**In vitro** evaluation of probiotic potential of five lactic acid bacteria and their antimicrobial activity against some enteric and food-borne pathogens

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Lactic acid bacteria (LAB) present many important properties in food manufacturing, such as improvement of physical characteristics and the production of lactic acid that aids in the increase of the shelf life of food products. Also, LAB can suppress growth of pathogens, control of serum cholesterol level, modulate immune system, and improve lactose digestion. Five standard (*Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, *Bifidobacterium longum* and *Lactococcus lactis*) lactic acid bacterial strains were screened for probiotic potential properties and their ability to antagonize the growth of some enteric pathogens isolated from patients suffering from acute gastroenteritis. The five strains of LAB were resistant to acidic pH and bile salts. *Lactobacillus* strains showed protein and starch digesting capability on agar plate while *B. longum* ATCC 15707 and *L. lactis* subsp. *lactis* ATCC 11454 showed only protein digestion. In addition, *Lactobacillus* strains showed antagonistic effects against all pathogenic strains tested. *L. paracasei* and *L. helveticus* [culture and cell-free culture supernatant (CFCS)] exhibited the highest antagonistic activity against the tested pathogens followed by *L. fermentum*. While *B. longum* and *L. lactis* subsp. *lactis* showed weak or no activity against the tested strains. *L. paracasei*, *L. helveticus*, and *L. fermentum* showed potential to be used as probiotic strains with considerable good antagonistic activity against the most important enteric pathogens.

**Key words:** *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, *Bifidobacterium longum* subsp. *longum*, *Lactococcus lactis* subsp. *lactis*, potential probiotic, antagonistic activity.

**INTRODUCTION**

Foodborne bacterial pathogens, such as *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia enterocolitica* and *Staphylococcus aureus* can cause diseases that ranged from mild diarrhea to severe illness with high mortality (Petri Jr. et al., 2008). Worldwide, diarrheal disease remains one of the most important causes of morbidity and mortality among infants and children (Ryan, 2004) with 15 billion episodes and 15 to 25 million deaths estimated to occur annually among children aged <5 years (Kosek et al., 2003; Black et al., 2003; Parashar et al., 2003). Although, the use of antimicrobials can limit the growth of enteric pathogens and the use of glucose-
electrolyte oral rehydration therapy (ORT) has dramatically reduced acute mortality from dehydration caused by diarrhea. Antimicrobial resistance becomes a common finding increasing over time and the rates of morbidity remain as high as ever (Kosek et al., 2003; Andersson and Hughes, 2010).

According to the working group FAO/WHO (2002), probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics can reduce the duration of diarrhea by 0.7 days as well as the frequency of diarrheal episodes already in the first hours (Van Niel et al., 2002) and their consumption is, therefore, recommended in case of acute gastroenteritis starting from the onset of symptoms (Aureli et al., 2011). Probiotics help keep up the balance between harmful and beneficial bacteria in the gut, thus maintaining a healthy digestive system (Ouwehand et al., 2002). Bifidobacterium and Lactobacillus are naturally inhabitants of the human intestinal microbiota, some strains have a satisfactory tolerance to the gastrointestinal transit, good survival in food or pharmaceutical supplements and health-promoting effects; so, the strategy of probiotic supplementation is the reinforcement of the intestinal microbiota, at least transitory, with health-promoting bacteria to benefit the intestinal balance (Farnworth, 2008).

The effect of probiotics ranges from regulation of bowel activity and well-being to more specific actions, such as, antagonistic effect on the gastroenteric pathogens like Clostridium difficile, Campylobacter jejuni, Helicobacter pylori, rotavirus, etc. (Doron and Gorbach, 2006).

