# *Chapter 2*

# Isolation, identification and biochemical characterization of *Lactobacillus* isolates from various sources.

*"Knowledge speaks, but wisdom listens"*

*-Jimi Hendrix*

# Chapter 2

## Isolation, identification and biochemical characterization of *Lactobacillus* isolates from various sources.

### 2.1 Introduction

In all the animal species including humans, the intestine hosts large number of microorganisms. Although microbes are present all along the gastrointestinal tract (GIT), by far the largest population is located in the caecum and colon (Guarner and Malagelada, 2003). The GIT of an adult human is estimated to harbour about $10^{14}$ viable bacteria (Luckey, 1972) i.e. 10 times the total number of eukaryotic cells in all tissues of man's body. Co-evolution of the microbiota and the host has led to a situation whereby the host fosters beneficial microbiota that increases host fitness by promoting stable functionality of the gut ecosystem (Bäckhed et al., 2005). The composition of the microbiota may evolve under the influence of a variety of factors, including age, genetic background, diet and health status of the host (Power et al., 2014). Among these microorganisms, lactic acid bacteria (LAB) are one of the major groups of microorganisms.

The importance of microorganisms for human health and longetivity was first hypothesized by Metchnikoff at the beginning of 20$^{th}$ century (Metchnikoff and Mitchell, 1907). According to the FAO of the UN and the WHO, 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' are considered as probiotics (FAO and WHO, 2002). There are numerous probiotic genera and species including lactobacilli. The benefits of lactobacilli have been reported

widely. One of them includes the production of organic acids and antimicrobial compounds which prevents the growth of pathogenic bacteria via competitive exclusion (Erkkilä and Petäjä, 2000; Fuller, 1989). The other health promoting effects attributed to lactobacilli are cholesterol reduction (Ejtahed et al., 2011), diarrhoea prevention (Allen et al., 2010), alleviation of lactose intolerance symptoms (Kim and Gilliland, 1983), anticancer effects (Choi et al., 2006), synthesis and enhancing the bioavailability of nutrients and immune-modulatory effects (Ejtahed et al., 2011; Lebeer et al., 2008; Marco et al., 2006).

To confer the health benefits, lactobacilli must survive in the GIT and also persist for a longer time in the host (De Vries et al., 2006). To survive in the gut, the organisms should have the ability to resist the digestion process in the stomach and the intestinal tract. Cellular stress begins in the stomach, which has a pH value as low as 1.5 (Charteris et al., 1998). After the bacteria pass through the stomach, they enter the upper intestinal tract where bile is secreted into the gut. Thus, strains selected for use as probiotic bacteria should be able to tolerate acid and bile. Antibacterial activity is also one of the desirable characteristics of probiotic bacteria. Lactobacilli produce a variety of substances that are inhibitory to both Gram positive and Gram-negative bacteria. These include organic acids (Gunal et al., 2006; Makras et al., 2006), hydrogen peroxide (Dahiya and Speck, 1968; Hawes et al., 1996) and bacteriocins (Larsen et al., 1993). These compounds may reduce not only the number of viable pathogenic organisms, but may also affect bacterial metabolism and toxin production (Rolfe, 2000). The resistance to various antibiotics by lactobacilli has raised the question against its safety in various applications as a potential probiotics. These resistant genes may get transferred horizontally or vertically to other organisms. In the other way around, resistance to

antibiotics is also considered as a desirable property of potential probiotics in treatment of the antibiotic associated diarrhoea.

The probiotic properties are highly variable and are both species and strain dependent (Sanz, 2007; Sultana et al., 2013). Moreover, any single strain does not possess all the probiotic properties. Thus, present study was aimed to isolate indigenous lactobacilli strains from various sources and characterise them for different probiotic properties like resistant to acid and bile, antimicrobial activity against pathogens and antibiotic susceptibility. These properties were compared with the standard established probiotic strain- *Lactobacillus rhamnosus* GG.

## 2.2 Materials and Methods

### 2.2.1 Standard *Lactobacillus* strain

An established probiotic stain *Lactobacillus rhamnosus* GG (LGG) was obtained as a kind from Dr. Shira Doron, MD, Department of Medicine, Tufts, New England Medical Center, USA.

