A hypothetic universal PCR: implication for the rapid detection of major pathogenic bacteria in milk☆

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Abstract

This paper presents a method of detecting potential major bacteria in milk and milk-related products based on primer-specific universal polymerase chain reaction (PCR). Using this universal PCR, five common bacteria are expected to be detected and be differentiated. The technique core and some special attentions of the hypothetic detection measure are addressed. [Life Science Journal. 2007; 4(2): 88 – 89] (ISSN: 1097 – 8135).

Keywords: PCR; rapid detection; pathogenic bacteria; milk product

1 Introduction

The detection of major pathogenic bacteria is an important measurement for providing quality guarantee for milk and milk-related products. It is particularly more important for rapid and sensitive detection to measure the milk product in large scale in modern factory. If such measures are available, the fresh milk products may be safely circulated in markets. Although some traditional techniques, such as bacteria culture, isolation, biochemical identification and phenotype analysis are used extensively, some deficiencies including low sensitivity, time-consuming and procedure complication are still existed. In China, the development of detection techniques for milk products is greatly slower than the update and improvement of milk products. Therefore, it is a major research direction to develop more simply, rapid and sensitive detection measures in field of the food hygiene. With the progress of molecular biology and biotechnology, some novel and effective techniques including PCR have been introduced [1–4]. Here we put forward to how to establish a set of universal, sensitive and rapid PCR-based detection in order to detect the major pathogenic bacteria in milk.

2 The Hypothetically Universal PCR

The universal PCR will be used to detect five more common bacteria in milk. They are *Escherichia coli* (*E. coli*), *Salmonella, Shigella, Listeria, Staphylococcus*. The following steps are being considered for designing the universal PCR.

First of all, two or more candidate genes (e.g. the virulence genes) will be selected from each bacterium for the PCR amplification. For example, the *uidA, eaeA* and Shiga-like toxin (SLT) genes of *E. coli*[5], *invA, ompC* and *oriC* genes of *Salmonella*[6], *ipaH, phoP* genes of *Shigella*[7], *hlyA*, internalin genes of *Listeria*[8], and *mecA*, *nuc* and *ent* C genes of *staphylococcus* can be selected for the preliminary analysis[9]. Secondly, the DNA sequences of such genes may be retrieved from public database (e.g. GenBank database), and then we can make a DNA sequence alignment to find out several pairs of the highest

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homologous short sequences that flank a different length fragment among these genes. These short sequences will be used as universal primers for all the candidate genes. Third, the genomic DNA of these bacteria will be extracted with biochemical method or commercially available kits and used as PCR templates. Fourth, the above-mentioned universal primers will be tested for their amplification ability and specificity for each template. Fifth, one or more high specific primers will be selected and subjected to further replicable experiment for testing the PCR feasibility and stability. Sixth, the PCR condition needs to be optimized for increasing the sensitivity. Finally, the confirmed PCR needs to be tested its suitability using various milk products. Using such a universal PCR, it may be easier to identify the bacteria pollution, according to the size of PCR product.

3 Analysis of the Hypothesis

Evidently, the universal PCR may benefit the rapid detection of the common bacteria in milk. However, we should pay more attention to some key points to this PCR. Firstly, we need compare these candidate gene sequences carefully in order to make it possible to sift one or more pairs of universal primers. At the same time, the length of amplification produced by the primers should be optimal, but different among the bacteria. Secondly, restriction enzyme analysis and sequencing are needed for confirming the authenticity of the PCR products prior to the extensive use of the detection measure. Thirdly, more negative controls should be included in this test to exclude potential false positive reaction. Fourthly, the genomic DNA extraction and purification protocol should be standardized to minimize the error difference of system. Fifthly, the set up of optimal condition of PCR is vital for bacteriological examination of the milk products. For example, the decreasing of PCR cycles means the saving of examination time. Finally but not least, the given milk products sometimes need to be treated to facilitate such a PCR reaction. For example, we may also need to incubate the milk (at best, in a liquid form) at optimal temperature for some time in order to get enough numbers of bacteria, once it fails to take a trace of milk or milk product as template for a successful PC.

4 Conclusions

It is a good idea to apply the modern molecular biological technique in the detection of the milk and milk products. Such a PCR has more advantages than other traditional bacteriological methods in terms of its rapidness, sensitivity and manipulation. However, the PCR amplification from milk is rather different from the direct amplification from plasmid DNA. Therefore, more factors existed in milk together with the PCR system should be considered to develop the rapid detection.

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