Chapter 3

Analysis of probiotic property of selected *Enterococcus* isolates

Introduction

*Enterococcus* is one of the several genera of lactic acid bacteria that are naturally found in the intestinal microbiota of human and other animals. To prevent the colonization of an unfavorable microbiota inside the host, enterococci often compete with these pathogenic microorganisms in the epithelial cells for adhesion (Araújo and Ferreira, 2013). Additionally, enterococci also harbor some antimicrobial and anti-listeral properties (Foulquié Moreno et al., 2003). Enterococcal probiotics are by and large not incorporated at once as starter cultures in food fermentation but are at times employed as food supplements in the form of pharmaceutical preparations to enrich the microbial balance of the intestine and to treat gastroenteritis in humans and animals (Banwo et al., 2013).

In order to provide health benefits, the major requirement of probiotic bacteria is to overcome the various barriers like acid and bile inside the gastrointestinal (GI) tract. A probiotic bacterial species should have high tolerance to acid in order to survive in the highly acidic condition of the stomach. This characteristic of a bacteria is measured by evaluating its ability to survive at lower pH for 2-3 h, which is an average passage time through the stomach (Araújo and Ferreira, 2013). Likewise bile
tolerance is also required by probiotic bacterial strains to facilitate their passage through the duodenum to their site of action in the GI tract (O’Sullivan et al., 2002). Enterococci offers resistance to gastric juices and bile salts and thus when they are administered to humans or animals, they reach the intestine and colonize it. Owing to these characteristics, many enterococcal strains have been commercialized as probiotics (Franz et al., 1999). Viability of probiotic bacteria in the gastrointestinal tract also depend on their ability to adhere to the intestinal tract. A probable mechanism for adherence and colonization of these bacteria involves binding of the microorganisms to the mucus layer protecting the epithelial cells of the gastrointestinal tract of the host (Jeronymo-Ceneviva et al., 2014). Adhesion between bacterial cell membrane and the various interacting surfaces in the host is a complex process. Cell surface hydrophobicity is an important property of probiotic bacteria which depends mainly on the chemical composition of the surface components of bacteria. While bacterial adhesion is an intricate interaction of various electrostatic and chemical forces, strains adhering well to hydrocarbons are regarded to be hydrophobic whereas the ones adhering poorly are considered hydrophilic (Duary et al., 2011).

There is an abundance of oxidative reactions that occur during regular metabolism inside biological system. In human body, free radicals and other reactive oxygen species (ROS) are generated by exogenous chemicals or endogenous metabolic processes causing damaging effect to tissues and cells (Pieniz et al., 2015). The antioxidant property and free radial scavenging ability of Enterococcus is in addition
a typify of probiotic trait. While the probiotic facet of some strains is well recognized, the emergence of antibiotic resistant strains of enterococci and their increased correlation with human diseases have raised concerns regarding their use as probiotics.

There have been reports which described the anticancer properties of various lactic acid bacteria (Kim et al., 2002). The different antitumor properties of LAB include inactivation or inhibition of carcinogenic compounds in the gastrointestinal tracts, stimulation of immune response and reduction of enzymatic activities of various enzymes such as β-glucuronidase, nitroreductases and azoreductases which have the ability to convert precarcinogens to carcinogens (Vamanu et al., 2006). Bacteriocins from LAB are of great importance in medicine as they inhibit the growth of pathogenic bacteria in human body. The most studied bacteriocin, nisin, has been recently tested for its anticancer properties against various cancer cells (Ahmadi et al., 2017).

The present chapter aimed to characterize the probiotic potential of the bacteriocin producing Enterococcus faecalis strains through studying their ability to survive in conditions resembling that of gastrointestinal tract, their cell surface hydrophobicity and also evaluating their antioxidant properties and susceptibility to antibiotics. Furthermore, the anti-proliferative activities of bacteriocins, extracted from the E. faecalis isolates were also screened and compared against a number of human cancer and normal cell lines. IC\textsubscript{50} values (the drug concentration required to kill 50% of the
cells) of the potent bacteriocin, which significantly affected the proliferation of the cancer cell lines, was also determined.

**Materials and Methods**

**Bacterial strains:** The five *Enterococcus faecalis* isolates (KBSRJ8, KBSRJ26, KBSRJ32, KBSRJ39, KBSRJ42) were cultured in MRS broth for 24 h at 37 °C and were used to investigate their probiotic properties.

