ABSTRACT: The capability of different probiotic strains of Lactobacillus spp. (L. acidophilus, L. bulgaricus, L. paraplantarum, L. plantarum, L. pentosus and L. rhamnosus) to ferment donkey milk was examined in order to investigate the possibility of formulating a probiotic milk beverage. Donkey milk was inoculated with different strains of Lactobacillus for 48 h. Microbial growth and fermentative aptitude was monitored as well as the microbial capability to produce short chain organic acids (SCOAs). All the strains were capable to grow in donkey milk, as demonstrated by the high microbial count (> 9 log colony forming units/ml) and low pH values. Chromatographic analysis evidenced that the fermented milk exhibited SCOAs other than lactic acid, ranging from 1.41 mmol/L of succinic acid (in milk fermented by L. paraplantarum) to 22.96 mmol/L of acetic acid (in product fermented by L. acidophilus). Butyric acid was present in all products. The possibility of using donkey milk as a growth medium for several probiotic strains, thereby giving rise to the production of a probiotic beverage with healthy SCOAs, can lead the way to a vast number of potential uses for this milk.

KEY WORDS: Donkey Milk, Fermented Food, Lactobacillus, Probiotic

INTRODUCTION

In recent years, the interest in donkey milk has considerably increased, mainly due to its composition. It may be considered for infant nutrition as a valid alternative to synthetic powdered milks, soybean milk or other formulas because its composition in poly-unsaturated fatty acids, optimal calcium/phosphorous ratio and protein content makes it more similar to human milk than are these current alternatives. In addition, donkey milk is very rich in the natural antimicrobial enzyme lysozyme, a glycosidase capable of hydrolyzing the polysaccharides of the microbial cell wall.

Several scientists have shown the nutritional and health importance of donkey milk. The high content of lactose has a positive effect on the intestinal absorption of calcium and is responsible for the pleasant taste. Even after the first months of life, donkey milk can enhance bone mineralization, counteracting osteoporosis in adults, and provide important nutritional support in children with severe Ig-E mediated cow milk protein allergies, thus contributing to the formation of a complete and efficient immune system (Carroccio et al., 2000; Iacono et al., 1992; Monti et al., 2007; Vita et al., 2007). The relevant percentage of essential amino acids makes this milk a potential new dietetic food and an excellent breast milk substitute (Guo et al., 2007). Donkey milk is characterised by a low content of saturated fatty acids that, together with a high amount of poly-unsaturated fatty acids, render it of great utility in the prevention of cardiovascular diseases in adults and the elderly (Salimei et al., 2004). In the elderly, donkey milk could also be an important support in ipo-cholesterolemic diets; furthermore it might reinforce the weaker immune system, and it seems to act against atherosclerosis (Tafaro et al., 2007). For these reasons, this product can certainly be included in the category of so-called “functional foods” that positively affect human health.

Generally, functional foods, which are always in higher demand in the markets, are considered to be of noticeable strategic importance and can contain microorganisms that meet a variety of health requirements. These microorganisms, referred to as “probiotics”, are live microorganisms that, when administered in adequate amounts, confer health benefits
to the host (FAO/WHO 2001). Probiotics have been clinically proven to have several benefits, including the improvement of irritable bowel syndrome, allergic conditions, and immune function. Also, many probiotics are promising candidates for the treatment of cancer (Saggiorgo 2004; Abrahamsen 2007; Rafter 2003; Salminen et al., 1998). Dairy products, such as yoghurt, fermented milk, cheese or ice cream can represent important vehicles to deliver these beneficial microorganisms to consumers (Rybka and Kailasapthy, 1995). Considering the potential, strains can have a positive effect on the composition of the fermented milk-based products with specific probiotic strains can have a positive effect on the composition of the gut microflora (Fuller, 1989). Considering the potential, specific health benefits offered by donkey milk, as well as its recognized pleasant taste, we evaluated the possibility to formulate a new type of fermented milk drink created with probiotic strains.

MATERIALS AND METHODS

Microorganisms

*Lactobacillus acidophilus* DSM 20079, *Lactobacillus bulgaricus* DSM 20081, *Lactobacillus paraplantarum* DSM 10667, *Lactobacillus plantarum* DSM 20174, *Lactobacillus pentosus* DSM 20314, and *Lactobacillus rhamnosus* DSM 20711 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany). Each strain was incubated for 18 hours in Man de Rogosa Sharpe (MRS) broth (Oxoid, Milan, Italy), using the temperature conditions suggested by the company.

Donkey milk fermentation

Donkey milk was obtained from an organic breeding in the Salerno province (Italy). 42 ml of milk was inoculated with 2% of a microbial culture from one of strains mentioned above and incubated for 48 h at temperatures recommended for that microbe.

Viable microorganisms

Samples were diluted in peptone–saline water (0.9%, w/v saline; 0.1%, w/v peptone) and viable counts of the probiotic strains were determined on MRS agar (Oxoid, Milan, Italy) after incubation for 48 h in an anaerobic system (Oxoid, Milan, Italy).