### 2.2.2 Isolation and identification of lactobacilli

Lactobacilli were isolated from different food and adult human stool samples. Approximately 1g of each sample was suspended in 10 ml sterile normal saline. The suspension was vigorously mixed, allowed to settle and 100 µl aliquot of the suspension was spread onto the Rogosa SL agar medium (Himedia, Mumbai, India) containing 100 µg/ml of cyclohexamide (Sisco research laboratories, Mumbai, India). Rogosa SL is a selective media for *Lactobacillus* isolation and cyclohexamide was added to prevent the growth of yeast (Rogosa et al., 1951). The plate was incubated at 37°C for sufficient growth of *Lactobacillus.* From each plate, around 5-6 colonies were transferred to de Man, Rogosa and Sharpe (MRS; Himedia) agar plate. The colonies were then examined

for their morphological and biochemical characteristics. The isolates with Gram-positive nature and negative for endospore and catalase phenotype were then subjected to molecular identification by amplification of 16S-23S rRNA gene intergenic spacer region (Tannock et al., 1999). The primers used for the PCR were the following: 16-1A (5′-GAATCGCTAGTAATCG-3′) and 23-1B (5′-GGGTTCCCCCATTCGGA-3′).

**Table 2. 1 PCR system for amplification of 16S-23S rRNA gene intergenic region**

| **COMPONENTS** | **VOLUME (µL)** |
|---|---|
| A | 16.9 |
| 10X Buffer for Taq DNA Polymerase | 2.5 |
| dNTP mix (2.5 mM each) | 2.0 |
| Forward primer (100 pmol/µl) | 0.8 |
| Reverse primer (100 pmol/µl) | 0.8 |
| Taq DNA polymerase (2.5 U/µl) (Sigma-Aldrich) | 1.0 |
| Colony suspension | 2 |
| **Total volume** | **25** |

**Table 2.2 PCR conditions used for amplification of 16S-23S rRNA gene intergenic region**

| **STEPS** | | **TEMPERATURE (°C)** | **TIME** | **NO. OF CYCLES** |
|---|---|---|---|---|
| **1** | Pre-cycle denaturartion | 94 | 5 min | 1 |
| **2** | Denaturation | 94 | 45 sec | 30 |
| **3** | Primer annealing | 55 | 30 sec | |
| **4** | Primer extension | 72 | 1 min | |
| **5** | Post-cycle elongation | 72 | 6 min | 1 |

**Agarose gel electrophoresis**

The PCR amplified products were analyzed by electrophoresis on 0.8% agarose gel in 0.5X TBE followed by staining with ethidium bromide (EtBr) and viewing under UV light.

**Composition of 0.5 X TBE (for 1 L)**

| | |
|---|---|
| Tris | 54 g |
| Boric acid | 27.5 g |
| EDTA (0.5 M) | 20 ml |

### 2.2.3 Bile and Acid tolerance

For bile tolerance assay, lactobacilli ($1\times10^6$ CFU/ml) were inoculated into the MRS containing 1%, 2% and 3% (w/v) bile salts (sodium cholate and sodium deoxycholate; Sigma; India) and incubated for 24 h to determine the minimum inhibitory concentration of bile for each strain. Based on this assay, the survival rate of each lactobacilli strain was assessed at 0.3% and 1% bile concentration. For this, overnight grown lactobacilli cells were washed with PBS and 20 µl of each lactobacilli ($1\times10^8$ CFU/ml) were inoculated into 980 µl of MRS containing 0.3% and 1% (w/v) ox-bile (Sigma; India) and incubated for 2 h at 37°C. The aliquots were collected at 0 h and 2 h and plated onto the MRS agar plate after appropriate dilution. The enumeration was done after 48 h of incubation at 37°C and the percentage survival rate was calculated from the mean of log10 CFU/mL of the cultures after 2 h of incubation with respect to their mean of log10 CFU/mL at 0 h. For determining acid survival rate of these isolates, the same procedure was followed as for bile survival assay except the inoculation was done in the acidic buffer (pH-2.5) (Casey et al., 2004).