**Acid tolerance of Enterococcus faecalis isolates:** To examine the ability of the *E. faecalis* isolates to survive in gastric juice, acid tolerance studies were carried out by following the protocols developed by Lim and Im (2009) with minor modifications. The *E. faecalis* isolates cultured in fresh MRS broth for 24 h at 37 °C were harvested by centrifugation (10,000 rpm for 15 min) and they were washed three times in phosphate buffered saline pH 7 (PBS). 1% of the *E. faecalis* suspension was inoculated into fresh 100 ml MRS broth containing 1000 units of pepsin (HiMedia, India) that had been acidified to pH 2 and pH 3 (with HCL), as well as in neutral MRS broth and incubated at 37 °C for 3 h. CFU of the isolates was determined by spreading 10-fold serial dilutions bacterial culture on MRS agar plates and counting the number of colonies after incubating for 24 h at 37 °C.

**Bile tolerance of Enterococcus faecalis isolates:** To assess the viability of the *E. faecalis* strains in intestinal conditions, their bile tolerance was determined following the method developed by Banwo *et al.* (2013) with minor modifications. The *E. faecalis* isolates were cultured in MRS broth for 24 h at 37 °C. Cells were then
harvested by centrifugation (10,000 rpm for 15 min) and washed three times with phosphate buffered saline (PBS) (pH 7). 1% of the *E. faecalis* suspension was inoculated into fresh MRS broth containing 0, 0.1, 0.2, 0.3, 0.4 and 0.5% Oxgall (HiMedia, India). Cultures were incubated at 37 °C for 4 h. Optical density (OD) of the cell suspension was determined by measuring the absorbance at 600 nm and was then compared with the absorbance of the control cells (grown without bile salts).

**Cell surface hydrophobicity:** The cell surface hydrophobicity of the *E. faecalis* isolates and a control culture (*Lactobacillus plantarum*) was determined by microbial adhesion to hydrocarbons following the method as described by (Todorov *et al.*, 2011). The control as well as the *E. faecalis* isolates were cultured in MRS broth for 24 h at 37 °C. Cultures were then harvested by centrifugation (10,000 rpm for 15 min), washed three times in Ringer's solution and resuspended in the same solution. Initial absorbance (*A*₀) of the suspension was measured at 580 nm. In 2 ml of the cell suspension, equal volume of n-hexadecane (Sigma- Aldrich) was added and vortexed thoroughly for 2 min. The tubes were left undisturbed for 30 min at room temperature and the aqueous phase was carefully recovered and absorbance (*A*₁) was recorded at 580 nm. Cell surface hydrophobicity was calculated using the following formula in terms of percentage (%):

\[
\text{Cell surface hydrophobicity (\%)} = \frac{A_0 - A_1}{A_0} \times 100
\]

**Free radical scavenging activity:** Free radical scavenging activity of *E. faecalis* strains was determined in accordance with the method developed by Shimada *et al.*
A control strain (*L. plantarum*) as well as the five *E. faecalis* isolates were cultured in MRS broth for 24 h at 37 °C and harvested by centrifugation (10,000 rpm for 15 min), washed three times in PBS (pH 7) and resuspended in the same buffer. 800 μl of each cell suspension and 1 ml of freshly prepared 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (HiMedia, India) solution (0.2 mM in methanol) were mixed and allowed to react for 30 min in dark. Blank samples contained deionized water with DPPH. The amount of scavenged DPPH was then determined by measuring the decrease in absorbance at 517 nm. The scavenging activity was calculated as follows:

\[
\text{DPPH scavenging activity (%) } = 1 - \frac{A_{\text{Sample}}}{A_{\text{Blank}}} \times 100
\]