There are various proposed mechanisms that describe how different probiotics work and they vary depending on the strain of probiotic used. The effects of probiotics also depend on the dosage and route of administration (Upadhay and Moudgal, 2012). Some of these mechanisms are the production of bacteriocins, such as nisin [approved by the US Food and Drug Administration (FDA) since last decade for food preservation and shelf life extension] (Yateem et al., 2008; Collins et al., 2012) or lowering the pH by producing acidic compounds like lactic acid (Psmosas et al., 2001). Competition with other infectious bacteria for nutrients and receptors mediating colonization to host cells (Piard and Desmazeaud, 1991). A few strains are also known to produce active enzymes which inhibit other pathogenic bacteria (Gotcheva et al., 2002). The most common application of probiotics is for dairy production such as yogurt, cheese, and ice cream. Recently, several studies were done for the use of probiotic bacteria in preventing antibiotic-associated diarrhea and C. difficile infections (McFarland, 2009). In addition, many species have been suggested to be effective in alleviating gastrointestinal pathogenic bacterial infections both in vitro and in vivo (Chung and Yousef, 2010; Forestier et al., 2001; Wong et al., 2013), without any pathogenic effect on human and animals.

Based on previously reported beneficial properties of probiotics, our study aimed to evaluate the probiotic properties of Lactobacillus paracasei ATCC 25598, Lactobacillus helveticus ATCC 15009, Lactobacillus fermentum EMCC 1346, Bifidobacterium longum subsp. longum ATCC 15707 and Lactococcus lactis subsp. lactis ATCC 11454, including their antagonistic activity against some enteric and food borne-pathogens.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions**

Five lactic acid bacterial strains (LAB) were supplied from Microbiological Resources Center (Cairo MIRCE), Faculty of Agriculture, Ain Shams University; these strains were as follows: L. paracasei ATCC 25598, L. helveticus ATCC 15009, L. fermentum EMCC 1346, B. longum subsp. longum ATCC 15707, and L. lactis subsp. lactis ATCC 11454. Lactobacillus strains were cultured in De Man, Rogosa and Sharpe (MRS) broth with tween 80 (Biolife Italiana, Milano, Italia) anaerobically at 37°C, B. longum subsp. longum ATCC 15707 were cultured in tryptone soy (TS) broth (Lab M Limited, United Kingdom) anaerobically at 37°C, however, L. lactis subsp. lactis ATCC 11454 were cultured in Brain heart infusion (BHI) broth (Lab M Limited, United Kingdom) aerobically at 37°C. Also, standard pathogenic bacterial strains were supplied from the same previous center. These strains were E. coli ATCC 8739 and S. aureus ATCC 6538 and were cultured in Nutrient broth (Lab M Limited, United Kingdom) aerobically at 37°C.

Gram-negative enteric bacteria were isolated from patients suffering from acute gastroenteritis attending Minia University Hospital, Minia, Egypt. These isolates were identified according to standard laboratory procedures (Collee et al., 1996; MacFaddin, 2000). Five isolates of E. coli (E1, E2, E3, E4 and E5), one isolate of Y. enterocolitica (Y1), three isolates of Citrobacter koseri (C1, C2, C3) and one isolate of Shigella sonnei (Sh1) were used in this work.

**Starch, protein and lipid digesting capabilities**

The protein, lipid and starch digesting capabilities of Lactobacillus strains were evaluated using modified MRS agar containing 2.8% skimmed milk (HiMedia Laboratories Pvt. Ltd, India), 1% tributytrin (Sigma-Aldrich, St Louis, USA), and 0.2% soluble starch (Oxford lab, India), respectively. The overnight cultures of Lactobacillus strains (10 µl) were dropped on the modified MRS agar and incubated at 41°C for 24 h. The diameters of the halo zone on the agar plate were then measured. The digesting capability of the tested strains was classified as positive when the diameters of the

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clear zone were more than 1 mm. Each assay was performed in triplicate. The same test was performed for *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* using TS agar and BHI agar, respectively instead of MRS agar (Thongsom, 2004).

