Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities.

ABSTRACT

The aim of this study is to optimize molecular detection and quantification methods of probiotic bacteria in complex microbial communities that have long been difficult for traditional culture-based methods. Traditional and real-time PCR were optimized to detect and quantify Lactobacillus spp. and Bifidobacterium spp. in complex microbial community. Fish and shrimp sauce were used as a model for complex microbial community. Directly form samples, 4 DNA extraction methods, primers specificity, PCR, and real-time PCR procedures were optimized, tested in comparison with samples, enriched bacteria and related standard bacterial strains, E. coli, Bacteroides, Enterococcus and Salmonella. Results showed that extracted genomic DNA using Wizard® Genomic DNA Purification Kit showed the highest yield, quality and performance. Moreover, the specificity of the primer set specific for Lactobacillus spp. and Bifidobacterium spp. was checked and found highly specific. The sensitivity of real-time PCR was higher than the conventional PCR and its quantifying potential is very precise for the detection and quantification of Lactobacillus spp. but not Bifidobacterium spp. which was absent in the tested samples. In conclusion, PCR and real-time PCR assays could be used very efficiently in quantifying and detecting Lactobacillus spp. that are present in very PCR-suppressive and complex microbial environment.

Keyword: PCR; Real-time PCR; DNA extraction; Bifidobacterium spp.; Lactobacillus spp.; Fermentation; Probiotic.