**Antibiotic susceptibility:** Disc diffusion method as described by Bauer *et al.* (1966) was adopted to detect the susceptibility of the *E. faecalis* isolates to various antibiotics. Overnight culture of each isolate was spread evenly on the surface of a MRS agar plate followed by placing the antibiotic discs. The plates were then incubated at 37 °C for 24 h. Commercially available antibiotic discs used were amoxycillin (10 mcg), streptomycin (15 mcg), erythromycin (15 mcg), tetracycline (10 mcg), penicillin G (2 units), ampicillin (10 mcg), chloramphenicol (30 mcg), gentamycin (10 mcg), kanamycin (30 mcg), polymyxin B (30 mcg), rifampicin (20 mcg) and vancomycin (30 mcg). The *E. faecalis* strains were categorized as sensitive (S; ≥ 21 mm zone of inhibition), intermediate (I; 16-20 mm zone of inhibition) and resistant (R; ≤ 15 mm zone of inhibition) according to CLSI guidelines based on the inhibition zone (Clinical Laboratory and Standards Institute, 2014).
Cell killing ability by *E. faecalis* bacteriocins in normal and cancer cell lines and clonogenic cell survival assay: Trypan blue cell exclusion assay was carried out to determine the cell killing ability of the bacteriocins. MCF-7 (human breast cancer cell line, ATCC, USA), OE33 (human caucasian esophageal carcinoma, Sigma, USA), HCT116 (human colon carcinoma cells, ATCC, USA) were used as cancer cell lines, HEK293 (human embryonic kidney fibroblast, ATCC, USA) and freshly collected human peripheral blood lymphocytes were used as normal cells for the experiment.

Peripheral blood was obtained from a healthy donor and the blood mononuclear cells were isolated by Ficoll-Hypaque (Sigma Diagnostics, St. Louis, MO) density gradient centrifugation for 30 min at 1250 rpm. MCF-7, HCT116 and HEK293 were cultured in DMEM high glucose medium (Invitrogen), OE33 and normal lymphocytes were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml Penicillin, 100 mg/ml Streptomycin (Invitrogen) and 2 mM L-Glutamine (Invitrogen). These cells were treated with 2.75 mg/ml of bacteriocin, extracted from the five *E. faecalis* isolates for 24h and harvested by centrifugation (1200 rpm, 10 min) for the assay. Cell pellets were washed with fresh medium and then incubated for 10 min at room temperature with 0.4% trypan blue (Sigma, India) and viewed under a microscope. Dead cells were stained blue, and live ones remained unstained. Cell death was calculated by using the following formula

\[
\text{Cell death (\%) } = \frac{\text{Total number of dead cells}}{\text{Total number of cells}} \times 100
\]
The cell survivability was evaluated using clonogenic assay in MCF-7 cell line. The cells were detached by adding trypsin and appropriate numbers of cells (500-2000 cells) were seeded into three 100 mm culture dish in triplicate for treated and untreated cells. Eight hours after seeding the cells were exposed for 24 h with 0.5-3 mg/ml of bacteriocin, extracted from *E. faecalis* isolate KBSRJ8. After the treatment, the cells were washed twice with DMEM medium and finally the dishes were incubated in a humidified incubator with 5% CO₂ at 37 °C with fresh DMEM medium supplemented with 10% fetal calf serum for 10 days. After incubation the colonies were fixed and stained with 0.2% crystal violet in 70% ethanol. Each assay was performed in triplicate and colonies containing at least 50 cells were counted.

**Statistical analysis:** All data were reported as mean ± Standard error of mean (SEM) of three replicates. The data were analyzed using one-way variance analysis (One Way ANOVA) and Tukeys post test. The results for trypan blue assay are expressed as mean ± Standard error of mean (SEM). Statistical analysis of the data was performed by paired t-test and p values <0.05 were considered to be significant. The data obtained from clonogenic survival assay are represented as a sigmoidal fit curve with a linear X scale, the function used was Boltzmann. The Y value at X₀ is considered to behalf way between the two limiting values on Y-axis and this is considered to be the inhibitory concentration 50 (IC₅₀) value.
Results

**Acid and bile tolerance of *Enterococcus faecalis* isolates:** All the *E. faecalis* isolates were able to tolerate pH 2 and pH 3 even after 3 h of incubation ($P<0.0001$) (Fig 3.1.1-3.1.2). One-way analysis of the variance showed that there was significant difference ($P <0.05$) in the CFU/ml count among isolates when subjected to pH 2 and pH 3. The *E. faecalis* isolates could tolerate 0.3% bile salt concentration which is considered as the mean bile salt concentration inside the human gastrointestinal tract (Fig 3.2). One-way ANOVA study revealed that there was no mean significant difference ($P=0.83$) among the isolates when they were subjected to different bile concentrations.

