In vitro evaluation of the probiotic potential of microorganisms isolated from functional commercial fermented milks

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Abstract

Fermented milk is one of the carriers of probiotics, which is a Greek derived term meaning “pro-life”. Probiotics are live microorganisms that, when administered in adequate and daily amounts, confer benefits to host’s health. In this work, four in vitro tests were performed with six lactobacilli samples from commercial fermented milks: sensitivity test to gastric pH, sensitivity test to intestinal bile salts, antagonism test against pathogens and susceptibility test to antimicrobials. The tested samples were resistant to gastric pH (2.0) and some suffered growth inhibition in the sensitivity tests to bile salts (0.3%), in two different methods, reducing growth in about 45% or 1 Log₁₀ reduction. All pathogenic bacteria tested (Escherichia coli, Salmonella enterica serovar Typhimurium and Staphylococcus aureus) were antagonized by the supernatant (in MRS) of Lactobacillus spp. isolated from commercial fermented milks as well as in spot on the lawn test. Regarding antimicrobial susceptibility, samples presented a diverse profile, with prevalence of sensitivity to antimicrobials of clinical relevance, although there was high resistance profile regarding cephalosporins. In view of the in vitro tests performed, samples showed a satisfactory probiotic potential, as expected. However, some samples showed a slightly superior performance, such as Lactobacillus casei Shirota from Yakult, L. casei Defensis from Actimel and L. paracasei ST11 from Chamyto.

Keywords: Antagonism. Lactobacillus. Resistance to gastrointestinal transit.

Avaliação in vitro do potencial probiótico de micro-organismos isolados de leites fermentados funcionais comerciais

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Introduction

Concerned about a healthier life, more and more people have been looking for better life habits, associating physical exercise with a balanced diet (Berti et al., 2017). Knowing this, food industry, aware of consumer preferences, has raised its concern over healthy and functional food and beverage market (Granato et al., 2010). In this context, fermented milk or fermented dairy product stand out as an attractive vehicle for probiotic cultures (Barat; Ozcan, 2017).

The International Dairy Federation defines fermented dairy product as a dairy product prepared from skimmed or non-skimmed milk with specific cultures (Panesar, 2011). The main bacteria used in its production are usually lactobacilli, being responsible for the sensory characteristics of the product and the reduction of its pH. Addition of probiotic microorganisms in the product reinforces its functional properties by increasing its nutritional and therapeutic value, also resulting in the improvement of its sensory characteristics (Sharifi-Rad et al., 2020).

Probiotics have its’ beneficial properties only when administered in adequate amounts and in viable conditions (FAO/WHO, 2002). So, a minimum viable quantity for probiotics was established, ranging from 10^6 and 10^8 Colony Forming Units (CFU) in the daily recommendation of the product (ready for consumption). In addition, for a product to be classified as a probiotic, used culture must have resistance to bile salts and gastric acidity confirmed by laboratory tests, according to Brazilian legislation (Brasil, 2018). Resistance to gastric and intestinal injuries is of great importance in probiotic selection, since a minimum of 10^6 CFU is required for a probiotic sample to exert is possible beneficial effects. If not resistant, bacterial count may reduce drastically and probiotic effect will not occur (Vinderola; Reinheimer, 2003).

The objective of this work was to evaluate the in vitro functional features of microorganisms isolated from commercial functional fermented milks. For this, in vitro sensitivity of microorganisms to gastric juice and bile salts was evaluated, as well as antagonistic activity of microorganisms against pathogenic bacteria and susceptibility to antimicrobials.

Material and Methods

Commercial functional fermented milks found in retail chain of Minas Gerais were evaluated, namely: Actimel from Danone (Danone, Paris, France), Batavinho from Batavo (Batavo, Carambeí, Brazil), Chamyto from Nestlé (Nestlé, Vevey, Switzerland), Itambé from Itambé (Itambé Alimentos, Belo Horizonte, Brazil), Vigor from Vigor Alimentos, São Paulo, Brazil), and Yakult from Yakult (Yakult, Tokyo, Japan), from which single species of probiotic Lactobacillus sp. were isolated after counter, which is described below. All products were bought within expiry date, under refrigeration (<7°C) and were maintained at this temperature until experimentation.