Sensitivity to bile salts and acidic pH

Resistance to bile salts was evaluated by the ability of *Lactobacillus* strains, *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* to grow on MRS agar, TS agar, and BHI agar, respectively, containing 2% (w/v) bile salt. The cultures were checked after 48 h incubation at 37°C. Resistance to acidic pH (pH 3.0) was analyzed by centrifuging the overnight cultures of the *Lactobacillus* strains at 6000 ×g for 15 min at 4°C and re-suspending the pellets in the same volume of 0.9% (w/v) NaCl, pH 3.0. The suspensions were then incubated at 37°C for 3 h. After incubation and culture centrifugation, the resulting pellets were plated onto MRS agar and incubated at 37°C for 48 h. Resistance to acidic pH (pH 3.0) was performed for *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* using TS agar and BHI agar, respectively instead of MRS agar (Nouri et al., 2010).

**Assay of LAB culture effect on the growth of the tested enteric pathogens**

Antibacterial activity of LAB was studied using the agar diffusion method (Makras and Vuyet, 2006). The indicator strains used in this study were Gram-negative enteric bacteria isolated from patients suffering from gastroenteritis. In addition, some standard bacterial species potentially pathogenic to humans, such as *S. aureus* and *E. coli* were used. Indicator strains were cultivated in nutrient broth at 41°C for 18 h. To evaluate the antibacterial activity, *Lactobacillus* strains were cultivated in MRS broth at 41°C for 18 h. However, TS broth and BHI broth were used for cultivation of *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* respectively. The culture containing 10 µl of LAB (10⁸ CFU/mL) was dropped on MRS agar for *Lactobacillus* strains, TS agar for *B. longum* subsp. *longum* and BHI agar for *L. lactis* subsp. *lactis* and incubated at 41°C for 18 h. The LAB on the agar plate were overlaid with 9 ml of soft nutrient agar with 1 ml of culture of indicator strains cultivated overnight (10⁶ CFU/ml). The agar plates were incubated at 41°C for 18 h and diameters of inhibition zones on the agar plate were measured. Each assay was performed in triplicate. The antibacterial activity was calculated as follows: antibacterial activity in mm = diameter of inhibition zone - diameter of LAB colony (Musikasang et al., 2009).

**Assay of cell-free culture supernatant (CFCS) of LAB on growth of the tested enteric pathogens**

Preparation of cell-free culture supernatants: Briefly, *Lactobacillus* strains were grown for 18 h at 37°C in 10 ml of MRS broth; however, TS broth and BHI broth were used for cultivation of *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis*, respectively. The culture containing 10⁸ CFU/ml was then centrifuged at 4000 ×g for 10 min. Then the supernatants were collected and sterilized by filtration using CHM® CA syringe filter of 0.20 µm pore size and the cell-free culture supernatants (CFCS) were stored in cryotubes at -20°C (Yu et al., 2013; Pehrson et al., 2015).

Effect of CFCS on growth of the tested pathogens: The antibacterial activity of LAB strains was investigated against indicator strains by a modified method of Wang et al. (2014) using cell-free culture supernatant (CFCS). Dried and sterilized filter paper discs (6 mm diameter) were then filled with known volume (10 µl per disc) of the CFCS using micropipette. Disc containing the test material were placed on the same agar plate overlaid with 9 ml of soft nutrient agar inoculated with 1 ml of culture of indicator strains cultivated overnight (10⁸ CFU/ml) mentioned earlier. After overnight incubation, the diameter of the inhibition zone around disc was measured. Sterilized MRS broth in case of *Lactobacillus* strains, BHI broth in case of *L. lactis* subsp. *lactis* and TS broth in case of *B. longum* subsp. *longum*, all having pH 7.0, were used as control to verify the presence of a possible inhibitory compound in the medium (for example sodium acetate, citrate) (Pehrson et al., 2015). Each assay was performed in triplicate.