Chemical analysis

The pH was measured during fermentation using an HI 8417 pH meter (Hanna Instr.® USA). The pH meter was calibrated using standard buffer solutions (Merck, Milano, Italy) at pH 4.0 and 7.0.

Production of organic acids

Organic acids were analysed by high-pressure liquid chromatography (Nazzaro et al., 2009; Vulevic et al., 2004). A 1 ml fermented sample was centrifuged (11,600 x g, 10 min) and filtered (0.2 lm, Millipore, USA). Twenty µl of sample was then injected into an HPLC (Beckman, USA) with an UV/Vis detector set at 210 nm. Separation was achieved using an Aminex HPX-87H cation exchange column (300-7.8 mm, Bio-Rad Laboratories, CA, USA). The mobile phase used was 5 mM H₂SO₄ at a flow rate of 0.6 ml min and the column temperature was 30°C. Organic acids were identified according to their retention times compared with standard solutions of the following acids: lactic, acetic, butyric and succinic (Sigma Aldrich, Milano, Italy).

Statistical analysis

All experiments were carried out in triplicate and are mean of two independent studies. The data are presented as mean±SD.

RESULTS

Growth and fermentation aptitude of probiotic strains in donkey milk.

The ability of probiotic strains to use donkey milk as a growth medium is shown in Figure 1. In our experiments, all strains of probiotic bacteria were able to grow well in donkey milk, attaining viable cell counts above 9 log cfu/mL after 48 h of incubation; the most effective growth was observed for the *L. plantarum* type strain (9.39 log cfu/mL); on the other hand, *L. bulgaricus* exhibited the lowest values, but always above 9 log cfu/mL. The strongest growth was observed during the first 24 hours of incubation, with a Δlog cfu/mL ranging from 1.58 (for *L. rhamnosus*) to 2.23 (for *L. pentosus*). The increase was much slower between 24 and 48 hours of incubation, never exceeding a Δ log cfu/mL of 1. On the whole, all strains showed good fermentative action. Depending on the probiotic strain used, the pH of donkey milk decreased from the initial level of 7.12 to 3.9–4.56 after 48 h of incubation (Fig.2).

Milk inoculated with *L. plantarum*, *L. pentosus* and *L. paraplantarum* had a pH ranging between 4.45 and 4.63 after 24 h of fermentation; after 48 h, the pH range observed in these samples fell between 3.90 and 3.92. *L. bulgaricus* and *L. rhamnosus* showed a slightly minor capability to ferment donkey milk, where the final pH was, after 48 h, 5.21 and 4.59, respectively. *L. plantarum* and *L. pentosus* exhibited the highest fermentative capability, with a ΔpH of 2.6-2.7 within the first 24 h of incubation and a final ΔpH of 3.24-3.26 at the end of the incubation; a slower trend was observed for *L. bulgaricus*, which displayed a final pH of 3.2–3.3 at the end of the incubation.
ΔpH of about 1.57 and 1.93, after 24 and 48 h of incubation, respectively.

FIGURE 1. Growth of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus paraplantarum*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, and *Lactobacillus rhamnosus* in donkey milk. On y axis are reported the log of colony forming units of microbial strains/mL of milk. Each strain was incubated for 48 hours. For the experimental details, see the Materials and Methods section.

Production of organic acids

The production of organic acids by probiotic strains used in our experimentation is shown in Table 1. The amount of lactic acid produced during the fermentation of donkey milk varied from 98.3 to 114.81 mmol/L after 48 h of incubation. Acetic acid levels were generally of about 19-23 mmol/L at the end of incubation. The least effective strain was *L. plantarum*, which produced about 40% less acetic acid. With regard to *L. rhamnosus*, our data are in contrast with Ostlie et al. (2003), but the results probably could depend on the type of milk and the strain used in the experiment. Succinic acid was present in all of the fermented milk samples, albeit at low values of approximately 1.4-2 mmol/L, except than in samples fermented by *L. acidophilus*, where the concentration was about 2-2.5 times higher. In our samples, butyric acid was present in all samples and ranged from 3.98 mmol/L in milk fermented with *L. paraplantarum* to 6.26 mmol/l in milk fermented with *L. acidophilus*.

**FIGURE 2.** The pH trend in donkey milk during 48 h of fermentation. For the experimental details, see the Materials and Methods section.

**TABLE 1.** The amount of select organic acids present in donkey milk after 48 h of fermentation. The data are expressed as the mean of mmoles/L ± standard deviation.

