HOUSEKEEPING GENE SEQUENCE ANALYSIS IS A REQUIRED PROCEDURE FOR EXPLORING NOVEL LAB STRAIN FROM TRADITIONAL FERMENTED FOODS

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Traditional fermented foods have a close relationship with the culture, tradition and climate of their localities of origin, and are produced by local techniques with different kinds of materials. Lactic Acid Bacteria (LAB) that are comprised a large bacterial group containing of about 370 species in 33 genera, are closely involved in the production of fermented foods. To reveal the distinctive features of traditional fermented foods, and to explore novel LAB strains in terms of their possible application as probiotics, it is very important to investigate the LAB diversity associated with the fermentation.

The 16S rRNA-based method has become a mainstream for classification and identification of bacteria, and it is now used as an alternative gold standard to DNA-DNA hybridization test. However, in many cases, members of a group of closely related species are impossible to discriminate on the basis of their 16S rRNA gene sequences alone, because the similarity values in these species are considerably higher than the recommended value for species differentiation (>97%).

During a study of biodiversity of LAB in stinky tofu brines and fu-tsai (fermented mustard) in Taiwan, we found the novel LAB strains which could not be clearly placed within any recognized species by means of 16S rRNA gene sequencing. On the basis of housekeeping genes (such as pheS and rpoA) similarities, these strains were clearly discriminated from the known validated species. From the results of the phenotypic and genotypic features, we have proposed that these strains represent the novel Lactobacillus species, as Lactobacillus odoratitofui sp. nov. and Lactobacillus futsaii sp. nov., respectively.

In conclusion, we confirmed that the analysis based on housekeeping gene sequences is a required procedure which enabled the detailed identification of LAB strains in species groups for which the identification by 16S rRNA-based methods is impossible.

keywords: housekeeping genes, Lactic acid bacteria, taxonomy

GENETIC DIVERSITY AND ACE-INHIBITORY PEPTIDES PRODUCTION IN LACTOBACILLUS HELVETICUS STRAINS ASSESSED BY MULTI-LOCUS SEQUENCE TYPING

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Lactobacillus helveticus is a homofermentative thermophilic lactic acid bacteria, which is used in the production of fermented drinks. In addition, part of Lactobacillus helveticus strains have properties associated with reduction of blood pressure after ingestion. This is due to the production of significant levels of bioactive peptides that are known to inhibit the enzyme angiotensin converting enzyme (ACE). In this study, to study the association of ACE inhibitory peptides and Lactobacillus helveticus, 249 Lactobacillus helveticus strains were examined by multi-locus sequence typing (MLST). The MLST scheme, based on the analysis of ten housekeeping genes (dnaA, pyrG, groEL, murC, pyrA, dnaK, uvrC, clpX, recA, and murE) and two peptidases genes (pepN and pepX) were developed. The 12 targeted loci were successfully amplified and sequenced for all strains. Phylogenetic analysis showed that all 249 strains belonged to 14 subgroups, including 132 sequence types. The results clearly showed that most of the strains isolated from different regions formed different subgroups, and these maybe because the strains isolated from the different ecological niches have experienced different evolution. Meanwhile, the association between ACE-inhibitory peptides production and multi-locus genes of Lactobacillus helveticus strains was analyzed. 39 Lactobacillus helveticus strains were divided into two groups, VPP/IPP-rich group and VPP/IPP-low group. Interestingly, the pyrA gene has been reported to be associated with the production of VPP and IPP. These results indicate that the production of VPP and IPP could be attributed to the evolution of Lactobacillus helveticus.

keywords: Lactobacillus helveticus, MLST, ACE, Phylogenetic