FT-IR spectroscopy is a powerful tool for the characterization of microflora and exopolysaccharides recovered from kefir grains

A. Bosch¹, M. Golowczyc², M. F. Rey³, D. E. Romanin³, N. Borst⁴, M. Wenning³, A. Abraham², G. L. Garrote², G. De Antoni and O. Yantorno¹

¹Centro de Investigación en Biotecnología aplicada, CINDEFI (CONICET)
²Centro de Investigación y Desarrollo en Criotermbología de Alimentos (CIDCA, CONICET)
³Cátedra de Microbiología, Facultad de Ciencias Exactas, UNLP
⁴Department of Microbiology, Technical University of Munich (ZIEL), Germany

Kefir is fermented milk with a slightly acidic taste obtained by incubating milk with kefir grains. These grains are white or lightly yellow gelatinous masses characterized by irregular forms, constituted by a complex mixture of lactococci, homofermentative and heterofermentative lactobacilli, yeasts and acetic acid bacteria living in a strong and specific symbiosis. This complex microflora is enclosed in a matrix containing mainly proteins and polysaccharides, known as kefiran.

Current investigations for the improvement of probiotic products based on kefir grains and kefiran have been focused on the study of both polysaccharides and microflora composition of the grains. Different grains obtained from various Argentinean sources were recently chemically and microbiologically analysed. Microbiological characterization at species level was carried out applying biochemical tests, whole-cell protein profiles, hydrophobicity, and cellular aggregation (¹). Although the results obtained in this study were suitable to distinguish the species isolated, these analyses are time consuming, expensive and sometimes of limited reliability. Therefore, in this work a different approach based on Fourier transform infrared (FT-IR) spectroscopy and Hierarchical Cluster Analysis (HCA) was applied to analyse entire grains, purified polysaccharides, and for the discrimination and identification of lactobacilli and yeasts recovered from local kefir grains.

Forty-four lactobacilli and 37 yeasts isolated from 7 different kefir grains and 23 reference strains were included in this study. First derivative spectra obtained from the above mentioned organisms were analyzed by HCA using Pearson’s product-moment correlation coefficient and Ward’s algorithm (²). Lactobacilli identification was performed by a sequential identification scheme based on HCA (³). We proposed a first level of discrimination between homo and hetero-fermentative lactobacilli using 4 windows: 1789-1700, 1059-935, 3000-2927 and 896-833 cm⁻¹. Lactobacilli classified as heterofermentative were then discriminated by a 2nd HCA model, which identified all these isolates as L.kefiri (20 isolates) except 1 that was discriminated as L. parakefiri. The 3rd HCA model developed for the discrimination among homofermentative lactobacilli grouped all these isolates as L. plantarum (23 isolates). Finally, we developed an independent model for the identification of yeasts isolates which indicated that three main genera were present in the grains analysed hereby: Saccharomyces, Kluyveromyces and Issatchenkinia. Eighteen isolates were identified as S. cerevisiae, 6 as S. unisporus, 10 as K. marxianus and 4 as belonging to Issatchenkinia genera. Additionally, yeast and lactobacilli identified by the developed models were tested against the corresponding spectral libraries of the Abteilung Mikrobiologie, Zentralinstitut für Ernährungs-_und Lebensmittelforschung (ZIEL), Technical University of Munich, Germany.

FT-IR proved to be a rapid and powerful tool for the discrimination and identification at the species level of lactobacilli and yeasts isolated from kefir grains.

References