**Composition of acidic buffer (for 1L)**

| | |
|---|---|
| D-glucose | 3.5 g |
| NaCl | 2.05 g |
| $KH_2PO_4$ | 0.6 g |
| $CaCl_2$ | 0.11 g |
| KCl | 0.37 g |

pH was adjusted to 2.5 using HCl

### 2.2.4 Antimicrobial activity

The antimicrobial activity of lactobacilli isolates was determined by agar spot method as described by Schillinger and Lücke (1989) with few modifications. Briefly, 2 µl of each *Lactobacillus* isolate ($1 \times 10^8$ CFU/mL) was spotted on MRS agar plate and allowed to grow for 24 h at 37 °C. Following the growth on the plate, 15 mL of Luria soft agar (0.6% *w*/*v*) containing 150 µl of overnight grown indicator bacteria was poured over the MRS agar plate. The plates were then incubated for 24 h at 37 °C, and the zone of inhibition was measured in accordance with Baccigalupi et al., (2005). The indicator organisms used to determine antimicrobial activity are following: *Shigella dysenteriae*, *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 25668), *Salmonella enteric* subsp. *enterica* serovar Typhi (MTCC 733), *Proteus vulgaris*, and *Escherichia coli* O26:H11. They were obtained from the culture collection facility at our department.

### 2.2.4 Antibiotic susceptibility assay

To assess the antibiotic susceptibility of lactobacilli isolates, disc diffusion method was used. Commercially available antibiotic discs (Himedia) containing chloramphenicol (30 µg), kanamycin (30 µg), gentamicin (10 µg), vancomycin (30 µg), bacitracin (10 U), ofloxacin (5 µg), cephalothin (30 µg), tobramycin (30 µg), and cloxacillin (5 µg) were used. Lactobacilli isolates were grown overnight and 100 µl of each isolate was

spread onto the MRS agar plate. Various antibiotic discs were placed on these MRS agar plates and incubated for 24 h at 37°C. The zone of inhibition was measured inclusive of disc diameter.

### 2.2.5 Statistics

Each experiment was performed in triplicates and the values are given as mean and standard deviation (SD). Significant ANOVA was followed by Dunnett's test for all the assays to compare with respect to standard strain (LGG) ($P<0.05$).

## 2.3 Results

### 2.3.1 Isolation and identification of lactobacilli

Total 77 isolates (45 from human stool samples and 32 from fermented food samples) were screened for microscopic and biochemical characters. Amongst them, 14 isolates (7 from human stool samples and 7 from fermented food samples) were found to be Gram positive rods and negative for catalase production, and which also could grow on Rogosa SL and MRS agar plates. These 14 isolates were further selected for their molecular identification by 16S-23S rRNA gene intergenic spacer region amplification. The agarose gel mobility of the amplification products of 10 isolates matched with that of the standard strain LGG. The smaller fragment from the amplified product of these 10 isolates was excised, eluted, and re-amplified using the same set of primers and sequenced. The sequence alignment with the NCBI database revealed that only 6 of them were belonged to the *Lactobacillus* genus. These sequences were then submitted to GeneBank and the corresponding accession numbers are reported in table 2.3.

**Table 2. 3 16S-23S rRNA gene intergenic sequence analysis and Genbank submisiion of selected isolates.**

| ISOLATES | SOURCE | GENBANK ACCESSION NO. OF 16S-23S SEQUENCE | 16-23S SEQUENCE BASED SPECIES IDENTIFICATION | % SIMILARITY OF 16-23S SEQUENCE TO THAT OF REFERENCE STRAIN IN GENBANK |
|---|---|---|---|---|
| **FA-1** | Fermented bamboo shoot (Iku) | KT337434 | *Lactobacillus fermentum* | 100% |
| **FA-5** | Fermented soybean seeds (Agya) | KT337435 | *Lactobacillus fermentum* | 99% |
| **FA-7** | Fermented rice (Nyogrin) | KT337436 | *Lactobacillus helveticus* | 99% |
| **IIS11.2** | Feces of adult human | KT337437 | *Lactobacillus fermentum* | 99% |
| **GPI-3** | Feces of adult human | JX118834 | *Lactobacillus fermentum* | 99% |
| **GKI-1** | Feces of adult human | JX118832 | *Lactobacillus fermentum* | 97% |

