Investigation of bile tolerance and deconjugation ability of various Lactobacillus Casei group strains

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INTRODUCTION

Gastrointestinal micro flora is now recognized as one of the factors that determine the state of health and diseases in human. This micro flora is in dynamic equilibrium between beneficial and harmful micro organisms that may be altered by diet, medication, stress, aging and various other environmental factors. Increasing the population of beneficial organisms is thought to be important in maintaining optimal intestinal health. One such approach for increasing beneficial organisms in gut is probiotics. Probiotic bacteria constitute a major part of the natural micro flora of human and animal intestine and when present in sufficient numbers create a healthy equilibrium between beneficial and potentially harmful microflora in the gut (Gilliland et al., 1977; Beck et al., 1961). The organisms most often included in the probiotic group are member of the Lactobacillus acidophilus complex, Lactobacillus casei complex and species of Bifidobacterium under the category Lactic Acid Bacteria. Traditionally probiotic bacteria have been utilized in dairy products such as milk or yoghurt and it has been hypothesized that milk enhances probiotic efficacy by providing lactose as a substrate (Succi et al., 2005). At present, a large number of products for humans are present in market and are being promoted with health claims based on several characteristics of selected strains of lactic acid bacteria, particularly belonging to the genera Lactobacillus and Bifidobacterium (Shah, 2000). The suggested concentration of probiotic bacteria to provide health benefits is 10^6 CFU/g of a product (Lankaputhra et al., 1995).

These probiotic bacteria favourably alter the intestinal microflora balance, promote intestinal integrity and mobility, inhibit the growth of harmful bacteria and increase resistance to infection (Veldman, 1992) and should possess the properties like survival in the GIT, persistence in the host, and proven safety for consumer (Tuomola, 2000 and De-Vries, et al., 2006). The survivability and colonization in the digestive tract are considered critical to ensure optimal functionality and expression of health promoting physiological functions by probiotics. A number of factors have been claimed to affect the viability of probiotic bacteria in dairy foods such as yoghurt and fermented milks, including low pH and refrigerated storage (Shah, 000). Microorganisms ingested with food begin their journey to the lower intestinal tract via the mouth and are exposed during their transit through the gastrointestinal tract to successive stress factors that influence their survival (Marteau et al., 1993; Simon and Gorbach, 1987). The time from entrance to release from the stomach is about 90 min, but further digestive processes have longer residence times (Berrada et al., 1991). These bacteria must overcome biological barriers like low pH in the stomach and bile in the intestine (Lankaputhra and Shah, 1995). Strains need to be resistant to the stressful

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conditions of the bile in the upper intestine (Çakır et al., 2003). Bile secreted in the small intestine reduces the survival of bacteria by destroying their cell membranes, whose major components are lipids and fatty acids and these modifications may affect not only the cell permeability and viability, but also the interactions between the membranes and the environment (Gilliland et al., 1984; Gilliland, 1987). Although the resistance mechanisms of these bacteria are still poorly understood, the inhibition of Lactobacillus growth by bile may be overcome in some cases by progressive adaptation to increasing concentrations of these compounds (Margolles et al., 2003).

Therefore before a probiotic can benefit human and animal health it must fulfill the criteria of ability to tolerate bile concentration (Pereira and Gibson, 2002). Hence the aim of this study was to evaluate the tolerance and conjugation ability of twenty-two strains of Lactobacillus to bile concentrations that commonly exist in the human intestine.

MATERIALS AND METHODS

Bacterial strains and growth conditions: Twenty-two strains of Lactobacillus casei group were obtained from Australian Starter Culture Research Centre (Werribee, Australia) and the research work was carried out in Shri Venkateshvara University (U.P., India). They were cultured using de Man, Rogosa, and Sharpe (MRS) broth at 37ºC. All strains grew well without the use of anaerobic conditions. Type strains (i.e., recognized reference strains) were included in the study. Stock cultures were stored in 40% glycerol at -80ºC. All of the organisms were sub cultured three times consequently prior to use in sterile MRS broth using 1% inoculum and 20 h incubation at 37ºC.