Count of viable microorganisms was carried out from six different samples of commercial fermented milk. Initially, a serial dilution of up to 10^5 of 0.1mL of each sample was made in 0.9mL of sterile peptone saline (0.9% NaCl, 0.1% peptone). Afterwards, 0.1mL of the solution obtained from each dilution was plated with the aid of the Drigalski’s loop. Solution was then distributed over the surface of petri dishes containing MRS (de Man Rogosa and Sharpe - MRS) agar (Difco, Detroit, USA). Plates were incubated aerobically for 24 hours at 37°C. Colonies with different morphotypes were subjected to Gram staining and catalase tests, in order to confirm it as lactobacilli.

Antibiogram was performed according to the technique of antimicrobial susceptibility by the principle

Resumo

O leite fermentado é um dos veiculadores dos probióticos, que é um termo derivado do grego que significa “a favor da vida”. Probióticos são microrganismos vivos, que quando administrados em quantidades adequadas e diárias, conferem benefícios à saúde do hospedeiro. Neste trabalho foram realizados quatro testes in vitro com amostras de lactobacilos de seis leites fermentados comerciais: teste de sensibilidade ao pH gástrico, teste de sensibilidade aos sais biliares intestinais, teste de antagonismo contra agentes patogênicos e teste de suscetibilidade aos antimicrobianos. As amostras testadas foram resistentes ao pH gástrico (2.0) e algumas sofreram inibição do crescimento nos testes de sensibilidade aos sais biliares (0,3%), em dois tipos de teste in vitro diferentes, com redução de aproximadamente 45% no crescimento ou cerca da 1 Log10. Todas as bactérias patogênicas testadas (Escherichia coli, Salmonella enterica sorovar Typhimurium e Staphylococcus aureus) foram antagonizadas pelo sobrenadante (em MRS) dos Lactobacillus spp. isolados de leites fermentados comerciais assim como em teste de antagonismo to tipo spot on the lawn. Quanto à suscetibilidade aos antimicrobianos, as amostras apresentaram perfil diversificado com predominância para a sensibilidade a antimicrobianos de importância clínica, apesar do elevado perfil de resistência às cefalosporinas observado. Frente aos testes in vitro realizados, as amostras apresentaram potencial probiótico satisfatório.

of drug diffusion in disc. Discs with pre-defined drug concentrations were used and the diameters of the halos formed by inhibition were measured following the one proposed by Charteris et al. (1998a).

Each microorganism was transferred to a test tube containing 3.5ml of 0.9% saline until the concentration equivalent to the 0.5 McFarland scale (corresponding to an estimated population of 10^8 CFU/mL). Culture was then spread on a pizza-type Petri dish (14cm diameter) with a sterile swab until the entire surface containing MRS agar (Difco) was covered. Then, in an equidistant manner, 10 disks (Labordrin, Pinhalis, Brasil) containing antimicrobials from different chemical groups were distributed. Drugs belonging to the following chemical groups were used:

- Cell wall synthesis inhibitors, with bactericidal action, such as beta-lactams: penicillin (PEN, 10U), ampicillin (AMP, 10μg). Glycopeptides: such as vancomycin (VA, 30μg).
- Third generation cephalosporins: ceftriaxone (CRO, 30μg), cefoxitin (CFO, 30μg).
- Protein synthesis inhibitors, with bacteriostatic action, such as tetracyclines: tetracycline (TE, 30μg); as well as aminoglycosides: gentamicin (GEN, 10μg), chloramphenicol (CLO, 30μg) and streptomycin (EST, 30μg).

