and 4). Isolate CP-12d and CP-8d showed the high growth rate in presence of different concentration of inulin as compared to other LABs isolates. Thus these isolates can be effectively used with inulin as synbiotic.

It may be concluded that the LABs isolated from *Desi* cows has high probiotic potential as compared to the cross-breed cows and they also shown satisfactory growth in presence of prebiotic.

MATERIALS AND METHODS

Isolation and characterization of LAB

Milk samples of lactating cows of both *Desi* (Tharparker, Badri, Kankrej) and cross-breed (Jersey, Holestian fries) were collected aseptically from different area of Haridwar district (Uttarakhand). Fifty two lactic acid bacteria were isolated from 14 milk samples by using serial dilution method. Selected dilutions of the milk samples were spread on the MRS medium and purified by following the method of De Man *et al.* (1960). The isolates were priorly identified on phenotypic traits according to the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Finally, isolates were identified, following different morphological and biochemical tests.

Acid, Bile and NaCl tolerance

Probiotic characteristics, such as tolerance to low pH, bile and NaCl were evaluated by following the method of Romos *et al*. (2013). The overnight grown cultures were separately harvested and suspended in 3 ml MRS broth having pH 2.0, 3.0 and 6.5, and MRS broth supplemented with 0.5 and 1.0% oxgall (Himedia) and NaCl. All the tubes were incubated at 37°C for 24 h and absorbance was measured at 600 nm using a spectrophotometer (Shimadzu, Japan).

Antibiotic resistance

Determination of the resistance of bacterial isolates against different antibiotics was carried out following the method described of Zonenschain *et al*. (2009). Freshly grown cultures of all isolates were separately spread on the MRS agar plates and antibiotic discs (Himedia) were placed on agar surface and incubated at 37°C for 48 h.

Auto-aggregation assay

All the isolates were analyzed for auto-aggregation ability qualitatively as well as quantitatively following the method of Rhaman *et al*. (2008).The overnight grown cultures were harvested by centrifugation, re-suspended in PBS and adjusted to an absorbance between 0.5 and 1.0 at 600 nm and incubated at 37°C. One ml of upper phase was removed carefully after 2 h and the absorbance was measured at 600 nm. Auto-aggregation rate was measured by using the following formula:

$$\text{Auto-aggregation (\%)} = OD_{Initial} - OD_{final} / OD_{Initial} \times 100$$

Bacterial adhesion to hydrocarbons (BATH) test

Adhesion potency of bacterial isolates to different hydrocarbons *i.e.* xylene, n-hexane and n-octane was determined by the modified method of Reniero *et al*. (1992). Bacterial isolates were harvested in log phase by centrifugation at 8000 rpm at 4ºC for 3-5 min. Cell pellets of isolates were washed 2-3 times in phosphate urea magnesium (PUM) buffer (pH 6.5) and absorbance of bacterial

suspension was adjusted near to 1.0 at 600 nm. Aliquots of 3 ml bacterial suspension were separately transferred into different tubes containing each hydrocarbon (1ml). The tubes containing the mixture of bacterial suspension and hydrocarbons were incubated at 37ºC for 10-15 min, and further vortex for 1min. Tubes were kept undisturbed for 1 h to allow the phase separation. Therefore, aqueous phase was separated and transferred carefully into another tube, and absorbance was measured at 600 nm by using spectrophotometer (Shimadzu, Japan). Hydrophobicity was calculated by the following formula:

Hydrophobicity (%) = Initial absorbance/final absorbance X 100

Antagonistic activity of isolates

Antimicrobial activity of the LAB isolates against enteric pathogens *viz*., *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 27853, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733 (procured from IMTECH, Chandigarh, India) was determined by agar well diffusion method using Mueller Hinton agar (MHA) plates following the method of Ridwan *et al*. (2008). Wells of MHA plates containing pathogens were filled with suspension of LAB. Plates were incubated at 37ºC for 24-48 h and the zone of inhibition was measured.