Lactobacilli strains:
1. Lactobacillus rhamnosus ASCC 192.
2. Lactobacillus rhamnosus ASCC 277.
3. Lactobacillus paracasei ASCC 279.
4. Lactobacillus casei ASCC 290.
5. Lactobacillus paracasei ASCC 292.
6. Lactobacillus paracasei ASCC 295.
7. Lactobacillus paracasei ASCC 526.
8. Lactobacillus rhamnosus ASCC 1002.
9. Lactobacillus paracasei ASCC 1180.
10. Lactobacillus rhamnosus ASCC 1181.
11. Lactobacillus rhamnosus ASCC 1519.
12. Lactobacillus rhamnosus ASCC 1520.
13. Lactobacillus rhamnosus ASCC 1521.
14. Lactobacillus rhamnosus ASCC 1522.
15. Lactobacillus rhamnosus ASCC 1523.
16. Lactobacillus rhamnosus CSCC 2603.
17. Lactobacillus rhamnosus CSCC 2605.
18. Lactobacillus rhamnosus CSCC 2607.
19. Lactobacillus rhamnosus CSCC 2627.
20. Lactobacillus casei CSCC 5203.
21. Lactobacillus rhamnosus CSCC 5376.
22. Lactobacillus zeae ATCC 15820.

Survival of Lactobacillus spp. in the presence of bile salts: he effects of bile on the growth of probiotic strains were examined using methods modified from those of Gilliland and Walker (1990) and Tsai et al. (2007). A series of bile concentrations were employed in this study considering the fluctuation of bile concentration at different times. Broth with 0% bile concentration serves as a control of the study. To evaluate the survival ability, aliquots of active cultures of twenty-two strains of Lactobacillus casei were grown in MRS broth for 20 h at 37ºC in the presence of bile salts, adjusted to pH 4.5 with sterile 1 N HCl or 1 N NaOH, depending on the final pH of the culture after 20 h of incubation. Concentrated bile solution was prepared separately by dissolving powdered bile extract and then it was filter sterilized by 5μ filter and was added to two of the cultures to achieve a final concentration of 1.0% and 1.5% and the third culture served as a control sample. The cultures were incubated at 37ºC for 3 h. Samples were taken prior to adding bile solution and then every hour for 3 h. Viable counts of L. casei strains were determined by pour plating of all the samples using 10-fold serial dilutions prepared in 0.1% peptone water. The experiment and analysis were carried out in triplicate. The results shown are the averages of at least two replicates. Data obtained from the study was expressed in terms of log_{10} CFU/ml and analysed as mean ± standard deviation (SD).

Enumeration of Lactobacillus spp: Enumeration of L. casei was done on MRS agar. Cultures were serially diluted from 10^{-1} to 10^{-4} in 0.1% sterile peptone water. One millilitre aliquots of the dilutions were pour plated with MRS agar and incubated aerobically at 37ºC for 72 h. All colonies were counted and recorded as colony forming units (CFU) per gram of culture. All the experiments and analyses were duplicated. The results shown are the average of at least two replicates. Data obtained from the study was expressed in terms of log_{10} CFU/ml and analysed as mean ± standard deviation (SD).

Deconjugation of sodium glycocholate and sodium taurocholate by Lactobacilli: Seven high bile resistant Lactobacillus strains were tested for their deconjugation activity. Ten ml volumes of MRS broth were supplemented with 6 mM sodium taurocholate, 6 mM sodium glycocholate or a combination of sodium glycocholate and sodium taurocholate at 2.8 and 1.2 mM respectively. Individual bile salt were added as 6 mM each, because it resembles the concentrations prevailing in the human small intestine (Brashears et al., 1998), while bile mixtures contained 2.8 mM sodium glycocholate and 1.2 mM sodium taurocholate, because they resemble the molar ratio of the two salts and
RESULTS AND DISCUSSION

Survival of *Lactobacillus* strains in the presence of bile:
The 80% of stomach contents (after consumption of fermented product) had passed through to the intestine after 90 min (Charteris et al., 1998). The concentration of bile varies from 0.5 to 2% during the first hour of digestion; the levels may decrease during the subsequent period. Davenport (1977) reported that while bile concentrations in the intestine range between 0.5 to 2.0% during first hour of digestion. The levels may decrease during the second hour. Hence the bile concentrations ranging from 0% to 1.5% have been used in several microbiological media for selective isolation of bile tolerant bacteria from mixed cultures.

