

**TITLE:** FERMENTED SHEEP'S MILK BY KEFIR: A SOURCE *Lactobacillus rhamnosus* RESISTANT TO  
*in vitro* HUMAN DIGESTION SIMULATION

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**ABSTRACT:**

The search for new probiotic strains has been increasing, probiotic food has gained more space in the market. A functional food characterized as probiotic, is kefir. Thus the fermented sheep's milk by kefir can be a food "carrier" of this beneficial microorganisms. For a microorganism to be considered probiotic, it must resist the food manufacturing conditions, to have viability and effectiveness when inserted in the food matrix, these should also be resistant to gastrointestinal tract (GIT), tolerating the acid, action of digestive enzymes such as pepsin and pancreatic and bile salts. Therefore, the purpose of this work is to isolate strains with probiotic characteristics. The strains are isolated after *in vitro* digestion simulation of fermented sheep's milk by kefir grains and the microorganisms are identified by molecular taxonomy. The milk fermented was made with the same method that Lima *et al.* (2017); human digestion simulation was based on the methods of Saito *et al.* (2014), at the presence of pepsin adjusted to pH 2.0, pancreatin at pH 8.0 and bile salts; at the end of simulation a 100 µL aliquot was pipetted out, serially diluted and spread-plate on MRS supplemented with 200 mg/L of Cicloxemide agar to isolate digestion resistant strains. After the growth period, 5 stains (gram positive e catalase negative, with rod-shaped morphology) were chosen to identification of molecular taxonomy. In the results, 3 strains were identified as *Lactobacillus rhamnosus* based on 100% identity of 16S rDNA sequences, compared to database sequences (GenBank accession no. CP020464.1). This work is an important contribution to the study of probiotics isolated from fermented sheep's milk by kefir grains, and this strains analyzed here can be useful in the production of dairy foods for potential human health benefits.

**Keywords:** fermented milk, in vitro digestion, kefir, *Lactobacillus rhamnosus*

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