Compatibility of isolates with prebiotics

Compatibility of bacterial isolates with prebiotic was determined following the method of Dhewa *et al*. (2009) with little modification. In MRS medium, glucose was replaced with different concentration of inulin and honey. Thereafter, active bacterial culture was inoculated in the modified MRS medium and viable cells were enumerated at different time intervals as earlier. The medium containing glucose as sole energy source acted as control.

ACKNOWLEDGEMENT

The authors wish to thanks the Head, Department of Botany and Microbiology Gurukula Kangri Vishwavidyalya for providing laboratory facilities.

REFERENCES

Battcock, M., Azam-Ali, S., Axtell, B., & Fellows, P. (1998). Training in food processing: successful approaches. Intermediate Technology Publications Ltd (ITP).

Brouillaud-Delattre, A., Maire, M., Collette, C., Mattei, C., & Lahellec, C. (1997). Predictive microbiology of dairy products: influence of biological factors affecting growth of Listeria monocytogenes. Journal of AOAC International, 80(4), 913-919.

Casey, P. G., Casey, G. D., Gardiner, G. E., Tangney, M., Stanton, C., Ross, R. P., ... & Fitzgerald, G. F. (2004). Isolation and characterization of anti-Salmonella lactic acid bacteria from the porcine gastrointestinal tract. Letters in Applied Microbiology, 39(5), 431-438.

Cesena, C., Morelli, L., Alander, M., Siljander, T., Tuomola, E., Salminen, S., ... & Von Wright, A. (2001). Lactobacillus crispatus and its nonaggregating mutant in human colonization trials. Journal of Dairy Science, 84(5), 1001-1010.

Chou, L. S., & Weimer, B. (1999). Isolation and Characterization of Acid-and Bile-Tolerant Isolates from Strains of Lactobacillus acidophilus1. Journal of Dairy Science, 82(1), 23-31.

Cormier, F., Raymond, Y., Champagne, C. P., & Morin, A. (1991). Analysis of odor-active volatiles from Pseudomonas fragi grown in milk. Journal of Agricultural and Food Chemistry, 39(1), 159-161.

De Man, J. C., Rogosa, D., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. Journal of Applied Microbiology, 23(1), 130-135.

De, S., Paradkar, P., Vaidya, A.D.B. (2015). Indian Breed Cow Milk-Powerhouse of Health. On https://www.researchgate.net/publication/281430281.(access on 14.02.2018).

Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic, T., & Shah, N. P. (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. International Dairy Journal, 17(6), 657-665.

Garabal, J. I., Rodríguez-Alonso, P., & Centeno, J. A. (2008). Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). LWT-Food Science and Technology, 41(8), 1452-1458.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews, 17(2), 259-275.

Gong, H. S., Meng, X. C., & Wang, H. (2010). Plantaricin MG active against Gram-negative bacteria produced by Lactobacillus plantarum KLDS1. 0391 isolated from "Jiaoke", a traditional fermented cream from China. Food Control, 21(1), 89-96.

Gupta, A., & Tiwari, S. K. (2014). Probiotic potential of Lactobacillus plantarum LD1 isolated from batter of Dosa, a South Indian fermented food. Probiotics and Antimicrobial Proteins, 6(2), 73-81.

Helland, M. H., Wicklund, T., & Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria in milk-and water-based cereal puddings. International Dairy Journal, 14(11), 957-965.

Herreros, M. A., Sandoval, H., González, L., Castro, J. M., Fresno, J. M., & Tornadijo, M. E. (2005). Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). Food Microbiology, 22(5), 455-459.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Aerobic chemolithotrophic bacteria and associated organisms. Williams and Williams (eds). Bergey's Manual of Determinative Bacteriology, 9th ed. Baltimore, USA 427-455.

Holzapfel, W. H., & Schillinger, U. (2002). Introduction to pre-and probiotics. Food Research International, 35(2-3), 109-116.