Tables 1 and 2 are showing the viable count of twenty two different *Lactobacillus* strains in the bile concentrations of 0.0% and 1.0%. All 22 *Lactobacillus* strains had shown moderate activity at 0% bile concentration up to 3 h of incubation. CSCC 2607 has shown highest growth followed by ATCC 15820 and ASCC 1521 at 1% bile concentration. Remaining *Lactobacillus* strains have shown fair growth up to 3 h of incubation. Results from 1.5% bile concentration are shown in Table 3. Only seven *Lactobacillus* strains showed growth up to 3 h of incubation. ASCC 1521 showed highest activity followed by ASCC 290 and ATCC 15820 up to 3 h of incubation. Furthermore ASCC

| Bacterial strain | Viable count (Log) cfu / g | 0h | 1h | 2h | 3h |
|------------------|--------------------------|----|----|----|----|
| ASCC 292         |                          | 159±3 | 171±4 | 192±2 | 205±2 |
| ASCC 290         |                          | 197±4 | 195±2 | 211±3 | 252±1 |
| ASCC 279         |                          | 162±2 | 178±2 | 187±2 | 211±3 |
| ASCC1521         |                          | 194±2 | 187±3 | 212±3 | 274±4 |
| ASCC1520         |                          | 161±1 | 182±4 | 193±1 | 208±5 |
| ATCC 15820       |                          | 212±3 | 219±2 | 231±7 | 241±6 |
| CSCC 2607        |                          | 184±2 | 188±3 | 204±5 | 233±3 |
| CSCC5203         |                          | 178±4 | 193±1 | 215±3 | 254±2 |
| ASC2295          |                          | 148±2 | 166±2 | 186±6 | 197±3 |
| ASCC 526         |                          | 143±5 | 151±5 | 168±4 | 175±4 |
| ASCC 1180        |                          | 113±1 | 124±3 | 147±2 | 171±6 |
| ASCC 277         |                          | 182±3 | 198±4 | 209±3 | 219±7 |
| ASCC 1002        |                          | 145±6 | 167±3 | 184±4 | 205±3 |
| ASCC1181         |                          | 184±2 | 193±6 | 211±1 | 227±8 |
| ASCC 1519        |                          | 165±3 | 175±2 | 194±5 | 205±6 |
| ASCC 1522        |                          | 117±4 | 134±3 | 152±7 | 182±2 |
| ASCC 1523        |                          | 156±3 | 179±4 | 196±4 | 209±4 |
| CSCC2603         |                          | 139±7 | 156±5 | 169±7 | 199±3 |
| CSCC 2605        |                          | 217±4 | 235±7 | 251±5 | 281±5 |
| CSCC 2627        |                          | 188±6 | 198±3 | 207±2 | 223±7 |
| CSCC 5376        |                          | 165±2 | 181±5 | 199±3 | 215±4 |
| ASCC 192         |                          | 187±3 | 191±3 | 200±2 | 217±6 |

± Values refer to standard error of means
Values are means of triplicates from two separate runs, n = 6.
1 Means viable count (CFU/g).

Table 1: Survival of twenty two different *Lactobacillus* strains during three hours in the presence of 0.0% bile solution.

Human bile (Sandine, 1979). Each strain was inoculated at the 1% level and incubated aerobically at 37°C for 20 h.