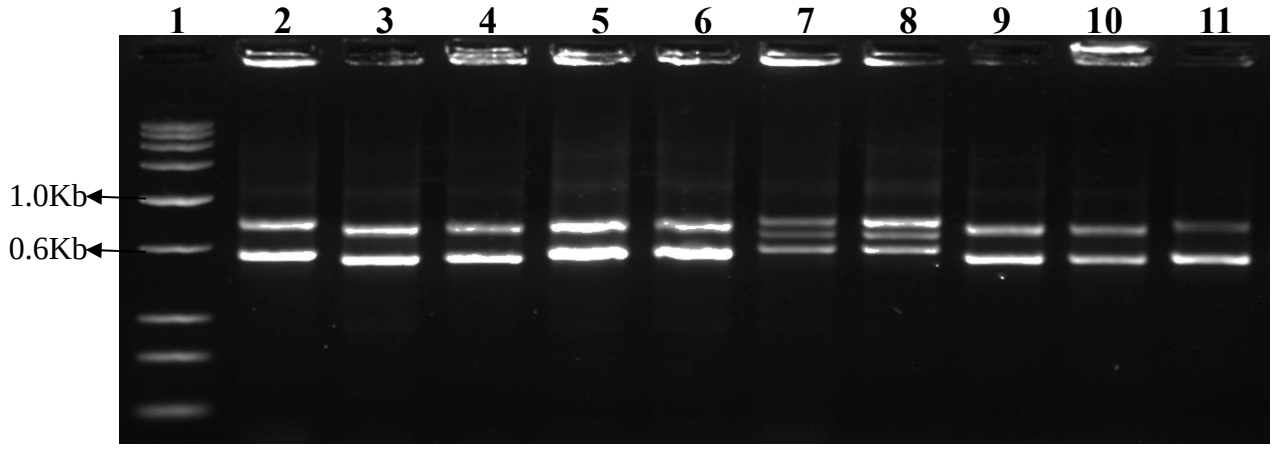


**Figure 2. 1 Ethidium bromide stained 0.8% agarose gel of 16s-23S rRNA gene intergenic region PCR amplification.**

**Lane 1:** Low range DNA marker
**Lane 2:** LGG (Positive control)
**Lane 3-11:** putative isolates (1-9)

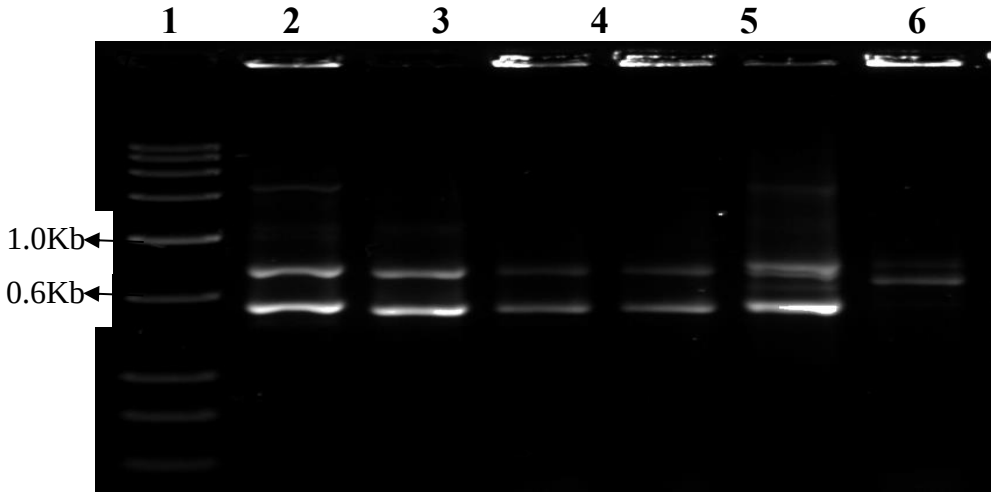


**Figure 2. 2 Ethidium bromide stained 0.8% agarose gel of 16s-23S rRNA gene intergenic region analysed following PCR amplification.**

**Lane 1:** Low range DNA marker
**Lane 2:** LGG (Positive control)
**Lane 3-7:** Putative isolates (10-14)

The 16S-23S rRNA gene intergenic region amplification profile of isolates was compared with that of standard *Lactobacillus* strain – LGG. The putative isolates – 1, 2, 3, 4, 7, 8, 9 and 10, 11, 12 in corresponding wells of Figure 2.1 (Lane 3-6, 9-11) and Figure 2.2 (Lane 3-5) showed the profile same as standard, respectively.