Jin, L. Z., Ho, Y. W., Abdullah, N., Ali, M. A., & Jalaludin, S. (1996). Antagonistic effects of intestinal Lactobacillus isolates on pathogens of chicken. Letters in Applied Microbiology, 23(2), 67-71.

Kos, B., Šušković, J., Beganović, J., Gjuračić, K., Frece, J., Iannaccone, C., & Canganella, F. (2008). Characterization of the three selected probiotic strains for the application in food

industry. World Journal of Microbiology and Biotechnology, 24(5), 699-707.

Kristo E, Biliaderis CG, Tzanetakis N (2003) Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of *Lactobacillus paracasei*. Int Dairy J 13:517–528

Kristo, E., Biliaderis, C. G., & Tzanetakis, N. (2003). Modelling of rheological, microbiological and acidification properties of a fermented milk product containing a probiotic strain of Lactobacillus paracasei. International Dairy Journal, 13(7), 517-528.

Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., & Yadav, H. (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Experimental Diabetes Research, 2012.

Lee, H., Yoon, H., Ji, Y., Kim, H., Park, H., Lee, J & Holzapfel, W. (2011). Functional properties of *Lactobacillus* strains isolated from kimchi. International Journal of Food Microbiology, 145(1), 155-161.

Oliveira, R. P. D. S., Perego, P., Converti, A., & De Oliveira, M. N. (2009). Effect of inulin on growth and acidification performance of different probiotic bacteria in co-cultures and mixed culture with *Streptococcus thermophilus*. Journal of Food Engineering, 91(1), 133-139.

Ouwehand, A. C., Kirjavainen, P. V., Grönlund, M. M., Isolauri, E., & Salminen, S. J. (1999). Adhesion of probiotic micro-organisms to intestinal mucus. International Dairy Journal, 9(9), 623-630.

Pennacchia, C., Ercolini, D., Blaiotta, G., Pepe, O., Mauriello, G., & Villani, F. (2004). Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. Meat Science, 67(2), 309-317.

Rahman, M. M., Kim, W. S., Kumura, H., & Shimazaki, K. I. (2008). Auto-aggregation and surface hydrophobicity of bifidobacteria. World Journal of Microbiology and Biotechnology, 24(8), 1593-1598.

Ramos, C. L., Thorsen, L., Schwan, R. F., & Jespersen, L. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum and Lactobacillus brevis* isolates from Brazilian food products. Food Microbiology, 36(1), 22-29.

Reniero, R., Cocconcelli, P., Bottazzi, V., & Morelli, L. (1992). High frequency of conjugation in *Lactobacillus* mediated by an aggregation-promoting factor. Microbiology, 138(4), 763-768.

Ridwan, B. U., Koning, C. J. M., Besselink, M. G. H., Timmerman, H. M., Brouwer, E. C., Verhoef, J., & Akkermans, L. M. A. (2008). Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. Letters in Applied Microbiology, 46(1), 61-67.

Saarela M, Morgensen G, Forden R, Matoto J, Mattla-Sanholm T (2000). Probiotic bacteria: safety, functional and technological properties. Journal of Bacteriology (84),197-215

Schiffrin, E. J., Brassart, D., Servin, A. L., Rochat, F., & Donnet-Hughes, A. (1997). Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. The American Journal of Clinical Nutrition, 66(2), 515S-520S.

Singh, K., Kallali, B., Kumar, A., & Thaker, V. (2011). Probiotics: A review. Asian Pacific Journal of Tropical Biomedicine, 1(2), S287-S290.

Walker, W. A., & Duffy, L. C. (1998). Diet and bacterial colonization: role of probiotics and prebiotics. The Journal of Nutritional Biochemistry, 9(12), 668-675.

Zonenschain, D. A. N. I. E. L. A., Rebecchi, A., & Morelli, L. O. R. E. N. Z. O. (2009). Erythromycin and tetracycline resistant lactobacilli in Italian fermented dry sausages. Journal of Applied Microbiology, 107(5), 1559-1568.