Bile salt deconjugation ability was based on release of deconjugated bile and the modified method of Irwin, Johnson, and Kopalo (1944) was used to measure the amount of free cholic acid released by the organisms. Briefly, 10 ml culture of each organism after the incubation period was adjusted to pH 7.0 with NaOH (1 N). Cells were centrifuged at 10000 *g* at 4°C for 10 min. Supernatant obtained was adjusted to pH 1.0 with 10 N HCl. One millilitre of the supernatant was added with 2 ml of ethyl acetate and the mixture was vortexed for 1 min. Two millilitre of the ethyl acetate layer was transferred into glass tube and evaporated under nitrogen at 60°C. The residue was immediately dissolved in 1 ml of NaOH (0.01N). After complete mixing, 1 ml of furfuraldehyde (1%) and 1 ml of 16N H₂SO₄ were added and the mixture was vortexed for 1 min. The sample was then heated in water bath at 65°C. After cooling 2 ml glacial acetic acid was added and the mixture was vortexed for 1 min. OD was taken at 660 nm. Standard cholic acid was used to determine the amount of cholic acid released from samples. All samples were analysed in duplicates, all experiments were repeated thrice. Data obtained from the study was expressed in terms of log₁₀ CFU/ml and analysed as mean ± standard deviation (SD).
Table 3: Survival of twenty two different Lactobacillus strains during three hours in the presence of 1.5% bile solution.

| Bacterial strain | Viable count (Log) cfu / g |   |   |   |
|------------------|---------------------------|---|---|---|
|                  | 0h                        | 1h | 2h | 3h |
| ASCC 292         | 269 ± 6                   | 269 ± 7 | 171 ± 4 | 166 ± 2 |
| ASCC 290         | 164 ± 3                   | 151 ± 4 | 159 ± 6 | 153 ± 2 |
| ASCC 279         | 168 ± 2                   | 167 ± 2 | 163 ± 2 | 157 ± 5 |
| ASCC1521         | 179 ± 3                   | 179 ± 3 | 178 ± 4 | 178 ± 5 |
| ASCC1520         | 153 ± 5                   | 154 ± 4 | 149 ± 5 | 154 ± 1 |
| ATCC 15820       | 226 ± 7                   | 187 ± 1 | 194 ± 2 | 187 ± 3 |
| CSCC 2607        | 227 ± 4                   | 225 ± 7 | 228 ± 6 | 241 ± 7 |
| CSCC5203         | 193 ± 6                   | 185 ± 3 | 179 ± 2 | 147 ± 3 |
| ASCC295          | 186 ± 3                   | 169 ± 2 | 165 ± 4 | 66 ± 2  |
| ASCC 526         | 162 ± 2                   | 157 ± 4 | 138 ± 3 | 89 ± 5  |
| ASCC 1180        | 184 ± 4                   | 118 ± 2 | 95 ± 2  | 77 ± 1  |
| ASCC 277         | 195 ± 3                   | 131 ± 2 | 107 ± 5 | 74 ± 3  |
| ASCC 1002        | 183 ± 6                   | 158 ± 5 | 101 ± 3 | 71 ± 1  |
| ASCC1811         | 175 ± 1                   | 139 ± 6 | 99 ± 1  | 84 ± 4  |
| ASCC 1519        | 172 ± 5                   | 102 ± 3 | 119 ± 2 | 45 ± 2  |
| ASCC 1522        | 218 ± 4                   | 152 ± 3 | 159 ± 4 | 63 ± 1  |
| ASCC 1523        | 171 ± 2                   | 63 ± 6  | 52 ± 4  | 40 ± 2  |
| CSCC2603         | 235 ± 7                   | 160 ± 4 | 152 ± 5 | 35 ± 2  |
| CSCC 2605        | 219 ± 5                   | 92 ± 5  | 79 ± 1  | 56 ± 3  |
| CSCC 2627        | 186 ± 6                   | 132 ± 3 | 122 ± 6 | 61 ± 4  |
| CSCC 5376        | 161 ± 3                   | 88 ± 4  | 70 ± 2  | 53 ± 5  |
| ASCC 192         | 168 ± 3                   | 155 ± 4 | 109 ± 1 | 90 ± 4  |

± Values refer to standard error of means
Values are means of triplicates from two separate runs, n = 6.
1 Means viable count (CFU/g).

