Differentiation of homofermentative Lactobacilli isolated from kefir grains by FT-IR spectroscopy

Alejandra Bosch[1]; Marina Golowczyc[2]; Graciela Garrote,[2]Analía Abraham[2]
Graciela De Antoni [2] and Osvaldo Yantorno[1]

[1]Centro de Investigación y Desarrollo de Fermentaciones Industriales (CINDEFI, CONICET),
[2]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, CONICET, CIC). Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata (1900), Argentina.
E-mail: yantorno@quimica.unlp.edu.ar.

Kefir is a type of sour fermented milk in which kefir grains are the starters employed. Despite the widely consumption of kefir, it has not been thoroughly studied, probably due to its complexity. Several reports indicated that kefir grain microflora strongly depends on the origin of the grains, local conditions of culture, storage and on the elaboration processes. In order to characterize and identify the organisms present in different kefir grains, phenotypic methods like API (BioMerieux, USA) and whole-cell protein profiles analysis were used [1]. Previously, we applied FT-IR spectroscopy to differentiate heterofermentative from homofermentative Lactobacilli. Besides, the isolations of the first group were further characterized. In this work FT-IR spectroscopy in combination with cluster analysis were used for the identification and characterization of homofermentative Lactobacilli isolated from kefir grains at species level. Twenty five isolations and 7 reference strains of 5 different species were analysed. The bacteria were grown in MRS agar plate for 48 h at 30^{o}C. Four independent parallels of each isolation and strain were prepared. The sample preparation was performed as described by Helm et al.[2]. Spectra were recorded between 4000 and 650 cm^{-1} in a Spectrum One FT-IR spectrometer (Perkin Elmer Inst. USA). Hierarchical cluster analysis was carried out using Ward´s algorithm with scaling to first range.

Our study demonstrated that FT-IR spectroscopy is suited for identification and differentiation of homofermentative Lactobacillus normally present in kefir grains: *L. plantarum* (2 strains), *L. acidophilus* (2 strains) *L. cassei*, *L. kefiranofaciens*, and *L. kefirgranum*. In our case, 95 % of the strains isolated from the kefir grains analysed were grouped in the *L. plantarum* cluster and 5 % in the *L. kefiranofaciens* cluster. These results were similar to the ones obtained by the conventional classification schemes. We concluded that this methodology is a powerful tool for an easy, fast and reliable identification of Lactobacillus spp.

[1] Garrote, G. L. et al., 2001, *J. Dairy Res*., 68: 639-652. [2]Helm D. et al, 1991, *J. Gen. Microb*iol., 137: 69-79.