**RESULTS AND DISCUSSION**

Starch, protein and lipid digesting capabilities

Of the tested LAB strains it was found that the three *Lactobacillus* strains exhibited both protein and starch digesting capability, but the lipid digestion was not encountered here. Some strains of LAB have the capability to digest protein, but they cannot digest starch or lipids (Musikasang et al., 2009). Other LAB could utilize protein, lipids and starch (Duangchitchareon, 2006; Kawai et al., 1999; Thongsom, 2004). However, *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* showed only protein digestion, LAB have developed a complex system of proteases and peptidases which enable them to utilize casein protein found in milk (Smid et al., 1991). Klaver et al. (1993) reported that *Bifidobacterium* strains were not as proteolytic as other LAB. This may explain why *Bifidobacterium* species grows slowly in milk and may require supplementation of peptides and amino acids from external sources (Dave and shah, 1998). LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids (Savijoki et al., 2006). In general, the exploitation of casein by LAB is initiated by a cell-envelope proteinase (CEP) that degrades the protein into oligopeptides that are subsequently taken up by the cells via specific peptide transport systems for further degradation into shorter peptides and amino acids by a concerted action of various intracellular peptidases (Kunji et al., 1996; Christensen et al., 1999). Although most LAB are unable to degrade starch because of the lack of the amylolytic activity, a few exhibit this activity and are qualified as amylolytic lactic acid bacteria (ALAB) which are able to decompose starchy material through the amylases production during the fermentation processes (Asoodeh et al., 2010). Most amylolytic LAB isolated belong to the *Lactobacillus* genus, while few studies reported the existence of amylolytic activity in some strains of *Bifidobacterium* isolated from the human large intestinal tract (Ji et al., 1992; Lee et al., 1997). Amylolytic LAB are mainly used in food fermentation, they are involved in cereal based fermented foods such as European sour rye.
bread, Asian salt bread, sour porridges, dumplings and non-alcoholic beverage production (Fossi and Tavera, 2013). Few of them are used for the production of lactic acid in single step fermentation of starch (Reddy et al., 2008).

**Sensitivity to bile salts and acidic pH**

In order to classify LAB as probiotic bacteria, they should be resistant to low pH environment of the stomach and bile salts of the intestinal tract to survive and grow in the GIT to exert their probiotic function effectively (Musikasang et al., 2009).

It was found that the three *Lactobacillus* strains used in the study were able to grow on the MRS agar containing 2% bile salts, and also resist the acidic pH (pH=3), similarly, *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* showed acceptable resistance to the effect of bile salts and acidic pH and remained viable as shown in Table 1. Resistance to bile salts is important for LAB to become able to colonize and be metabolically active in the small intestine of the host (Strompfova and Laukova, 2007). Bile resistance may be due to expression of bile-resistance related proteins in the bacterial cells (Hamon et al., 2011). In a previous work, *L. paracasei* was included in human pilot studies, as part of a multicentre European project named PROB-DEMO, to assess the ability of probiotics to survive intestinal transit and to examine their influence on the native microbiota of consumers. It was found that it has a proven ability to survive gastric transit and to persist in the colonic environment of humans (Crittenden et al., 2002).

Lankaputhra and Shah (1995) have reported that among nine strains of *Bifidobacterium* spp., *B. longum*, *B. pseudolongum* and *B. infantis* showed the best tolerance to bile salt (1 to 15%). Also, it was reported by Clark et al. (1993) that *B. longum* shows better survival in acidic conditions compared with other *Bifidobacterium* spp. (*Bifidobacterium infantis*, *Bifidobacterium adolescentis*, and *Bifidobacterium bifidum*).