Table 2: Survival of twenty two different Lactobacillus strains during three hours in the presence of 1.0% bile solution.

| Bacterial strain | Viable count (Log) cfu / g |   |   |   |
|------------------|---------------------------|---|---|---|
|                  | 0h                        | 1h | 2h | 3h |
| ASCC 292         | 269 ± 6                   | 269 ± 7 | 171 ± 3 | 166 ± 4 |
| ASCC 290         | 164 ± 3                   | 151 ± 4 | 159 ± 6 | 153 ± 2 |
| ASCC 279         | 168 ± 2                   | 167 ± 2 | 163 ± 2 | 157 ± 5 |
| ASCC1521         | 179 ± 3                   | 179 ± 3 | 178 ± 4 | 178 ± 5 |
| ASCC1520         | 153 ± 5                   | 154 ± 4 | 149 ± 5 | 154 ± 1 |
| ATCC 15820       | 226 ± 7                   | 187 ± 1 | 194 ± 2 | 187 ± 3 |
| CSCC 2607        | 227 ± 4                   | 225 ± 7 | 228 ± 6 | 241 ± 7 |
| CSCC5203         | 193 ± 6                   | 185 ± 3 | 179 ± 2 | 147 ± 3 |
| ASCC295          | 186 ± 3                   | 169 ± 2 | 165 ± 4 | 66 ± 2  |
| ASCC 526         | 162 ± 2                   | 157 ± 4 | 138 ± 3 | 89 ± 5  |
| ASCC 1180        | 184 ± 4                   | 118 ± 2 | 95 ± 2  | 77 ± 1  |
| ASCC 277         | 195 ± 3                   | 131 ± 2 | 107 ± 5 | 74 ± 3  |
| ASCC 1002        | 183 ± 6                   | 158 ± 5 | 101 ± 3 | 71 ± 1  |
| ASCC1811         | 175 ± 1                   | 139 ± 6 | 99 ± 1  | 84 ± 4  |
| ASCC 1519        | 172 ± 5                   | 102 ± 3 | 119 ± 2 | 45 ± 2  |
| ASCC 1522        | 218 ± 4                   | 152 ± 3 | 159 ± 4 | 63 ± 1  |
| ASCC 1523        | 171 ± 2                   | 63 ± 6  | 52 ± 4  | 40 ± 2  |
| CSCC2603         | 235 ± 7                   | 160 ± 4 | 152 ± 5 | 35 ± 2  |
| CSCC 2605        | 219 ± 5                   | 92 ± 5  | 79 ± 1  | 56 ± 3  |
| CSCC 2627        | 186 ± 6                   | 132 ± 3 | 122 ± 6 | 61 ± 4  |
| CSCC 5376        | 161 ± 3                   | 88 ± 4  | 70 ± 2  | 53 ± 5  |
| ASCC 192         | 168 ± 3                   | 155 ± 4 | 109 ± 1 | 90 ± 4  |

± Values refer to standard error of means
Values are means of triplicates from two separate runs, n = 6.
1 Means viable count (CFU/g).
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Table 4: Deconjugation of sodium glycocholate and sodium taurocholate by Lactobacillus strains.

| Strains   | Sodium glycocholate | Sodium taurocholate | Sodium glycocholate + sodium taurocholate |
|-----------|---------------------|---------------------|------------------------------------------|
| CSCC 2607 | 1.82 ± 0.11c, A     | 1.40 ± 0.15 b, A    | 1.34 ± 0.35bc, A                         |
| ATCC 15820| 3.40 ± 0.17a, A     | 2.44 ± 0.16ab, A    | 2.11 ± 0.30ac, A                         |
| ASCC 290  | 1.93 ± 0.50c, A     | 1.22 ± 0.14b, A     | 1.67 ± 0.17ac, A                         |
| ASCC 292  | 2.33 ± 0.42bc, A    | 1.31 ± 0.13b, A     | 2.06 ± 0.33ac, A                         |
| ASCC 1520 | 1.55 ± 0.14c, A     | 1.27 ± 0.12b, A     | 1.14 ± 0.09c, A                          |
| ASCC 1521 | 4.76 ± 0.45a, A     | 3.29 ± 0.35a, AB    | 2.71 ± 0.35ab, A                         |
| ASCC 279  | 1.83 ± 0.33c, A     | 1.46 ± 0.12b, A     | 1.93 ± 0.19ac, A                         |

± Values refer to standard error. Values are means of triplicates from two separate runs, n = 6.
abc Means within a column with different lowercase letters are significantly different (p< 0.05)
AB Means within a row with different uppercase letters are significantly different (p< 0.05).