After the distribution of disks, Petri dishes were incubated in aerobiosis at 37°C for 48 hours. For quality control of disks containing antimicrobials, a sample of Escherichia coli ATCC 25922 was used. Finally, diameters of the inhibition halos were read using a Mitutoyo digimatic caliper (Mitutoyo). This test was performed in duplicate - the same revealing microorganism applied to two plates with the same isolated microorganism tested (spot) - with two replicates.

Second method: in vitro antagonism methodology by Acucio et al. (2017) was used, with adaptations. Initially, microorganisms isolated from the six samples of fermented milk were grown in BHI broth (Difco) at a temperature of 37°C for 24 hours, under aerobic conditions. Then, 1mL of each probiotic sample was pipetted into three different microtubes. Microtubes were centrifuged at 5.000g for 5 minutes and, supernatants were obtained. The pH of each supernatant was measured and its microfiltration was carried out, in order to ensure the exclusive action of culture supernatants.

The reference microorganisms (Escherichia coli ATCC 25922, Salmonella enterica serovar Typhimurium ATCC 14028 and Staphylococcus aureus ATCC 29213) were inoculated in BHI broth (Brain Heart Infusion) and subsequently incubated at 37°C for 24 hours. Afterwards, in three microtubes of each pathogenic sample, 4% (v/v) solutions were prepared containing 960μl of BHI broth (Difco) added, individually with 40μl of one of the pathogenic microorganisms. Then, 100μl of the probiotic supernatant was transferred to an ELISA plate with 96 wells and another 100μl of the solutions described above. The plate was then incubated in a spectrophotometer (Microplate Spectrophotometer System SpectraMax 340 - Molecular Devices, San Jose, USA) for 12 hours at 37°C. The OD 620nm reading at every 30 minutes determined the absorbance of the culture.

To calculate the percentage of growth inhibition, the GraphPad Prism 6.01 (GraphPad, San Diego, USA) program was used. The formula (1-AT/ CT) x 100, with AT corresponding to the area under the growth curve of the revealing pathogenic microorganism in probiotic supernatant and CT correspond to the area under the growth curve of the control pathogenic microorganism, was adapted form Andrade et al. (2014). The control consisted of adding the solutions of pathogenic samples in pure MRS broth (Difco), in which there was no previous
growth of any probiotic microorganism. The tests were performed in triplicate, with two repetitions.

Evaluation of the sensitivity or resistance of microorganisms to gastric pH and intestinal bile salts were also carried out in two different ways.

First technique (gastric simulation): To measure the sensitivity to gastric pH, the technique described by Santos et al. (2016) was primarily used. The isolated microorganisms were activated in MRS broth (Difco) and subsequently incubated for 48 hours at 37°C under aerobic conditions. In order to define the control growth of each of the lactobacilli samples, the microorganisms were plated prior to the test in the serial dilutions at 10^4 and 10^6 on MRS agar (Difco) (incubation at 37°C, for 48 hours, aerobically). Later, 1ml of each sample was centrifuged in 2ml microtubes at 5.000g for 5 minutes. The obtained supernatant was discarded and the samples were exposed to 1 ml of 0.9% saline solution (pH 2.0, 0.3 w/v of pepsin), for one hour. After the determined time, a plating based on serial dilutions at 10^4 and 10^6 on MRS agar (Difco) was performed, with the purpose of evaluating the viability of the cells. Finally, the plates were incubated at 37°C for 48 hours, aerobically. The results obtained were compared with the results of the plating done before the test to observe the behavior of the microorganisms studied in an artificial stomach environment.