**Fig 3.1.1:** Acid tolerance by *E. faecalis* isolates represented by their viable count at pH 2 (Values are mean ± SEM of three independent experiments)
**Fig 3.1.2:** Acid tolerance by *E. faecalis* isolates represented by their viable count at pH 3 (Values are mean ± SEM of three independent experiments)

**Fig 3.2:** Bile tolerance by studied *E. faecalis* isolates at different bile concentrations. (Values are mean ± SEM of three independent experiments)
**Cell surface hydrophobicity:** The *E. faecalis* isolates were investigated for their *in vitro* cell surface hydrophobicity and the result was compared to the hydrophobicity of a probiotic strain of *L. plantarum* MTCC 1325. In the present study, all the strains of *E. faecalis* showed high levels of hydrophobicity ranging between 55-65% (**Fig 3.3**). One-way analysis of the variance revealed that there was no mean significant difference \((P=0.142)\) among the isolates when their hydrophobicity was compared to that of *L. plantarum*.

![Figure 3.3](image.png)

**Fig 3.3:** Cell surface hydrophobicity of the *E. faecalis* isolates.
(Values are mean ± SEM of three independent experiments)

**2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:** **Fig 3.4** shows the *in vitro* free radical scavenging activity of the five *E. faecalis* and the control *L.*
*L. plantarum* MTCC 1325 strains. All the *E. faecalis* isolates showed similar scavenging activity as compared to the *L. plantarum* (*P*=0.0078) and there was no mean significant difference among them except the isolate KBSRJ32 which showed comparatively lower scavenging activity when compared to the standard (*P*<0.05) (Fig 3.4).

![Graph showing DPPH scavenging activity of the E. faecalis isolates](image)

**Fig 3.4:** DPPH scavenging activity of the *E. faecalis* isolates (Values are mean ± SEM of three independent experiments).

**Antibiotic susceptibility:** For the antibiotic susceptibility test the *E. faecalis* isolates were found to be susceptible to amoxicillin, streptomycin, erythromycin, penicillin, ciprofloxacin, chloramphenicol, tetracycline and polymyxin B with a zone of inhibition of 20 mm or more (Fig 3.5-3.6; Table 3.1). However, the isolates showed intermediate resistance to gentamicin, rifampicin and vancomycin with the zone of inhibition
ranging between 7-15 mm. KBSRJ42 on the other hand was resistant to vancomycin. All the isolates were resistant to ampicillin and kanamycin.

![Graphical representation of average zone of inhibition shown by the E. faecalis isolates for different antibiotics](image1)

**Fig 3.5:** Graphical representation of average zone of inhibition shown by the *E. faecalis* isolates for different antibiotics

![Antibiotic sensitivity test for representative E. faecalis isolates](image2)

**Fig 3.6:** Antibiotic sensitivity test for representative *E. faecalis* isolates
Table 3.1: Antibiotic sensitivity pattern conferred by the *E. faecalis* isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>KBSRJ8</th>
<th>KBSRJ26</th>
<th>KBSRJ32</th>
<th>KNSRJ39</th>
<th>KBSRJ42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>S</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

[Sensitive ‘S’: (≥ 21 mm), Intermediate ‘I’: (16-20 mm), Resistant ‘R’: (≤ 15 mm)]

**Cell killing ability by *E. faecalis* bacteriocins in normal and cancer cells and clonogenic cell survival assay:** The *E. faecalis* extracts were found to exhibit cytotoxicity on various cancer cell lines, whereas, low cytotoxicity was seen against normal cell line (Fig 3.7; Table 3.2). Significant increase in cell death percentage in cancer cell lines (p<0.001) compared to the normal cells after treatment with bacteriocins was recorded. Bacteriocin extract from KBSRJ8 appeared to inhibit the viable cells of MCF-7 and HCT116 to the highest extent. The plot on **Figure 3.8** was generated using Graphpad Prism software along with the calculated IC\(_{50}\) values for the bacteriocin extract from KBSRJ8 against HCT116 cell line. Based on the sigmoidal fit
graph estimation, IC$_{50}$ was found to be 1.34 mg/ml in case of bacteriocin extract of KBSRJ8 for HCT116 cell line.

