

4th International Conference and Exhibition on

Probiotics, Functional and Baby Foods

November 03-05, 2015 Valencia, Spain

Subspecies-specific identification of *Bifidobacterium animalis* subsp. *lactis* using 16S-23S ribosomal RNA intergenic spacer gene

Fahimdokht Mokhtari¹, Amir Hossein Mohseni² and Mojgan Heidarpour¹

¹Standard Research Institute, Iran

²Zist Takhmir Company, Tehran, Iran

Introduction: *Bifidobacterium lactis* is used as a probiotic in dairy products or supplied in infant food. This species has been characterized by its high oxygen resistance and production of considerable amounts of formate. In view of the widespread interest in *B. lactis* and *B. animalis* because of the expanding application of these species as probiotics, it is highly desirable to develop molecular approach to clear and reliable species identification. It has been recently suggested that sequencing of the internally transcribed spacer (ITS) region could be for molecular recognition of microbial agent since ITS regions might be under less evolutionary pressure and could provide greater genetic variation. Indeed, analysis of this region has already successfully differentiated strains and species of many bacterial groups. The purpose of the study was to use ITS region for molecular identification of *Bifidobacterium animalis* subsp. *lactis*.

Materials & methods: Human fecal samples were collected. All samples were cultivated in MRS agar and morphological assessments of colonies were confirmed by gram staining. Suspected microorganisms were subjected for genomic DNA extraction. 16S-23S ribosomal RNA intergenic spacer gene from *Bifidobacterium animalis* subsp. *lactis* was chosen for primer design based on Primer-BLAST online tool of NCBI database and PCR assay were setup on the genomic DNA. Final approval of the assay was performed with sequencing of PCR product.

Results: Genomic DNA amplification of samples along with the 16S-23S ribosomal RNA intergenic spacer gene primers exhibited the presence of 226 bp band as expected. Sequencing data analysis was performed by BLAST software of NCBI database. The results of BLAST showed 100% homology of its ITS DNA sequence with the sequence obtained from Genbank for *Bifidobacterium animalis* subsp. *lactis*. The sequences are deposited in the Gene Bank with the accession number: KJ558387.

Discussion: Specific identification of lactobacilli species and subspecies with microbiological tests are laborious and time-consuming. Molecular techniques can be replaced by microbial tests. The rRNA gene (rDNA) has been used widely to infer phylogenetic relationships among bacteria. However, as evolutionary distances decrease, the diversity found in the 16S rDNA is often insufficient and genetic relationships of closely related species cannot be accurately defined. The results of the present study demonstrated that PCR amplification of 16S-23S ribosomal RNA intergenic spacer gene is the appropriate test for subspecies-specific identification of *Bifidobacterium animalis* subsp. *lactis*.

Biography

Fahimdokht Mokhtari has completed her MS from Tehran Medical University about 20 years ago and has been working as a full expert in the field of microbiology and molecular biology, and Head of Biology and Microbiology research groups in Faculty of Food Industry and Agriculture. She has prepared more than 50 national standards in the field of microbiology and biotechnology as well as more than 20 articles in national conferences and journals.

mfahimdokht@gmail.com

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