

Identification and typing of lactic acid bacteria using intact-cell matrix-associated laser desorption/ionization time-of-flight mass spectrometry

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Matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been used for identifying and typing several bacteria such as *Salmonella* spp., *Legionella* spp., *Vibrio parahaemolyticus*, and *Staphylococcus aureus*. In this study, we established a intact-cell MALDI-TOF MS method for the rapid identification and typing of lactic acid bacteria at the species level. Total 60 strains of lactic acid bacteria (11 reference strains and 49 strains isolated in our laboratory) were used for this study. The bacterial proteins were extracted from intact cells with trifluoroacetic acid and analyzed by MALDI-TOF MS. In the spectrum data between 2,000-12,000 Da, specific identifying biomarker proteins (SIBPs) consisting of distinguishable and prominent mass peaks among the species of lactic acid bacteria were searched using gel-view representation of which software program has been developed in our laboratory. By this search, we successfully identified SIBPs for the classification of 7 species of lactic acid bacteria (*Lactobacillus sakei*, *L. curvatus*, *L. plantarum*, *L. pentosus*, *Lactococcus lactis*, *Weissella cibaria*, and *Leuconostoc citreum*) and 1 group of 3 closely related bacteria (*L. casei*, *L. paracasei*, and *L. rhamnosus*). Hierarchical cluster analysis revealed that respective strains were clustered into the corresponding species or group, supporting the results of SIBP classification.

Thus, the combination of SIBPs and the cluster analysis resulted in the accurate classification of lactic acid bacteria. These findings suggest that our method will be useful for the species identification and typing of unidentified isolates in lactic acid bacteria.