

Development of macroarray system for the detection of milk pathogenic microorganisms

陳信硯、李世傑

E-mail: 319690@mail.dyu.edu.tw

ABSTRACT

Milk is a high nutritional value of food, there is a lot of dairy products popular among consumers. However, there might have a variety of zoonotic and environmental pollution associated with the presence of pathogenic microorganisms, it will be considerable economic losses which are caused by microbes; freshness is also the focus of emphasis on milk, it has a very short time from process to market, so development of rapid and accurate identification method is also very important. The purpose of this study is to design PCR primers and DNA probes for detection of *Brucella* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Aeromonas* spp., *Enterobacter* spp., *Moraxella catarrhalis* and *Pasteurella multocida*. By using the macroarray, several pathogenic environmental microbes which might affect the quality of milk can be simultaneously detected, and no cross reactions were observed. Adding more oligonucleotide probes to the array may allow the detection of more bacterial genus in the future. In addition, food poisoning cases occur for many reasons. *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* are often detected from the food. Among the cases of food poisoning usually involved more than one pathogen, there is generally compounded by environmental pathogens indirectly contaminated food. Therefore, this study tested artificially contaminated food, in the testing of PCR sensitivity, without the pre-enrichment, the detection limit were $N \times 10^3 \sim 10^4$ cfu/ml, and using multiplex PCR with the microarray for testing hybrid experiments. It was successful in discriminating *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterobacter sakazakii*, *Escherichia coli* O157:H7, *Vibrio parahaemolyticus*, *Salmonella* spp. and *Pseudomonas fluorescens*, even in the presence of other non-target strains, whether it is for a single or mixed-strains experiment, the specificity can be obtained without interference signals, that can be used on food for the detection of pathogenic bacteria to achieve rapid and accurate test results.

Keywords : milk、 pathogenic microorganisms、 microarray

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