**Effect of culture suspension and cell-free culture supernatant (CFCS) of LAB on the growth of the tested enteric pathogens**

The antibacterial activity of LAB may often be due to the production of organic acids, with a consequent reduction in pH, or to the production of hydrogen peroxide (González et al., 2007). LAB could produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin during lactic fermentations. Levels and types of organic acids produced during the fermentation process depended on LAB species or strains, culture compositions and growth conditions (Lindgren and Dobrogosz, 1990). Lactic acid is the major organic acid in LAB fermentation where it is in equilibrium with its un-dissociated and dissociated forms, and the extent of the dissociation depends on pH.

The antimicrobial effect of organic acids lies in the reduction of pH, as well as the un-dissociated form of the molecules. It has been proposed that the low external pH causes acidification of the cell cytoplasm, while the un-dissociated acid, being lipophilic, can diffuse passively across the membrane. The un-dissociated acid acts by collapsing the electrochemical proton gradient, or by altering the cell membrane permeability, which results in disruption of substrate transport systems (Ammor et al.,
In general, organic acids have a strong inhibitory activity against Gram negative bacteria (Makras and Vuyst, 2006). Hydrogen peroxide is produced by LAB in the presence of oxygen as a result of the action of flavoprotein oxidases or nicotinamide adenine dinucleotide (NADH) peroxidase. The antimicrobial effect of hydrogen peroxide may result from the oxidation of sulfhydryl groups causing denaturing of a number of enzymes, and from the peroxidation of membrane lipids, thus increasing membrane permeability (Condon, 1987; Kong and Davison, 1980). LAB strains were reported to inhibit the growth of pathogenic bacteria in many studies (Ammor et al., 2006; Bernbom et al., 2006; Collado et al., 2005; Olkowski et al., 2008; Santos et al., 2003). It may also be due to the production of bacteriocins or bacteriocin-like compounds (González et al., 2007). The bacteriocins from the generally recognized as safe (GRAS) LAB have generated a great deal of attention as a novel approach to control pathogens in food-stuffs (Savadogo et al., 2004).

The results of antagonistic effects of the Lactobacillus strains, B. longum subsp. longum and L. lactis subsp. lactis against 5 types of pathogenic strains are shown in Tables 2, 3, 4 and 5. The three Lactobacillus strains showed antagonistic effects against all pathogenic strains tested, but the degrees of antagonism varied among the Lactobacillus strains. L. paracasei and L. helveticus (culture and CFCS) exhibited strong inhibition on the growth of E. coli ATCC 8739 (Figure 1a and 1b), and the other tested strains followed by L. fermentum (Figure 1c), while B. longum subsp. longum and L. lactis subsp. lactis showed weak or no activity against the tested strains.

L. paracasei showed the highest activity against S. aureus ATCC 6538, Y. enterocolitica and S. sonnei followed by L. fermentum while B. longum subsp. longum showed no activity against S. aureus ATCC 6538, weak activity against Y. enterocolitica and good activity against S. sonnei. L. lactis subsp. lactis CFCS showed weak activity against the tested strains (Tables 3 and 4).

The tested Lactobacillus species (culture and CFCS) showed excellent activity against the tested C. koseri. B. longum subsp. longum culture exhibited weak activity against 2 strains of C. koseri, while CFCS of B. longum subsp. longum showed good activity against the tested strains. On the other hand, L. lactis subsp. lactis culture showed no activity against C. koseri but its cell-free extract showed good activity against 2 strains of the tested C. koseri strains (Table 5).

Many authors reported the production of bacteriocin-like compounds by different species of Lactobacilli that exhibit broad activities against Gram-positive and Gram-negative bacteria (Coconnier et al., 1997; Silva et al., 1987; Ocaña et al., 1999; Schillinger et al., 1996). The antibacterial activity of L. paracasei was reported by Bendali et al. (2011) as they found that L. paracasei showed high activity against Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus, S. aureus and Enterococcus faecalis. Also, in a study done by Ashokkumar et al. (2011), it was found that L. paracasei strain, isolated from donkey milk, showed a maximum activity against E. coli and optimum activity against S. aureus.

