

## Identification of intestinal lactobacilli by automated ribotyping and MALDI-TOF MS

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### Abstract:

The human gastrointestinal tract contains a diverse microbial community. Among them lactobacilli are important members of the natural intestinal microbiota playing a beneficial role in balancing of the intestinal environment. A collection of 51 *Lactobacillus* isolates originating from children intestinal mucous tissues has been studied. Analysed biopsy samples were retrieved during a routine endoscopic examination of different parts of the large intestine in patients revealing various disorders. A combined approach, which included automated ribotyping and MALDI-TOF MS analysis, was applied for identification of isolated *Lactobacillus* spp. in order to compare the discriminatory power of these methods. Automated ribotyping using the RiboPrinter® Microbial Characterization System (DuPont Qualicon) assigned 38 lactobacilli to the species level: *Lactobacillus salivarius* (29 isolates), *Lactobacillus paracasei* subsp. *paracasei* (7) and *Lactobacillus rhamnosus* (2). The remaining 13 isolates were not identified due to low degree of similarity (85% threshold) with the reference ribotype patterns. Additional cluster analysis of the ribotype patterns was performed by BioNumerics v. 6.0 software (Applied-Maths) and enabled to assign 10 isolates as *L. salivarius*. Three remaining strains were not identified by the RiboPrinter/BioNumerics approach. MALDI-TOF MS analysis was performed on an Ultraflex III instrument (Bruker Daltonik) according to a standard sample preparation protocol of Bruker Daltonik. MALDI-TOF mass spectra were subjected to numerical analysis (BioTyper 1.1 software, Bruker Daltonik), which revealed the formation of a cluster harbouring all 39 isolates identified as *L. salivarius* and identified all *L. paracasei* and *L. rhamnosus* isolates being separated in species-specific clusters in agreement with the ribotyping results. Three isolates unidentified with the RiboPrinter system were assigned as *Lactobacillus mucosae* by the MALDI-TOF MS. In conclusion, the MALDI-TOF MS enabled reliable identification of all analysed *Lactobacillus* isolates to the species level. In contrast, automatic identification performed by the RiboPrinter system did not identify 10 *L. salivarius* and all *L. mucosae* isolates. Supported by the projects of the Ministry of Education, Youth and Sports of the Czech Republic (2B08068, LC06034, MSM0021622415 and MSM0021622416).

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