Isolation of potential probiotics obtained from fermented foods and human gastrointestinal tract products.
Rangel Ramos D., Vazquez Bucheli J., Arellano Ayala K., Lim J., Ji, Y., Holzapfel, W.
Graduate School of Advanced Green Energy and Environment, Handong Global University, Pohang, Gyungbuk 791-708, South Korea. Tel +82-10-4333-2672. Correo: cris88_27poison

Keywords: (Lactic acid bacteria, Lactobacillus spp., probiotics).

Introduction
Fermented food is one of the most promising sources of functional probiotics. The term probiotic can be defined as "a nutritional supplement of living microbes that beneficially affects the host by reestablishing its microbial balance." [1] Probiotics contribute on the preservation of food through the fermentation process by inhibiting the growth of pathogens, in addition to improving the balance of the host gut microbiota [1].

Probiotics contribute to quality, safety and positive sensory flavor characteristics of the foods [2]. In general, lactic acid bacteria (LAB) are Gram-positive, non-sporulating, anaerobic, microaerophilic, oxidase and catalase-negative coccis or rods that produce either lactic acid or lactic acid plus acetic acid and CO₂ as major product(s) of carbohydrate fermentation [1,2].

In this study 72 different fermented foods, insects, plants and human feces were used to isolate potential probiotics. Among the lactic acid bacteria 3 different genera (Enterococcus, Streptococcus, and Lactobacillus) were represented, in addition to Bifidobacterium spp.

The isolations were made with the objective of knowing the variety of probable probiotics present in samples of diverse origin to establish a database that serves as a reference to researchers for the selection and elaboration of products for human consumption at a commercial level.

Methodology
The 72 samples of multiple foods, plants and specimens of the gastrointestinal tract originated from different parts of the world, were collected and stored on -20°C. They include dairy products, pickles, fermentis, leaves, fruits, insects and stool samples.

1gr of each sample was taken and suspended 9:1 in phosphate buffered saline solution (PBS), and serial dilutions were made till reaching the concentration of 10⁻⁶; 100μl were taken from this last dilution and the plate surface seeding technique was used to extend the sample’s dilution on MRS agar to grow bacteria under aerobic conditions and on BL agar to grow bacteria under anaerobic conditions; the samples were incubated at 37 ° C for 24 h.

Four varieties of colonies were selected from each sample, they were isolated two more times on MRS agar or on BL agar using the streaking technique, and incubated at 37 ° C for 24 hours for each pass; later the microorganisms were seeded in 5 ml of MRS medium or BL medium (according to their requirement) in a test tube with reversed Durham tube and incubated for growth at 37 ° C for 24 h.

The bacterial strains were identified according to their type of fermentation and safety studies were performed, which included biogenic amino tests, hemolysis, catalase and microscopic analysis to characterize each bacterium morphologically. (Table 1).

Each one of the samples was subcultured using MRS or BL agar and by streaking for subsequent 16S rRNA sequencing. In order to identify each bacterium, the Solgent Sanger type sequencing service was used.
The selected strains were added to the culture collection in cryotubes and were identified and labeled numerically. Each sample was homogenized, 900 μl of the culture were taken (BL or MRS), incorporated with 500 μl of Glycerol 30% into 4 different cryotubes, each one with 1,400 μl in total and finally they were stored at -80 °C.

**Results and Discussion**

Of the total of 72 samples, 3 genera of lactic acid bacteria were identified, including 39 species (Streptococcus, Enterococcus, and Lactobacillus) and Bifidobacterium as the one genus of the Bifidobacteriaceae representing 7 species (spp.). (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Variety and proportions of bacterial isolates obtained from samples of diverse origin.

In the isolation of the samples from the gastrointestinal tract (Figure 1), the genera Streptococcus and Enterococcus were predominant; from the first one, 5 species were identified and from the second one 7 species (Figure 2).

![Streptococcus spp.](image2a.png)  
**Figure 2.** Prevalence of bacterial species in various samples. **Streptococcus spp.** (a); **Enterococcus spp.** (b)

Streptococcus lutetiensis was the species with the highest frequency among the streptococci encompassing 66.66%, while Enterococcus faecalis was the most frequently isolated among the enterococci (48.44%). (Figure 2)
Despite being part of the order Lactobacillales some strains (and particularly some enterococci) are grouped as Type 2 Biosafety level bacteria, that is, they are potential pathogens (considered to be moderate health hazard agents); some strains may be mainly found in baby feces. Therefore, there may be a probability that some of the babies from whom the samples were obtained might have been suffering from an underlying gastrointestinal infection causing dysbiosis [3, 4]. The genus Lactobacillus was the most representative among the samples, with 27 species and a presence of 57.45%. Lactobacillus plantarum was the most predominant species (Figure 3) with a percentage of 46.13%; this is possibly due to the great resistance of this bacterium to stress conditions in gastrointestinal tract and food, both osmotic and oxidative and its adaptability to different temperature gradients and the environment [5,6].

From the Bifidobacterium genus 7 species were isolated from mainly gastrointestinal tract samples. Bifidobacterium longum was isolated with the highest frequency (40%). It is a saprophytic bacterium typical of the microbiota of babies and infants, explaining why it was found in a greater proportion, since the obtained gastrointestinal tract samples were almost entirely baby feces (Figure 4) [1,7].

**Figure 3.** Prevalence of species of the genus Lactobacillus in samples of different origin (a); Proportion of isolated bacterial genera (b).

**Figure 4.** Prevalence of species of the genus Bifidobacterium in samples from different origin.
Conclusions
Of the 72 samples that were collected from different parts of the world, a total of 487 bacteria were isolated and Lactobacillus was the most representative genus of the study due to its ability to adapt to different environments, in particular the L. plantarum species.
Some of these isolated bacterial strains have demonstrated to be of importance in the treatment of gastrointestinal disorders and metabolic diseases [8]; thus, this collection of isolates serves as a reference for future research projects and further functional studies using animal models.

Table 1. Safety tests results.

<table>
<thead>
<tr>
<th></th>
<th>Hemolysis positive</th>
<th>Hemolysis negative</th>
<th>Lysine positive</th>
<th>Ornithine positive</th>
<th>Histidine positive</th>
<th>Tyrosine positive</th>
<th>Catalase negative</th>
<th>Catalase positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.</td>
<td>1</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>22</td>
<td>42</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>40</td>
<td>64</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>84</td>
<td>239</td>
<td>-</td>
<td>10</td>
<td>2</td>
<td>12</td>
<td>323</td>
<td>-</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>12</td>
<td>58</td>
<td>6</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
</tbody>
</table>

Bibliography