### 2.3.2 Bile tolerance

The isolates were inoculated into MRS containing 1%, 2% and 3% bile salts to check the minimal inhibitory concentration. 3% bile salt was maximum tolerant concentration for most of the isolates. The bile survivability was assessed at 0.3% and 1% bile concentration after 2 h incubation. Isolate GKI-1 exhibited the maximum survival at both 0.3% and 1% bile concentration (90% and 83% respectively) which is significantly higher than the standard strain LGG. Two of the other isolates FA-1 (85% and 82%) and FA-5 (83% and 82%) also had significantly higher survival rate than that of LGG at 0.3% and 1% bile salt concentration respectively ($P<0.05$) (Table 2.4).

**Table 2. 4 Survival rate of *Lactobacillus* strains in the presence of 0.3% and 1% bile salts.**

| ***Lactobacillus* isolates** | **Pre- incubation *Lactobacillus* concentration ($log_{10}$ cfu/ml)** | | | **Bile tolerance (0.3%) *Lactobacillus* concentration ($log_{10}$ cfu/ml)** | | | **Bile tolerance (1%) *Lactobacillus* concentration ($log_{10}$ cfu/ml)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Survival rate (%) | Mean | SD | Survival rate (%) | Mean | SD | Survival rate (%) |
| **LGG** | 8.36 | 0.01 | 100 | 6.78 | 0.03 | 81 | 6.76 | 0.16 | 81 |
| **FA-1** | 8.31 | 0.01 | 100 | 7.05* | 0.03 | 85 | 6.81* | 0.04 | 82 |
| **FA-5** | 6.79 | 0.02 | 100 | 5.63* | 0.21 | 83 | 5.66* | 0.03 | 83 |
| **FA-7** | 7.86 | 0.13 | 100 | 5.46 | 0.07 | 69 | 4.36 | 0.06 | 55 |
| **IIs11.2** | 7.93 | 0.02 | 100 | 6.14 | 0.04 | 77 | 5.82 | 0.31 | 73 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **GPI-3** | 7.96 | 0.01 | 100 | 6.13 | 0.13 | 77 | 6.09 | 0.02 | 77 |
| **GKI-1** | 6.82 | 0.05 | 100 | 6.14* | 0.04 | 90 | 5.65* | 0.02 | 83 |

LGG: *L. rhamnosus* GG; FA-1: *L. fermentum* FA-1; FA-5: *L. fermentum* FA-5; FA-7: *L. helveticus;* FA-7; IIS11.2: *L. fermentum* IIS11.2; GPI-3: *L. fermentum* GPI-3; GKI-1: *L. fermentum* GKI-1. Results were obtained from three independent experiments. Significant ANOVA was followed by Dunnett's test for all the assays to compare with respect to standard strain (LGG). Asterisks (*) indicates Mean value of isolates was significantly different from that of *L. rhamnosus* GG ($P < 0.05$).

### 2.3.3 Acid tolerance

The survival rate of isolates under acidic condition was determined by measuring the difference in the viable count at 0 h and 2 h incubated in acidic buffer (pH-2.5) (Table 2.5). As observed from the table, the survival rate of FA-5 (80%) and FA-7 (81%) were significantly higher than that of LGG (73%) ($P<0.05$). The survival rate of other isolates were observed to be in the range of 68% to 79%.

**Table 2. 5 Survival rate of *Lactobacillus* strains under acidic condition.**

| ***Lactobacillus* isolates** | **Pre- incubation *Lactobacillus* concentration ($log_{10}$ cfu/ml)** | | | **Acid tolerance ( pH 2.5) *Lactobacillus* concentration ($log_{10}$ cfu/ml)** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Survival rate (%) | Mean | SD | Survival rate (%) |
| **LGG** | 6.76 | 0.39 | 100 | 4.93 | 0.06 | 73 |
| **FA-1** | 6.99 | 0.08 | 100 | 4.74 | 0.08 | 68 |
| **FA-5** | 6.86 | 0.15 | 100 | 5.48* | 0.01 | 80 |
| **FA-7** | 6.72 | 0.14 | 100 | 5.46* | 0.08 | 81 |
| **IIS11.2** | 6.30 | 0.15 | 100 | 4.97 | 0.01 | 79 |
| **GPI-3** | 6.41 | 0.41 | 100 | 4.99 | 0.09 | 78 |
| **GKI-1** | 6.76 | 0.04 | 100 | 4.67 | 0.17 | 69 |

LGG: *L. rhamnosus* GG; FA-1: *L. fermentum* FA-1; FA-5: *L. fermentum* FA-5; FA-7: *L. helveticus;* FA-7; IIS11.2: *L. fermentum* IIS11.2; GPI-3: *L. fermentum* GPI-3; GKI-1: *L. fermentum* GKI-1. Results were obtained from three independent experiments. Significant ANOVA was followed by Dunnett's test for all the assays to compare with

respect to standard strain (LGG). Asterisks (*) indicates Mean value of isolates was significantly different from that of *L. rhamnosus* GG ($P < 0.05$).