292, ASCC 279, ASCC 1520 and CSCC 2607 showed reasonable growth during incubation. Bile resistance is necessary for an organism that is expected to grow in an intestine (Gilliland et al., 1984) and hence an important characteristic to be considered in the selection of a culture as dietary adjunct (Walker and Gilliland, 1993). Millette et al., (2008) reported loss of activity at bile concentration below 0.5%. While the viable counts were between 6-8 Log cfu/ml at 2% bile salt conc. and decrease drastically below this conc. as reported by Succi and Coppola, (2005), Hamon et al., (2011) checked bile tolerance in nine strains of Lactobacillus and reported lesser decrease in viable counts at 0.5-3.6% conc. of bile salts. Saran et al., (2012) showed that the viable counts of both the strains decreased more in 2% bile concentration (upto 3.82-3.93 log cfu/ml) than in 1% bile concentration (upto 4.83-5.81log cfu/ml) after 3 and 12 hr of exposure.

Deconjugation of bile salt by Lactobacilli: Bile salt deconjugation activity by Lactobacillus strains is shown in Table 4. Bile salt deconjugation was determined by the quantity of cholic acid released, which ranged from 1.14 to 4.76 mM. All seven strains were able to deconjugate both sodium glycocholate and sodium taurocholate at varying levels. Deconjugation activity in broth containing sodium glycocholate was observed. Overall deconjugation was observed to be higher by the strains ASCC 1521 followed by ATCC 15820 and ASCC 292. ASCC 1520 showed lowest deconjugation followed by CSCC 2607. All seven strains showed lower deconjugation of sodium taurocholate compared to sodium glycocholate. ASCC 1521 conjugated highest level followed by ATCC 15820. ASCC 290 showed least capable of deconjugating sodium taurocholate followed by CSCC 2607. Mixture of sodium glycocholate and sodium taurocholate at similar molar ratio in human bile, all seven strains showed consistent cholic acid secretion. ASCC 1521 also showed highest cholic acid liberation while ASCC 1520 showed lowest deconjugation ability.

This study was carried out only on best seven strains, which were bile resistant in our initial studies. Ljong and Shah, (2005) also showed that more cholic acid was liberated from the deconjugation of sodium glycocholate than sodium taurocholate, and Lactobacillus acidophilus strains had higher deconjugation ability than L. casei strains. While Ahn et al., (2003) showed that L. acidophilus strains deconjugated both taurocholate and glycocholate at similar rates. So our results confirmed the results of Ljong and Shah, (2005).

CONCLUSION
Results obtained in our study have shown that all twenty two strains of Lactobacillus spp. showed good tolerance against bile concentration. ASCC 1520, ASCC 1521, ASCC 279, ASCC 290, ASCC292, ATCC 15820 & CSCC 2607 have shown excellent bile tolerance. So these seven robust Lactobacillus strains were selected for their deconjugation activity to further confirm their tolerance against bile in order to study further for their therapeutic benefits.

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REFERENCES
Ahn, Y.T., Kim, G.B., Lim, K.S., Baek, Y.J. and Kim, H.U. (2003). Deconjugation of bile salts by Lactobacillus isolates. International Dairy Journal; 13(4): 303-311.
Beck, C. and Necheles, H. (1961). Beneficial effects of administration of Lactobacillus acidophilus in diarrhoea and other intestinal disorders Am J Gastroenterol; 35: 522-533.
Berrada, N., Lemeland, J. F., Laroche, G., Thovenot, P. and Piaia, M. (1991). Bifidobacterium from fermented milk: Survival during gastric transit. J Dairy Sci; 74: 409.
Brashears, M.M., Jaromi, D., Trimble, J., (1998). Isolation, selection and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of Escherichia coli O157:H7 in cattle. J. Food Prot. 66: 355–363.

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

Charteris, W.P., Kelly, P.M., Morelli, L., Collins, K. (1998). Antibiotic susceptibility of potential probiotic Lactobacillus species. J. Food Protect.; 61:1636-1643.