Second technique (gastric simulation): Adaptations were performed over methodology proposed by Acurcio et al. (2014). First, the isolated microorganisms were activated in MRS broth (Difco) and incubated for 24 hours at 37°C, under aerobic conditions. After incubation, 1ml of each sample of the activated microorganisms was added in two different microtubes that were centrifuged at 5.000g for 5 minutes. The supernatants formed after centrifugation were discarded and the samples were exposed to 1 ml of 0.9% saline solution (pH 2.0, 0.3 w/v of pepsin) or 7.0 (control). After that, the samples were incubated at 37°C for one hour and centrifuged again under the same conditions mentioned above. After centrifugation, the pellet was suspended in MRS (Difco). 2% (v/v) inoculum was also prepared in MRS broth (Difco). The sample was then distributed in a 96-well ELISA plate, with 200μl of each sample after passage through pH 2.0 (gastric pH) and pH 7.0 (control).

The plate was incubated in a spectrophotometer (Microplate Spectrophotometer System SpectraMax 340 - Molecular Devices) at 37°C for 12 hours and the absorbance of the culture determined by reading in OD 620nm every 30 minutes. The percentage of growth inhibition was calculated using the GraphPad Prism 6.01 program (GraphPad Software) as a tool using the formula (1-SG / CT) x 100, with SG corresponding to the area under the growth curve of bacteria treated with artificial gastric juice and CT, the control. The tests were performed in triplicate, with two repetitions.

First technique (intestinal bile salts): To measure sensitivity to bile salts, the technique described by Santos et al. (2016) was adapted. The isolated microorganisms were activated in MRS broth (Difco) and incubated at 37°C under anaerobiosis for 48 hours. Previously to the assay, the microorganisms were plated at serial decimal dilutions at 10^4 and 10^6 on MRS agar (Difco) in order to define the control growth of each of the lactobacilli samples (incubation at 37°C, for 48 hours, aerobically). Subsequently, 1ml of each sample was centrifuged in 2ml microtubes at 5.000g for 5 minutes. The supernatant formed was discarded and the samples exposed to 1ml of MRS broth solution (Difco) containing 0.3% (w/v) of bile salts (Oxgall, Difco) for six hours. At the end of the six hours, plating was done from decimal dilutions serialized at 10^4 and 10^6 on MRS agar (Difco) in order to assess the viability of the cells. Finally, plates were incubated at 37°C for 48 hours in anaerobiosis. The results found were compared with the plating results made prior to the test to observe the behavior of the microorganisms studied in an artificial intestinal environment.

Second technique (intestinal bile salts): To assess the resistance of microorganisms isolated from fermented milk to bile salts, the tests were based on the methodology described by Acurcio et al. (2017). First, the isolated microorganisms were activated in MRS broth (Difco) and incubated in an aerobic condition at 37°C for 24 hours. After incubation, the activated microorganisms were placed in microtubes at a 4% (v/v) dilution in MRS broth (Difco). Then, 100μL were transferred to one well on the 96-well ELISA plate containing 100μl of pure MRS broth (Difco) and another 100μL were transferred to another well on the same plate containing MRS broth (Difco) with 0.6% (w/v) of bile salts (Oxgall). To determine the absorbance of the culture, the reading of OD 620nm was performed, every 30 minutes for 12 hours.

In the end, the inoculum of samples was 2% and the concentration of bile salts 0.3% in the wells, simulating, in vitro, intestinal environment. To calculate the percentage of growth inhibition, the program GraphPad Prism 6.01 (GraphPad Software) was used, which carried out the determination by the formula (1-SB / CT) x 100, with SB corresponding to the area under the growth curve of the control bacteria treated with bile salts and CT, the control. The tests were performed in triplicate, with two repetitions.

Results and discussion

Regarding microorganisms’ count (Table 1) it was possible to observe if counts were between 10⁴ to 10⁹ CFU/ml. According to Vinderola and Reinheimer (2003), recommend dose would be approximately 10⁸ CFU per dose. Smaller values are accepted, once their effectiveness is proven (Minelli and Benini, 2008). Considering the approximate consumption of 100g of product, the product with the lowest count would be able to achieve at least the necessary count for its functional claim.
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Table 1 – Average count in Log_{10} (CFU/mL on MRS agar) of lactobacilli isolated from functional fermented milks