### 2.3.4 Antimicrobial activity

The antimicrobial activity of the isolates was analysed against both Gram-positive and negative bacteria. Amongst all the isolates, GKI-1 had highest zone of inhibition against all the test organisms except *E.coli*. Moreover, all the isolates were able to inhibit the growth of *P. vulgaris* except LGG and FA-7. The other isolates were also able to inhibit the growth of test organisms to a more or less extent. (Table-2.6)

**Table 2. 6 Spectrum of antimicrobial activity exhibited by various lactobacilli isolates**

| ***Lactobacillus* isolates** | **Zone of inhibition (in mm)†** | | | | | |
|---|---|---|---|---|---|---|
| | *E.coli* | *P. aeruginosa* | *S.typhi* | *S.aureus* | *S.dysenterriae* | *P.vulgaris* |
| **LGG** | 21.67±1.15 (+++) | 16.33± 2.08 (++) | 14.67±0.58 (+) | 21.33± 0.58 (+++) | 17.00± 1.00 (++) | 11.33±0.58 (+/-) |
| **FA-1** | 11.67±1.53* (+/-) | 10.33±0.58* (+/-) | 11.67±0.58* (+/-) | 14.67±2.52* (+) | 11.67± 0.58* (+/-) | 14.33± 0.58 (+) |
| **FA-5** | 10.67± 1.15* (+/-) | 10.67±0.58* (+/-) | 14.33±1.15 (+) | 14.67±2.31* (+) | 11.33±1.15* (+/-) | 17.3± 2.89* (++) |
| **FA-7** | 10.33±0.58* (+/-) | 10.67±0.58* (+/-) | 10.33± 1.15* (+/-) | 11.33±1.15* (+/-) | 16.67±1.53 (++) | 11.33±0.58 (+/-) |
| **IIS11.2** | 10.67±0.58* (+/-) | 11.33±1.15* (+/-) | 10.33±0.58* (+/-) | 10.67±0.58* (+/-) | 11.67±1.15* (+/-) | 16.67±1.53* (++) |
| **GPI-3** | 13.33±1.15* (+) | 13.33±1.53 (+) | 11.33±1.15* (+/-) | 11.67±0.58* (+/-) | 14.00±2.00 (+) | 14.67± 0.58 (+) |
| **GKI-1** | 11.33±1.15* (+/-) | 16.00±2.0 (++) | 18.33±0.58* (++) | 18.33± 2.89 (++) | 17.67±3.51 (++) | 19.33± 1.53* (++) |

†The inhibition zones ≤ 12 mm, 13-15 mm, 16-20 mm and more than 20 mm were classified as strains of no (+/-), mild (+), strong (++) and very strong (+++) inhibition, respectively. Results were obtained from three independent experiments. Significant ANOVA was followed by Dunnett's test for all the assays to compare with respect to standard strain (LGG). Asterisks (*) indicates Mean value of isolates was significantly different from that of *L. rhamnosus* GG ($P < 0.05$).

### 2.3.5 Antibiotic susceptibility

The isolates were analysed for their susceptibility towards various antibiotics. Isolates FA-1, FA-5 and GPI-3 were found to be less susceptible to all the antibiotics tested compared to the standard strain LGG ($P<0.05$). Moreover, all the isolates were less susceptible to ofloxacin and cloxicillin antibiotics compared to LGG as indicated by less zone of inhibition (Table 2.7). Except FA-7 which exhibited an intermediate susceptibility towards vancomycin, the other isolates were less susceptible to vancomycin.