The results of this study show that B. longum showed activity lesser than that shown by the tested Lactobacillus spp. It showed activity against...
Table 3. Effect of culture suspension and cell-free culture supernatant (CFCS) of LAB on the growth of *S. aureus* ATCC 6538.

<table>
<thead>
<tr>
<th>LAB</th>
<th><em>S. aureus</em> ATCC 6538</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAB culture*</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>29±0.46</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>16±0.55</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>30±0.36</td>
</tr>
<tr>
<td><em>B. longum</em> subsp. <em>longum</em></td>
<td>-</td>
</tr>
<tr>
<td><em>L. lactis</em> subsp. <em>lactis</em></td>
<td>-</td>
</tr>
</tbody>
</table>

*Inhibition zones ± standard deviation (SD) in mm. **Culture supernatant was used for investigating antimicrobial activity. Diameters of inhibition zone are the mean of three replicates.+ diameter of inhibition zone < 2 mm, ++ diameter of inhibition zone between 2 and 7 mm, +++ diameter of inhibition zone between 8 and 12 mm, ++++ diameter of inhibition zone between 13 and 17 mm NE no effect detected.

Table 4. Effect of culture suspension and cell-free culture supernatant (CFCS) of LAB on the growth of *Yersinia enterocolitica* and *Shigella sonnei*.

<table>
<thead>
<tr>
<th>LAB</th>
<th><em>Yersinia enterocolitica</em></th>
<th><em>Shigella Sonnei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAB culture*</td>
<td>CFCS**</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>27±0.8</td>
<td>+++</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>21±0.82</td>
<td>+++</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>32±1.4</td>
<td>+++</td>
</tr>
<tr>
<td><em>B. longum</em> subsp. <em>longum</em></td>
<td>5±0.5</td>
<td>++</td>
</tr>
<tr>
<td><em>L. lactis</em> subsp. <em>lactis</em></td>
<td>-</td>
<td>NE</td>
</tr>
</tbody>
</table>

*Inhibition zones ± standard deviation (SD) in mm. **Culture supernatant was used for investigating antimicrobial activity. Diameters of inhibition zone are the mean of three replicates.+ diameter of inhibition zone < 2 mm, ++ diameter of inhibition zone between 2 and 7 mm, +++ diameter of inhibition zone between 8 and 12 mm, ++++ diameter of inhibition zone between 13 and 17 mm NE no effect detected.

Table 5. Effect of culture suspension and cell-free culture supernatant (CFCS) of LAB on the growth of *Citrobacter koseri*.

<table>
<thead>
<tr>
<th>LAB</th>
<th><em>Citrobacter koseri</em> (C1)</th>
<th><em>Citrobacter koseri</em> (C2)</th>
<th><em>Citrobacter koseri</em> (C3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAB culture*</td>
<td>CFCS**</td>
<td>LAB culture*</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>50±1.6</td>
<td>+++</td>
<td>19±0.66</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>32±0.93</td>
<td>++++</td>
<td>19±1.4</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>38±0.75</td>
<td>+++</td>
<td>29±1.2</td>
</tr>
<tr>
<td><em>B. longum</em> subsp. <em>longum</em></td>
<td>8±0.4</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>L. lactis</em> subsp. <em>lactis</em></td>
<td>-</td>
<td>NE</td>
<td>-</td>
</tr>
</tbody>
</table>

*Inhibition zones ± standard deviation (SD) in mm. **Culture supernatant was used for investigating antimicrobial activity. Diameters of inhibition zone are the mean of three replicates.+ diameter of inhibition zone < 2 mm, ++ diameter of inhibition zone between 2 and 7 mm, +++ diameter of inhibition zone between 8 and 12 mm, ++++ diameter of inhibition zone between 13 and 17 mm NE no effect detected.