**Table 3.2:** Trypan Blue Exclusion Assay in human lymphocytes and in cancer and normal cell lines after treatment with 2.75 mg/ml of bacteriocin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MCF7 (%)</th>
<th>HCT116 (%)</th>
<th>OE33 (%)</th>
<th>HEK293 (%)</th>
<th>Lymphocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7.21 ± 0.1</td>
<td>7.60 ± 0.1</td>
<td>9.1 ± 0.4</td>
<td>8.25 ± 0.3</td>
<td>11.5 ± 0.1</td>
</tr>
<tr>
<td>KBSRJ8</td>
<td>29.35 ± 0.4</td>
<td>31.40 ± 0.7</td>
<td>27.5 ± 0.3</td>
<td>13.40 ± 0.4</td>
<td>18.3 ± 0.3</td>
</tr>
<tr>
<td>KBSRJ32</td>
<td>27.37 ± 0.5</td>
<td>28.50 ± 0.8</td>
<td>28.1 ± 0.3</td>
<td>10.70 ± 0.1</td>
<td>18.6 ± 0.2</td>
</tr>
<tr>
<td>KBSRJ39</td>
<td>27.50 ± 0.6</td>
<td>30.20 ± 0.5</td>
<td>32.1 ± 0.4</td>
<td>13.30 ± 0.3</td>
<td>16.4 ± 0.3</td>
</tr>
<tr>
<td>KBSRJ42</td>
<td>24.80 ± 0.3</td>
<td>22.50 ± 0.5</td>
<td>25.6 ± 0.1</td>
<td>9.80 ± 0.2</td>
<td>15.4 ± 0.1</td>
</tr>
<tr>
<td>KBSRJ26</td>
<td>22.08 ± 0.4</td>
<td>23.70 ± 0.5</td>
<td>25.4 ± 0.1</td>
<td>9.50 ± 0.1</td>
<td>18.2 ± 0.4</td>
</tr>
</tbody>
</table>

**Fig 3.7:** IC$_{50}$ calculation in KBSRJ8 treated HCT116 cell lines
Discussion

A high prevalence of enterococci in fermented and processed foods may be credited to their ability to grow at high temperatures, extreme salinity and harsh conditions. *Lactobacilli* are the most common probiotics when it comes to human nutrition, but *Enterococcus* spp. and *Saccharomyces* are often used as probiotics in animal nutrition (Lauková *et al.*, 2008). Probiotic bacteria must first survive their transit through the stomach before reaching the gastrointestinal tract where they can promote various health benefits as metabolically viable cells (Nueno-Palop and Narbad, 2011). In order to assess the probiotic characteristics of the *E. faecalis* isolates, their resistance to pH and bile salts were studied. Results from this study showed that all the *E. faecalis* isolates showed good survivability at pH 2 and pH 3 and likewise they could also proliferate in all concentrations of bile tested (0.1-0.5%) which is in accordance with the study reported by Cebrián *et al.* (2012) and Nueno-Palop and Narbad (2011). As compared to the study on lactobacilli, reports concerning probiotic characteristics of enterococci are relatively scarce. As reported by Sun *et al.* (2010) *E. faecium* strain SF68 maintained its viability when exposed to bovine bile and gastric juices for 60 min.

Stomach pH generally ranges between 1.5 to 3.5, although it may alter during prolonged fasting or immediately after a meal (Huang and Adams, 2004). Thus, the ability of *E. faecalis* isolates to survive at lower pH for almost 3 h can be classified as tolerant to gastrointestinal conditions. Acid tolerance of LAB depends on the pH profile of H⁺-ATPase and the composition of the cytoplasmic membrane (Pieniz *et al.*, 2014) but this is mostly influenced by the type of bacterium, the composition of growth
medium and conditions at which the bacteria is incubated (Madureira et al., 2005). Another important characteristic of probiotic bacteria is their ability to tolerance bile which helps them to survive, grow and carry out their beneficial activities in the small intestine (Nueno-Palop and Narbad, 2011). Ability of LAB to withstand 0.3% Oxgall bile concentration has been consistently used to evaluate the bile tolerances of bacteria (Gilliland and Walker, 2018) and in the present study it was noted that all *E. faecalis* isolates were able to grow at 0.3% bile concentration. Thus, tolerance of the *E. faecalis* isolates to simulated gastric and intestinal conditions is important to be considered as probiotic supplements.