**Table 2. 7 Spectrum of antibiotic susceptibility of various lactobacilli isolates.**

| | Zone of inhibition in mm† | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cultures** | Chloramphenicol | Kanamycin | Gentamicin | Vancomycin | Bacitracin | Ofloxacin | Cephalosporin | Cloxicillin | Tobramycin |
| **LGG** | 26.33±1.53 (S) | 10.00±1.00 (R) | 20.00±1.00 (I) | 10.67±1.15 (R) | 17.6±0.58 (I) | 19.0±1.00 (I) | 21.67±1.53 (S) | 20.6±2.52 (S) | 25.33±2.52 (S) |
| **FA-1** | 11.33±1.53 (R) * | 11.00±1.00 (R) | 11.33±1.53 (R) * | 11.67±1.53 (R) | 11.33±1.15 (R) * | 11.67±1.15 (R) * | 12.00±2.00 (R) * | 11.00±1.00 (R) * | 8.67±1.15 (R) * |
| **FA-5** | 12.00±2.00 (R) * | 11.33±1.53 (R) | 11.67±1.53 (R) * | 12.00±2.00 (R) | 12.67±1.53 (R) * | 10.67±0.58 (R) * | 10.67±1.15 (R) * | 10.67±0.58 (R) * | 11.33±0.58 (R) * |
| **FA-7** | 16,33±1.53 (I) * | 19.33±2.52 (I) * | 19.00±3.61 (I) | 17.33±1.53 (I) * | 19.00±1.00 (I) | 15.33±0.58 (R) * | 20.67±1.15 (S) | 14.67±2.31 (R) * | 18.67±2.31 (I) * |
| **IIS11.2** | 12.33±1.53 (R) * | 12.00±2.67 (R) | 11.00±1.00 (R) * | 12.33±2.08 (R) | 19.67±0.58 (I) | 11.33±1.15 (R) * | 11.67±0.58 (R) * | 10.33±0.58 (R) * | 12.00±1.73 (R) * |
| **GPI-3** | 12.33±1.53 (R) * | 12.00±2.00 (R) | 12.33±1.53 (R) * | 12.33±1.53 (R) | 11.67±1.15 (R) * | 14.67±1.53 (R) * | 12.00±2.00 (R) * | 14.33±0.58 (R) * | 13.00±2.00 (R) * |
| **GKI-1** | 12.67±1.53 (R) * | 11.67±2.08 (R) | 17.67±1.15 (I) | 10.67±1.15 (R) | 25.67±1.15 (S) * | 14.33±0.58 (R) * | 27.67±3.51 (S) | 11.33±1.53 (R) * | 11.33±1.53 (R) * |

†The inhibition zones ≤ 15 mm, between 16-20 mm and >20 mm indicates Resistant (R), Intermediate (I) and Sensitive (S) strains, respectively. Results were obtained from three independent experiments. Significant ANOVA was followed by Dunnett's test for all the assays to compare with respect to standard strain (LGG). Asterisks (*) indicates Mean value of isolates was significantly different from that of *L. rhamnosus* GG ($P < 0.05$).

## 2.4 Discussion

A potential probiotic organism must survive in the GIT for a long period of time in order to confer any health promoting effects. The survivability of any potential probiotic organism is dependent on their ability to tolerate bile and acidic environment of the GIT as well as on the origin of the strain. The persistence of the autochthounous strains into the GIT is longer than the allochthounous strains (Tannock et al., 2000). Most of the commercially available probiotic strains are from fermented foods of Indian origin and human origin. With this background, the isolation of *Lactobacillus* was carried out from fermented foods and human faeces of Indian population. The isolates have been then assessed for potential probiotic properties like tolerance to acid and bile, antibiotic susceptibility and antibacterial activity.

There are a number of different *in vitro* assays described to screen for acid resistance property of lactobacilli strains. Some of these are exposure of strains to pH-adjusted acidic buffer (Casey et al., 2004), incubation in gastric contents (Conway et al., 1987; Goldin et al., 1992), and also the use of a dynamic model of the stomach (Marteau et al., 1997). In the present study, the isolates were incubated in acidic pH of 2.5 for 2 h. Although, in the stomach, pH can be as low as 1.5 and as high as 4.5, the pH is mostly maintained at the value of above 2.0 (Charteris et al., 1998). Moreover, in most of the *in vitro* assays pH 2.5 has been preferred (Saran et al., 2012; Tulumoglu et al., 2013). This is due to the fact that a substantial decrease in the viability of strains is often observed at pH 2.0 or below (Gupta et al., 1996; Hood and Zoitola, 1988; Lancaputhra and Shah, 1995). In addition, the probiotic strains are likely to be buffered by food or other carrier matrix molecules following consumption and are thus not likely to be exposed to the extremes of pH in the stomach. Health effect of probiotic organisms is generally strain dependent and is achieved when the organism is used at $10^8$–$10^{11}$