Davenport, H.W. (1997). Physiology of the digestive tract. Chicago: Year Book Medical Publishers Inc.

De-Vries, M.C., Vaughan, E.E., Kleerebezem, M. and de Vos, W.M. (2006). Lactobacillus plantarum survival, functional and potential probiotic properties in the human intestinal tract. International Dairy Journal; 16: 1018–1028.

Gilliland, S. E. and Speck, M. L. (1977). Deconjugation of bile acids by intestinal Lactobacilli. Appl Environ Microbiol; 33(1): 15-18.

Gilliland, S.E. and Kim, H.S. (1984). Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. Journal of Dairy Science; 67: 1–6.

Gilliland, S.E. (1987). Importance of bile tolerance in lactobacilli used as dietary adjunct: In: Biotechnology in the feed industry. [T. P. Lyons]. Alttech Feed Co, Lexington, KY: 149-155.

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: 905-911.

Hamon, E., Horvatovich, P., Izquierdo, E., Bringel, F., Marchioni, E., Aoudé-Werner, D. and Ennahar, S. (2011). Comparative proteomic analysis of Lactobacillus plantarum for the identification of key proteins in bile tolerance. BMC Microbiology; 11: 63.

Irwin, J. L., Johnson, C. G., and Kopalo, J. (1944). A photometric method of the determination of cholates in bile and blood. Journal of Biological Chemistry; 153: 439–457.

Lankaputhra, W. E. V. and Shah, N. P. (1995). Survival of Lactobacillus acidophilus and Bifidobacterium spp. in the presence of acid and bile salts. Cultured Dairy Products J; 30: 2-7.

Liong, M. T. and Shah, N. P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. Journal of Dairy Science 88:55-66.

Margolles, A., García, L., Sánchez, B., Guéimonde, M. and de los Reyes-Gavilán, C.G. (2003). Characterisation of a Bifidobacterium strain with acquired resistance to cholate—a preliminary study. International Journal of Food Microbiology; 82:191–198.

Marteau, P., Pochart, P., Bouhnik, Y. and Rambaud, J. C. (1993). Fate and effects of some transiting microorganisms in the human gastrointestinal tract. World Review of Nutrition and Dietetics; 74:1–21.

Millette, M., Luquet, F.-M., Marcia Teresa Ruiz, M.T. and Lacroix, M. (2008). Characterization of probiotic properties of Lactobacillus strains. Dairy Science Technology; 88:695–705.

Pereira, D.I.A and Gibson, G.R. (2002) Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl Environ Microbiol, 68: 4689–4693.

Sandine, W. E. (1979). Roles of Lactobacillus in the intestinal tract. Journal of Food Protection; 42: 259–262.

Saran, S., Singh, K., Bisht, M.S. and Teotia, U.V.S. (2012). Analyzing probiotic attributes to assess comparatively two isolates of Lactobacillus acidophilus in probiotics, honey and inulin. Double Helix Research’s-International Journal of Biomedical and Life Sciences; 2(1):26-34.

Shah, N. P. (2000). Probiotic Bacteria: Selective enumeration and survival in dairy foods J Dairy Sci; 83: 894-907.

Simon, G. L. and Gorbach, L. (1987). Intestinal flora and gastrointestinal function: In: Physiology of gastrointestinal Tract. [L. R. Johnson. (ed)] Raven Press, New York, NY; 2: 1729.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., Pacifico, S. and Coppola, R. (2005). Bile salt and acid tolerance of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese. FEMS Microbiology Letters; 244:129–137.

Tsai, C-C., Lin, P-P. and Hsieh, Y. M. (2007). Three Lactobacillus strains from healthy infant stool inhibit enterotoxigenic Escherichia coli grown in vitro. Anaerobe 14: 1-7.

Tuomola, E.M., Ouwehand, A.C. and Salminen, S.J. (2000). Chemical, physical and enzymatic pre-treatments of probiotic lactobacilli alter their adhesion to human intestinal mucus glycoproteins. International Journal of Food Microbiology; 60(1): 75-81.

Veldman, A., (1992). Probiotics. Tijdschr Diergeneesk. 15: 117; 345-348.

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