Development and application of PCR primers for Clostridium tyrobutyricum and Listeria monocytogenes using heat shock ...

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ABSTRACT

Listeria monocytogenes is one of the common food pathogens. Contamination of the bacteria species on raw foods and cooked or processed foods, including low temperature sterilized milk, cheese and ice cream, smoked fish or meat is common, on the other hand, clostridium tyrobutyricum, which is often isolated from dry cheese and row milk, may spoil the quality of cheese and milk. Since conventional method for detection of these bacteria species is laborious and time consuming, rapid method is very important. In the study, we designed the PCR primers from the conserved region of hsp gene of clostridium spp. and used the primer set for the amplification of hsp gene of C. tyrobutyricum followed by sequencing to obtain the hsp gene sequence of C. tyrobutyricum. Through the alignment and comparison of the hsp gene of C. tyrobutyricum and L. monocytogenes to those genes available in Genebank, primers CT-1/CT-2 and Lm1/Lm2 specific for C. tyrobutyricum and L. monocytogenes were obtained. Using CT-1/CT-2, a PCR product with molecular weight of 104 bp could be obtained. Bacteria speices other than C. tyrobutyricum including other clostridium spp. would not generate any false positive reaction. On the other hand, Lm1/Lm2 allowed the amplification of a 120 bp PCR product. None of other bacteria species including the Listeria spp. would not generate positive reaction. As CT-1/CT-2 was used for PCR detection of target cells in dry cheese, the detection limit was N×100 CFU/g if the target cells were pre enrichment for 24 hrs, anaerobic. Similarly, the detection limit for milk and dry cheese samples using Lm1/Lm2 was N×100 CFU/ml or g is a 12 hrs pre enrichment step was performed prior to PCR. The this study, CT-1/CT-2 and Lm1/Lm2 were also used for real-time PCR detection of C. tyrobutyricum and L. monocytogenes. Under such condition, if a 24 hrs or 12 hrs per enrichment steps were performed prior to PCR, a detection limit of N×100 CFU/ml or g for milk or dry cheese could be obtained for both, detection cases.

Keywords : pathogens ; heat shock protein gene ; specific ; milk ; dry cheese

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