cfu/day (Vanderhoof and Young, 1998). In the present study using $10^8$ cfu/ml of each isolate, the survival rate was between 69% to 81% after 2 h of incubation in pH-2.5. Earlier reports on lactobacilli at pH 2.5 showed survival rates similar to those observed with the isolates used in this study (Saran et al., 2012; Tulumoglu et al., 2013). However, some of the isolates (FA-5 and FA-7) had significantly higher survival rate than the standard probiotic strain LGG.

The physiological concentration of human bile ranges from 0.3% to 0.5% (Dunne C. et al., 1999; Zavaglia et al., 1998). Various lactobacilli were resistant to bile (Charteris et al., 1998). Lactobacilli do possess bile salt hydrolases (BSH; (De Smet et al., 1995)) which deconjugate the bile salts into lesser toxic form which help them to survive in the presence of toxic conjugated bile salts. Here, the survivability of the isolates was assessed at 0.3% and 1% bile concentration. Interestingly, the isolates in the present study were able to survive even at higher (1% ox-bile) bile concentration than the usual bile concentration present in the human stomach. Moreover, some isolates (GKI-1, FA-1 and FA-5) had survivability significantly higher than the standard strain LGG at both the concentrations.

Lactobacilli are known for their production of various antimicrobial compounds (Ouwehand and Vesterlund, 2004; Pangallo et al., 2008). The production of these compounds by lactobacilli is probably one of the most important mechanisms responsible for the antagonistic phenomenon (Gomes D. A. et al., 2006) and therefore it is essential to examine this property in probiotic candidates. Moreover, the ability to produce antimicrobial substances by lactic acid bacteria has led to their potential use as natural preservatives to combat the growth of pathogenic microorganisms in the food industry (Barnby-Smith, 1992) and thereby to control the spread of infectious diseases. The antimicrobial activity of the isolates was analysed against both Gram-positive and

-negative bacteria. The isolates inhibited the growth of both Gram-positive and Gram-negative organisms to a more or less extent. Amongst all the isolates, GKI-1 inhibited the growth of most of the test bacteria except *E.coli.* Antimicrobial action of these lactobacilli isolates may be manifested by one or combination of the following actions including competition for nutrients, adhesion and production of different antimicrobial metabolites such as organic acids, $H_2O_2$, bacteriocins, etc.(Garneau et al., 2002; Midolo et al., 1995; Reid and Burton, 2002). To assess the antibiotic susceptibility of lactobacilli is considered as one of the crucial criteria from the safety point of view for potential probiotics. This is because bacteria used as probiotics may serve as hosts of antibiotic resistance genes, which can be transferred to pathogenic bacteria (Sharma P. et al., 2014). However, probiotics with known antibiotic resistance may also be used in antibiotic-associated diarrhoea (Siitonen et al., 1990). Additionally, based on the knowledge of antibiotic resistance, corresponding antibiotics may be used to washout the probiotic once it out lives its utility as a deliver vehicle. Zhou et al., (2005) and Liasi, S. A. et al., (2009) reported that most of the *Lactobacillus*, *Enterococcus*, and *Pediococcus* strains used as a probiotic were resistant to Gram-negative spectrum antibiotics and aminoglycoside antibiotics. In the present study, all the isolates were found to be less susceptible to ofloxacin and cloxicillin antibiotics compared to the other antibiotics studied. Ofloxacin antibiotic inhibits the nucleic acid synthesis and cloxicillin inhibits the bacterial growth by inhibiting cell-wall synthesis. Ammor et al., (2007) reported that resistance towards antibiotics that target on nucleic acid synthesis is an inherent property of lactobacilli. Except FA-7, the other isolates were resistance to another cell-wall synthesis inhibitory antibiotic vancomycin, while FA-7 was found to be less susceptible to vancomycin. It has been reported that lactobacilli are also intrinsically resistance to vancomycin (D'Aimmo et al., 2007; Swenson et al., 1990).

Moreover, compared to the other isolates, *L. fermentum* isolates FA-1, FA-5 and GPI-3 were found to be less susceptible to all the antibiotics.