<table>
<thead>
<tr>
<th>Sample</th>
<th>MRS count in Log_{10} (CFU/mL) ± SD *</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA – L. casei Shirota (Yakult)</td>
<td>8,78±0,02</td>
</tr>
<tr>
<td>AC – L. casei Defensis (Actimel)</td>
<td>8,60±0,01</td>
</tr>
<tr>
<td>CH – L. paracasei ST11 (Chamyto)</td>
<td>8,40±0,07</td>
</tr>
<tr>
<td>IT – L. casei (Itambé)</td>
<td>7,28±0,02</td>
</tr>
<tr>
<td>VI – L. casei (Vigor)</td>
<td>6,34±0,02</td>
</tr>
<tr>
<td>BA – L. acidophilus (Batavo)</td>
<td>5,96±0,04</td>
</tr>
</tbody>
</table>

* SD = Standard Deviation.

According to Forssten and Ouwehand (2020), cell viability is the fundamental test for assessing the effectiveness of lactic acid bacteria as probiotics. Probiotic bacteria must be able to withstand stress from gastrointestinal tract, such as gastric pH and bile salts in the small intestine. These bacteria need to resist such injuries to be able to exercise their therapeutic benefits.

Regarding in vitro resistance test to artificial gastric juice, it was possible to observe that all samples (Table 2) were able to survive artificial gastric juice (pH 2.0). Similar results were found by Hoque et al. (2010), where all isolated and evaluated microorganisms were able to survive at pH 2.2. Costa et al. (2013), in a similar study, found a low percentage of inhibition to artificial gastric juice and a high percentage of inhibition to bile salts, pointing the satisfactory results of this study. Maragkoudakis et al. (2006) also observed that, within two hours, with pepsin, artificial gastric juice did not have a great effect on the count of probiotic microorganisms in commercial functional fermented milks.

Table 2 – Percentage (%) of in vitro inhibition of gastric juice (pH 2.0) and bile salts (0.3% Oxgall) of lactobacilli isolated from functional fermented milks

<table>
<thead>
<tr>
<th>Samples</th>
<th>In vitro inhibition (functional test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric juice (%)</td>
</tr>
<tr>
<td>YA</td>
<td>No inhibition</td>
</tr>
<tr>
<td>AC</td>
<td>No inhibition</td>
</tr>
<tr>
<td>CH</td>
<td>No inhibition</td>
</tr>
<tr>
<td>IT</td>
<td>No inhibition</td>
</tr>
<tr>
<td>VI</td>
<td>No inhibition</td>
</tr>
<tr>
<td>BA</td>
<td>No inhibition</td>
</tr>
</tbody>
</table>

Table 2 showed, when tested by turbidimetry in spectrophotometry, something around inhibition of about 40% of the growth of lactobacilli with probiotic claim isolated from fermented milks. In the plating before and after exposure to bile salts at 0.3%, we can see an even more encouraging result, with a low reduction in counts (average of 13% reduction – Table 3). With regard to artificial gastric juice (pH 2.0), there was also good resistance to this TGI challenge (average of 8% reduction – Table 3). Silva et al. (2013) found higher percentages of inhibition from bile salts of lactobacilli candidates. Costa et al. (2013), in turn, observed in samples of lactic acid bacteria evaluated as to their potential, mostly, sensitivity to bile salts. This reinforces the individuality of the probiotic potential of the sample, despite the similarity of the tested species. Caillard and Lapointe (2017) found samples of Lactobacillus spp. with probiotic potential tolerant and not tolerant to gastric acid (pH 2.0).