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>LACTIC ACID</th>
<th>ACETIC ACID</th>
<th>BUTYRIC ACID</th>
<th>SUCCINIC ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td>114.81 (± 9.65)</td>
<td>22.96 (± 2.01)</td>
<td>6.26 (± 1.01)</td>
<td>4.57 (± 0.56)</td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td>104.32 (± 6.81)</td>
<td>19.54 (± 1.35)</td>
<td>5.47 (± 0.33)</td>
<td>1.75 (± 0.74)</td>
</tr>
<tr>
<td><em>L. paraplantarum</em></td>
<td>101.21 (± 8.01)</td>
<td>21.01 (± 1.64)</td>
<td>3.98 (± 0.21)</td>
<td>1.41 (± 0.41)</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>98.32 (± 5.11)</td>
<td>14.69 (± 1.43)</td>
<td>5.14 (± 0.45)</td>
<td>1.58 (± 0.04)</td>
</tr>
<tr>
<td><em>L. pentosus</em></td>
<td>101.34 (± 8.43)</td>
<td>22.12 (± 1.12)</td>
<td>5.76 (± 0.63)</td>
<td>1.98 (± 0.21)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>98.78 (± 3.05)</td>
<td>19.02 (± 1.56)</td>
<td>5.02 (± 0.35)</td>
<td>2.09 (± 0.84)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In order to exert beneficial effects in the host, it is essential that lactic acid bacteria be both alive and abundant in the product at the time of consumption. No general agreement has been reached regarding the concentration of probiotics necessary to achieve beneficial effects, but counts from 10^6 to 10^8 cfu/mL are usually recommended (Shah, 2001). Our results confirmed that donkey milk, in addition to being a natural source of probiotic strains (Nazzaro et al., 2008) may also allow the growth of different lactic acid bacteria with probiotic properties, at the sufficient doses to be evaluated as ‘probiotic concentration’. The excellent ability of these strains to grow in donkey milk could provide a strong basis for the production of new milk-based functional foods with lower allergenic properties than foods from cow’s milk. They also could provide superior therapeutic values in human nutrition. Indeed, as resulting by the no excessive low value of pH reached after fermentation, manufacturing of fermented donkey milk does not present the problem of over-acidification, probably due to the good buffering capability of this milk: in fact, in other milk-based fermented formulas containing, for example, goat milk (Alferez et al., 2002; Barriomueuo et al., 2001; Spuergin et al., 1997), over-acidification might create problems with microbial viability (Martin-Diana et al., 2003; Ryssstad and Abrahamsen, 1983). The fermentation of this milk with probiotic strains gave arise also to the interesting production of some short chain organic acids (SCOAs), like butyric and acetic acid, of interest to health, that usually are metabolized by the colonic epithelium (i.e. butyrate) and muscle (i.e. acetate) (Cummings and Mc Farlane, 1997); in addition, the milk was also source of lactic and succinic acid, that are important intermediates that are also degraded to SCOAs, CO₂, and H². SCOAs have been associated with a reduced risk of some diseases, including irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, and cancer (Roediger, 1980; Wong et al., 2006). By influencing the composition and activity of the human gut microbiota, the eating of specific food, such as fermented donkey milk, might exert an indirect effect on the gastrointestinal function of the host and thereby on the health of the host. The presence of...
SCOAs, especially butyrate, found in donkey milk fermented by the different probiotic Lactobacillus spp used in our experimentation, could contribute to the maintenance of the morphological and functional integrity of the colonic epithelium. Butyric acid promotes epithelial cell healing and improves the comfort of patients with distal UC by increasing colonocyte protein synthesis (Frankel, 1994); in addition, it reverses the sulphide-induced pathogenic effects caused by sulphate reducing bacteria (Christl, 1996). A deficiency in the availability or utilization of SCOAs like butyric acid is in fact the principal cause of colitis and has been demonstrated to be involved in colonic carcinogenesis and idiopathic ulcerative colitis (UC) treatment. Lactic acid, present in donkey milk after fermentation and generally produced as a byproduct of the fermentation process in dairy products, may act as a preservative by reducing pH, promotes digestibility, improves the utilization of calcium and other minerals and inhibits the growth of potentially harmful bacteria. The presence of acetic acid could result very important if we would think of using fermented donkey milk as adjuvant in the digestive cancer prophylaxis, being demonstrated the crucial role of some probiotics to kill colon cancer cells through apoptosis in vitro via their metabolites, SCOAs, acetate and propionate (Lan et al., 2007); in addition, the presence of acetate and lactate, known inhibitors of the growth of potential enteropathogens by probiotics (Gibson and Roberfroid, 1995; Salminen et al., 1998; Fooks and Gibson, 2002) might improve the antimicrobial activity of donkey milk.

CONCLUSIONS

Consumers are beginning to believe that diet has a powerful influence on health and well being, and the food industry has taken advantage of these new possibilities. Fermented milks containing probiotic bacteria may represent the best-known examples of functional food. Milk is much more than the sum of its nutrients. It is a natural source of a variety of beneficial nutrients and biologically-active compounds with a potential impact on human health. Additionally, probiotic bacteria beneficially affect the host by improving the intestinal microbial balance. Based on the results obtained in this study, donkey milk can be valued as an excellent base ingredient for many probiotic and therapeutic food preparations because of its lot beneficial properties. The possibility of using donkey milk as a growth medium for several probiotic strains, thereby giving rise to the production of a probiotic beverage with a high content of SCOAs, can lead the way to a vast number of potential uses for donkey milk. Our efforts contribute to improving the views of donkey milk, which could be presented at the market in different products for a wider range of consumers, offering new products with excellent nutritional and health properties. The production of donkey milk-based products presents interesting marketing perspectives and can create an innovative production choice for those agricultural enterprises in search of new outlets.

REFERENCES


Fermented donkey milk

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