Cell surface hydrophobicity can be described as a nonspecific interaction between the microbial cells and host. The initial interaction among the host and microbial cell may be weak and the process can be reversible, but certain mechanisms involving various cell surface proteins may help in subsequent adhesion of these microbes to the host (Todorov et al., 2011). Greater hydrophobicity may imply strong interaction of bacterial cells with mucosal surfaces. Based on the results of this study, the *E. faecalis* isolates can be considered as potential probiotic strains as they showed evidence of a strong hydrophobicity which was as good as the hydrophobicity of the *L. plantrum* strain used as a control.

Antioxidant molecules helps in reducing the effect of free radicals by removing them from the body and avert from diseases and conditions like cancer, diabetes as well as lowering the process of ageing (Pieniz et al., 2015). The intact cells of *E. faecalis* showed good antioxidant activity against DPPH. Various *in vitro* studies have reported
that intact cells of LAB also possess antioxidant activity (Lin and Yen, 1999). In the human body and food systems, various oxygen-centered free radicals and other reactive oxygen species are frequently produced (Lobo et al., 2010). Thus, consuming *E. faecalis* containing foods or supplements may be beneficial.

Antibiotic resistance in enterococci is an increasing worldwide health related issue. The present study determined the susceptibility of the *E. faecalis* isolates to several antibiotics. The isolates were sensitive to clinically relevant antibiotics such as tetracycline, erythromycin and exhibited intermediate resistance towards vancomycin. However, the isolates were found to be resistant to kanamycin and ampicillin. Nueno-Palop and Narbad (2011) also reported the susceptibility of *E. faecalis* towards tetracycline, erythromycin, and chloramphenicol and is in agreement with the present study.

One of the initial studies describing the toxic effects of bacteriocins on eukaryotic cells was reported by Farkas-Himsley and Cheung (1976). Various effects of bacteriocins on mammalian cells have also been reviewed over the years (Cornut et al., 2008). In our study, we evaluated the anti-proliferative activity of *E. faecalis* bacteriocins on MCF-7, HCT116, OE33, HEK293, and lymphocytes. The bacteriocins of all the *E. faecalis* isolates revealed their cytotoxicity potential on the cancer cells as described by the trypan blue assay. The effect of the bacteriocins on all the examined cancer cell lines significantly differed from that of the untreated or the normal cells. The *E. faecalis* bacteriocins showed no toxic effect on the normal cells and more than 90% of the cells grew normally. Thus, the bacteriocins reduced the viability of all the cancer cells but
did not negatively affect the normal cells indicating that these bacteriocins are non-toxic and have a selective action against cancer cells.

Although the exact mechanism for specificity of bacteriocins towards cancer cells is not well understood, the factors that may account for such selective action may possibly be explained based on the differences in the cell surface of cancer and normal cells (Kaur and Kaur, 2015). In normal mammalian cells, the phospholipid membrane is asymmetric; the outer surface consists of neutral choline-containing zwitterionic phospholipids, while the inner membrane is made up of aminophospholipids. But in cancer cells there is a loss of asymmetry. Due to high levels of anionic phosphatidylinerine, sialylated gangliosides, heparin sulphates and O-glycosylated mucins (Kaur and Kaur, 2015) cancer cell membranes are known to possess a negative charge. Since bacteriocins are cationic peptides by nature therefore they tend to bind better to the negatively charged membranes of the cancer cells as compared to the normal cells. Secondly, higher cell membrane fluidity in cancer cells as compared to normal cells facilitates easier membrane destabilization and thus explaining the specificity of bacteriocins to such cancer cells (Sok et al., 1999). Finally, the surface area of cancer cells is increased due to the presence of higher number of microvilli compared to the normal cells which results in binding of more antimicrobial peptides to the cancer cell membrane (Chiu et al., 1999).

The findings of this chapter reveal that the five *E. faecalis* isolates are resistant towards acidic conditions and bile salts. With good cell surface hydrophobicity, antioxidant activity and lower antibiotic resistance these isolates displayed desirable probiotic
properties in *in vitro* conditions making them promising candidates for further investigations concerning their use as probiotic starter cultures or food supplements. Furthermore, as the bacteriocins from these isolates showed cancer cell specific toxicities, their potential to assist as synergistic agents to standard cancer drugs open vistas for in-depth investigations.