The ability of certain samples of lactic acid bacteria, such as Lactobacillus casei, to survive in acidic environments is extremely important for the functionality of these microorganisms during bioprocessing. Acid adaptation experiments with L. casei ATCC 334 demonstrated that the induction of acid tolerance response can be triggered by transient exposure to various sub-lethal pH values. When faced with acid stress, bacteria can act to counter the influx of protons, increasing the rigidity and compactness of the cytoplasmic membrane (Broadbent et al., 2010).
Table 3 – Percent inhibition (CFU/mL and %) of six samples of lactic acid bacteria isolated from commercial fermented milks, in gastric pH (2.0) and bile salts (0.3% Oxgall)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Count (CFU/mL) - Percentage reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>YA</td>
<td>3.95x10⁹</td>
</tr>
<tr>
<td>AC</td>
<td>6.00x10⁹</td>
</tr>
<tr>
<td>CH</td>
<td>6.55x10⁹</td>
</tr>
<tr>
<td>IT</td>
<td>5.55x10⁹</td>
</tr>
<tr>
<td>VI</td>
<td>5.30x10⁹</td>
</tr>
<tr>
<td>BA</td>
<td>5.05x10⁹</td>
</tr>
</tbody>
</table>

Another factor that has an important effect on the gastric tolerance of some samples is the presence of milk proteins, alone and/or in combination. Studies have shown that L. casei 212.3 was able to survive gastric juice in the presence of sodium caseinate, protein whey and a combination of these (Charteris et al., 1998b).

The in vitro antimicrobial susceptibility test performed on some lactic acid bacteria (Table 4) showed that all microorganisms were resistant to three antimicrobials (ceftriaxone, gentamicin and vancomycin) and sensitive to five others (ampicillin, ciprofloxacin, chloramphenicol, penicillin and tetracycline). It is desirable that a probiotic candidate is sensible to all antimicrobials, in order to avoid genetic transference do pathogenic microorganisms. However, presence of resistance, if acquired (not intrinsic) brings concern, as it may be involved in transference of resistance of antimicrobials of clinical use, such as cephalosporins, to gut pathobiont bacteria (Sharma et al., 2014). For the other antimicrobials tested (streptomycin and cefoxitin), there was a diverse susceptibility profile. Divergent results were observed by Cebeci and Gurakam (2003), in which only 27% of the evaluated Lactobacillus plantarum samples were resistant to gentamicin. Costa et al. (2013) revealed that vancomycin resistance occurred in all samples of Lactobacillus spp. tested, which was also observed in other studies (Anisimova; Yarrulina, 2019; Guo et al., 2017), corroborating the statement that lactobacilli can have intrinsic resistance to vancomycin (Teuber et al., 1999).

Table 4 – Antimicrobial susceptibility profile of six samples of lactic acid bacteria isolated from commercial fermented milks

<table>
<thead>
<tr>
<th>Samples</th>
<th>TET</th>
<th>AMP</th>
<th>EST</th>
<th>CRO</th>
<th>CLO</th>
<th>CIP</th>
<th>CFO</th>
<th>GEN</th>
<th>VAN</th>
<th>PEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>AC</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>CH</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>IT</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>VI</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>M</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>BA</td>
<td>S</td>
<td>S</td>
<td>M</td>
<td>M</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

TET = tetracycline, AMP = ampicillin, EST = streptomycin, CRO = ceftriaxone, CLO = chloramphenicol, CIP = ciprofloxacin, CFO = cefoxitin, GEN = gentamicin, VAN = vancomycin, PEN = penicillin. S = sensitive, M = moderately sensitive, R = resistant.

The in vitro antagonism test (from supernatant) (Table 5) showed antagonistic activity of all samples evaluated against the three pathogenic bacteria tested (Escherichia coli, Salmonella Typhimurium and Staphylococcus aureus). In sample BA there was a higher percentage of antagonism (77.21%) against E. coli, while sample YA showed a high percentage of inhibition (86.65%) against Salmonella Typhimurium. In turn, samples CH and VI showed 72.85 and 72.66% inhibition against S. aureus, respectively. Results by Silva et al. (2013) also showed strong antagonistic activity against seven pathogenic bacteria tested (which included pathogenic samples of the species selected here), as well as those of Maragkoudakis et al. (2006), who observed the inhibition of certain probiotic samples of Lactobacillus spp., as well as pathogenic E. coli and S. Typhimurium.
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Table 5 – Percentage (%) of antagonism (inhibition) by the supernatant (in MRS) of lactobacilli isolated from functional fermented milks against relevant pathogenic microorganisms

