

利用熱休克蛋白基因設計Clostridium tyrobutyricum及Listeria monocytogenes之PCR引子組及其應用

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摘要

Listeria monocytogenes 廣泛分布自然界的病原菌，而傳染給人類，其為現代食品衛生上極為重要的一株病原菌，也經常出現在生的食品，以及許多熟食或加工食品，尤其低溫殺菌的牛乳、乾酪和冰淇淋、煙燻的魚或肉類製品。另外 Clostridium tyrobutyricum 雖然非病原菌，然其常在乾酪及生乳中會分離出來。因該菌會破壞乾酪及牛乳品質，因此快速檢測是很重要的，因為在傳統檢測方法通常很費力費時。而本研究中建立兩對新穎特異性引子組，分別針對 C. tyrobutyricum 及 L. monocytogenes 進行檢測。而因為 C. tyrobutyricum 之熱休克蛋白未發表，本研究因此先利用 Clostridium 屬內菌株之已發表之熱休克蛋白基因比對，分析並選擇基因序列之保守區段設計引子組，嘗試增幅出 C. tyrobutyricum 之熱休克蛋白基因片段並進行定序確認。之後利用定序結果之熱休克蛋白基因序列，設計引子 CT-1/CT-2，以及 L. monocytogenes 之類熱休克蛋白基因設計引子 Lm1/Lm2，分別針對 C. tyrobutyricum 及 L. monocytogenes 使用 PCR 增幅進行 PCR 引子組特異性檢測。使用引子組 CT-1/CT-2 進行檢測，除了 C. tyrobutyricum 會產生 104 bp 之預期產物外，其他菌株，包含其他 Clostridium 屬菌株，皆不會產生偽陽性之干擾。而由 L. monocytogenes 之類熱休克蛋白基因引子進行 PCR 增幅，可得到 120 bp 之預期產物外，其他菌株，包含其他 Listeria 屬，皆不會產生偽陽性之干擾。當引子組 CT-1/CT-2 應用於檢測鮮乳樣品中 C. tyrobutyricum 時，在 PCR 增幅前，先進行 24 小時之預培養後，其檢測靈敏度為 $N \times 100$ CFU/ml；而應用於檢測乾酪樣品，其靈敏度為 $N \times 100$ CFU/g。而引子組 Lm1/Lm2 應用於食品樣品中 L. monocytogenes 檢測，經 12 小時預培養後，鮮乳及乾酪樣品的檢測靈敏度均可達到 $N \times 100$ CFU/ml 及 $N \times 100$ CFU/g。本研究亦利用 Real-time PCR 方法，以引子組 CT-1/CT-2 及 Lm1/Lm2 檢測鮮乳及乾酪樣品中之 C. tyrobutyricum 及 L. monocytogenes。當引子組 CT-1/CT-2 應用於檢測鮮乳樣品中 C. tyrobutyricum 時，在 Real-time PCR 增幅前進行 24 小時之預培養後，其檢測靈敏度為 $N \times 100$ CFU/ml；而應用於檢測乾酪樣品，其靈敏度為 $N \times 100$ CFU/g。而引子組 Lm1/Lm2 應用於食品樣品中 L. monocytogenes 檢測，經 12 小時預培養後，鮮乳及乾酪樣品的檢測靈敏度可達到 $N \times 100$ CFU/ml 及 $N \times 100$ CFU/g。

關鍵詞：病原菌；熱休克蛋白基因；特異性；鮮乳；乾酪

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