REAL-TIME PCR ANÁLISIS OF HUMAN INTESTINAL MICROFLORA WITH 16S RRNA-GENE-TARGETED GENUS- AND SPECIES-SPECIFIC PRIMERS

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Background: The human intestinal tract harbors a large, active, and complex community of microbes.
Intestinal microflora play several significant roles in digestion of food, metabolism of endogenous and exogenous compounds, production of essential vitamins, immunopotentiation, and prevention of the colonization of the gastrointestinal tract by pathogens, and hence is involved in maintaining human health.
Analyses of human intestinal microflora have been performed using classical culture methods; these methods generally entail the isolation, identification, and enumeration of these species, processes that are labor-intensive and time-consuming. In recent years, analyses of bacterial flora have been performed using molecular techniques. 16S rRNA-targeted hybridization probes of PCR primers enable rapid and specific detection of a wide range of bacterial species. In order to develop an accurate and convenient method for the characterization of microorganisms in intestinal flora, we developed 16S rRNA-gene-targeted genus- and species-specific primers for the predominant inhabitants of the human intestinal tract. In the present study, the genus- and species-specific PCR technique using fecal DNA was also used to investigate the distribution of bacteria in the human intestinal microflora.

Materials and Methods: On the basis of 16S rRNA sequences, genus- and group-specific primers for 12 predominant inhabitants of human intestinal microflora were developed. In addition, we developed and evaluated species-specific primers for 35 predominant species that inhabit the human intestinal tract, to analyze the bacterial composition of human intestinal microflora in high detail. Real-time PCR analyses for these bacteria were then conducted for 46 healthy volunteers using DNA extracted from fecal samples.

Results: Real-time PCR analysis of 12 bacterial groups for 46 healthy adults revealed that the C. coccoides group, the C. leptum subgroup, the Bacteroides fragilis group, Bifidobacterium, the Atopobium cluster, the Eubacterium cylindroides group, and Prevotella were the predominant bacterial groups (Matsuki et al., Appl Environ Microbiol 70: 7220-8; 2004: Matsuki et al., IUMS in San Francisco, 2005). Real-time PCR analysis of these 35 bacterial species for 46 healthy adults revealed that the following bacterial species are distributed widely and are predominant: Ruminococcus obeum, Eubacterium rectale, Faecalibacterium prausnitzii, Bacteroides vulgatus, Bacteroides ovatus, Bacteroides uniformis, Bifidobacterium adolescentis, the Bifidobacterium catenulatum group, and Collinsella aerofaciens (Matsuki et al., Appl Environ Microbiol 70: 167-73; 2004: Matsuki et al., 104th ASM General Meeting in New Orleans, 2005).

Discussion: For real-time PCR analysis of human intestinal microflora, we developed and evaluated 12 sets of genus-specific primers and 35 sets of species-specific primers. The present 12 sets of genus- and group-specific primers cover most of the predominant bacteria that have been detected in human feces, and therefore they provide an effective way to analyze microflora at the genus level. Further, the present 35 sets of species-specific primers enable us to analyze human intestinal microflora at the species level an analysis too difficult to achieve using conventional culture methods. Thus, real-time PCR with genus- and species-specific primers will provide more extensive and detailed information about intestinal microflora, facilitating understanding of the effects of probiotics or prebiotics, the side effects of antibiotics, and the relationship between microflora and human health.