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH ± SD*</th>
<th>E. coli</th>
<th>S. Typhimurium</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA</td>
<td>3.96±0,10</td>
<td>76,88</td>
<td>86,65</td>
<td>71,92</td>
</tr>
<tr>
<td>AC</td>
<td>4.08±0,06</td>
<td>70,72</td>
<td>82,74</td>
<td>70,16</td>
</tr>
<tr>
<td>CH</td>
<td>4,00±0,09</td>
<td>73,35</td>
<td>83,72</td>
<td>72,85</td>
</tr>
<tr>
<td>IT</td>
<td>4,04±0,07</td>
<td>69,50</td>
<td>82,27</td>
<td>56,85</td>
</tr>
<tr>
<td>VI</td>
<td>3,96±0,08</td>
<td>72,08</td>
<td>84,15</td>
<td>72,66</td>
</tr>
<tr>
<td>BA</td>
<td>4,03±0,11</td>
<td>77,21</td>
<td>81,72</td>
<td>70,82</td>
</tr>
</tbody>
</table>

*Inhibition values are represented as a percentage (%). SD = Standard Deviation.

Some strains of lactobacilli are capable of producing potent antimicrobial compounds, such as bacteriocins, antimicrobial peptides and organic acids, such as lactic acid (Mohanty; Saini; Mohapatra, 2017), which can inhibit bacteria with a deteriorating and/or pathogenic character, including those from the following genera: Salmonella, Escherichia, Pseudomonas and Staphylococcus (Castro et al., 2011).

The in vitro antagonistic effect, by the spot on the lawn technique, of samples of lactic acid bacteria against pathogenic microorganisms (Escherichia coli, Staphylococcus aureus and Salmonella Typhimurium) was also measured, showing that all tested samples were able to produce halos of inhibition against pathogens (Graph 1).

Average of inhibition halos for each pathogenic bacteria were more expressive over Staphylococcus aureus and Escherichia coli. Salmonella Typhimurium, in turn, showed a quantitative lower inhibition by all probiotic tested samples, which is not necessarily a sign for lower effectiveness. Slightly different results were found by Costa et al. (2013), in which the lowest quantitative inhibition against pathogenic microorganisms occurred against S. aureus. Guedes Neto et al. (2005), in turn, found a diversified response on the part of the lactobacilli prospecting regarding its probiotic characteristics. These data reinforce that, in the context of probiotic potential against the inhibition of pathogens, lactobacilli tested in vitro in the present study were able to inhibit pathogenic microorganisms of relevance to food and diseases associated with food, especially those of animal origin.

Conclusion

Microorganisms carried by commercial fermented milks confirmed, as expected, a favorable probiotic potential. Tested samples presented outstanding resistance do in vitro gastric environment and great resistance to biliary salts in vitro challenge (average of 40% of inhibition). Intestinal injury challenge did not reduce more than one Log_{10} of probiotic population, which is desirable. Samples performed remarkable in vitro antagonism against pathogenic tested strains. Resistance to cephalosporins is a concern since they are probably not intrinsic, with the risk of horizontal transmission to pathobiont microorganisms. So, probiotic features of commercial strains performed accordingly, with a discrete quantitative superiority of samples from Actimel, Chamyto and Yakult.
Graph 1 – Average results of *in vitro* antagonism tests (mm of inhibition halo) of probiotic lactic acid bacteria against pathogenic microorganisms

**Graph**

EC = *Escherichia coli*; SA = *Staphylococcus aureus*; ST = *Salmonella Typhimurium*. Bars represent standard deviation. Sample 1: BA, Sample 2: YA, Sample 3: VI, Sample 4: CH, Sample 5: IT, Sample 6: AC.

**References**


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