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A PCR-based Method Using D-Xylulose 5-Phosphate/D-frutose 6-phosphate Phosphoketolase(xfp) Gene for Identification of *Bifidobacteria* at the Species Level

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Introduction

The genus *Bifidobacterium* includes Gram-positive pleomorphic strict anaerobes, and certain species are predominant inhabitants of human intestines. The beneficial effects of *Bifidobacteria* on human health have been demonstrated with regard to immunopotentiation, nutrition, the prevention of intestinal infection, and the reduction of intestinal putrefaction. Analyses of *Bifidobacteria* in intestinal microflora require labor-intensive and time-consuming techniques such as the single-colony isolation of candidate isolates from a specific selective medium, followed by testing for multiple physiological and biochemical traits. Moreover, these tests do not always provide clear-cut results, and are sometimes unreliable. Therefore, there is a need for practical techniques that enable rapid and accurate analysis of intestinal *Bifidobacteria*. For some years now, the comparison of 16S rRNA sequences has attracted attention as a reliable method for the classification and identification of several bacterial species. 16S rRNA- targeted hybridization probes or PCR primers enable rapid and specific detection of a wide range of bacterial species, and have become key procedure in the detection of microorganisms. Phosphoketolase are thiamine diphosphate dependent key-enzyme of pentose phosphate pathway of *Bifidobacteria*. Sequence of D-Xylulose 5-Phosphate/ D-frutose-6-phosphate Phosphoketolase Gene(xfp) are obtained from GenBank and aligned with Clustal W software. The present report describes the rapid identification by using a colony-PCR of xfp gene-targeted genus and species-specific primer for human intestinal *Bifidobacteria*, and applying this technique to identify from human fecal *Bifidobacteria*.

Materials and Methods

1. Identification of the suspect *Bifidobacteria* Primer design and PCR conditions

The DNA was extracted according to the modification of a boiling lysis method (Sambrook et al., 2001): The DNA was extracted from the suspect using a modified boiling lysis method and used for a PCR experiment with the species-specific primers to confirm whether or not these colonies are a member of *Bifidobacterium*. Species-specific primer was consisted a pair of primer: forward primer was 5'-TCA TCG ACG GCA AGA AGA C-3' and reverse primer 5'-GAC GGC CGG TGA GCA GGT A-3'. The amplification consisted of one cycle at 94℃ for 2 min, 40 cycles of 94℃ for 1 min, 50℃ for 30 sec (increase 0.3 ℃ each cycle), and 72℃ for 2min, and a final cycle of 72℃ for 5 min and then 4℃ for 1 min. After 40 cycles of PCR, amplified products were run on a 2% agarose gel, stained with ethidium bromide, and visualized under UV transillumination. The results as a whole show that a single amplified product of genus size (about 560 bp) and various size was observed.

2. Design of xfp gene targeted primer

Sequence of xfp gene gained from GenBank (http://wwwncbi.nlm.nih.gov/Genbank/index.html). Primer was designed from alignment of sequence of xfp gene were performed by clustal W (DNA star, DNAstar Inc., Ver 5.0, USA) and comparison with conserved region of DNA sequence.

3. Comparison with restriction enzyme treatment methods

Compare result of Restriction enzyme *RsaI*, *Sau*3AI and *Taq*I (Promega, USA) treatment after amplification with *Bifidobacterium* genus specific Bif164 and Bif662 (Ruben G. Kok et al., 1995).

Conclusion

This study was carried out by using a colony-PCR with a pair of xfp gene-targeted primers (xfp2349 and xfp2912) for confirming *Bifidobacterium* species rapidly and major results were obtained as follows.

1. A pair of the genus-specific primers, xfp2349 (2349-2368) and xfp2912 (2893-2912), were developed based on the sequence of the XFP gene of *Bifidobacterium*.

2. These primers allow us to identify *Bifidobacterium* strains at both genus-and species-level by controlling annealing temperature in PCR amplification. The results were consistent throughout the experiments.

3. For detection and isolation, colonies on MRS agar surface, typically shown as *Bifidobacterium* under microscopic observations (data not shown), were directly amplified with the xfp-based primers, resulting in the distinct and consistent banding patterns for the different *Bifidobacterium* isolates (Fig. 1).

4. Reference banding patterns for 6 type strains of *Bifidobacterium* were established using the xfp-based primers (Fig. 2).

5. The suspicious isolates as *B. longum* by this method were able to be confirmed by digestion of a restriction enzyme (*Rsa*I, *Sau*3AI, *Taq*I) for obtaining correct identification result with *B. longum* ATCC 15707 (Fig. 3~4).

6. Thus this method could be primarily applied to the identification of *Bifidobacterium* strains from human fecal samples.

References

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