*E. coli*, *Y. enterocolitica*, *S. sonnei* and *C. koseri*. Kailasapathy and Chin (2000) reported that the main therapeutic and health benefits of *L. acidophilus* and bifidobacteria are: (i) enhancement of immunity against intestinal infections, (ii) immune enhancement, (iii) prevention of diarrheal diseases, (iv) prevention of colon cancer, (v) prevention of hypercholesterolaemia, (vi) improvement in lactose utilization, (vii) prevention of
upper gastrointestinal tract diseases, and (viii) stabilization of the gut mucosal barrier. Lactobacillus acidophilus and bifidobacteria exert antagonistic effects on the growth of pathogens such as S. aureus, Salmonella typhimurium, Y. enterocolitica and Clostridium perfringens (Gilliland and Speck, 1977; Oezbas and Aytac, 1995), but in this study, B. longum subsp. longum showed no activity against S. aureus. In addition, B. longum (human origin) was found to stabilize the digestive system (Samona and Robinson, 1994).

L. helveticus is one of Lactobacillus strains that obtained from fermented foods and it was found to have technological importance and health promoting properties (Taverniti and Guglielmetti, 2012). L. helveticus can display efficient epithelium adhesion and pathogen inhibition in body sites other than the gut as, it was found that L. helveticus strain MIMLh5, isolated from Grana Padano natural whey starter, adheres deficiently to both, the human hypopharyngeal epithelial cell line Fa Duand HaCat keratinocytes, and inhibited the adhesion of Streptococcus pyogenes (the etiological agent of numerous diseases, including sore throat and acute rheumatic fever) better than the 10 probiotic and dairy lactic acid bacterial strains tested (Guglielmetti et al, 2010).

L. fermentum is one of the most important Lactobacillus species that have been identified in the gastric microbiota. Singh et al. (2013) reported that L. fermentum SBS001 of marine source showed maximum inhibitory activity to the human pathogens such as S. aureus, K. oxytoca, Pseudomonas aeruginosa and E. coli and minimum towards Salmonella paratyphi, Proteus mirabilis, Vibrio cholerae and K. pneumoniae.

Many studies reported that L. lactis has a probiotic

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**Figure 1.** Antimicrobial activity of the culture suspension and the cell-free culture supernatants (CFCS) of L. helveticus (a), L. paracasei (b) and L. fermentum (c) against E. coli strain.

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activity that agree with our results and has antagonistic activity against some food-borne pathogens as *Listeria monocytogenes* and *Enterococcus faecalis* (Furtado et al., 2014). Our results show that *L. lactis* subsp. *lactis* CFCS showed weak activity against the tested Gram-negative bacteria but no activity was shown against *S. aureus*. The antimicrobial activity of *L. lactis* DF04Mi against Gram-negative bacteria due to the production of bacteriocins was previously reported by Furtado et al. (2009).

*Lactobacillus* species in the human intestinal system act as a barrier to infection and contribute to the control of the enteric microbiota by competing with other microorganisms for adherence to epithelial cells and inhibiting the growth of potential pathogens. Hence, the use of probiotic strains of lactobacilli is potentially interesting both as preventive and curative agents.

Delivery of viable bifidobacteria in yogurt to the consumers remains a problem. Insufficient survival of *Bifidobacterium* spp. in commercial and experimental products has been reported by a number of authors. However, possible areas for improvement in enhancing the survival and viability of these organisms are in the selection and use of strains that are resistant to acids, bile, and oxygen and possess better in vivo colonizing ability such as *B. longum*.

Conclusion

Conclusively, it was found out that *Lactobacillus* strains showed the highest antagonistic activity against the tested enteric pathogens in comparison to *B. longum* and *L. lactis* and it was concluded that *L. paracasei*, *L. helveticus*, *L. fermentum*, *B. longum* subsp. *longum* and *L. lactis* subsp. *lactis* are good candidates for further in vivo studies to elucidate their potential health benefits to be used as promising probiotic bacteria.

Conflict of interest

The authors have not declared any conflict of interest.

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