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Review

DNA chips for detection of bacterial pathogens

Dušan Blaškovič & Imrich Barák*

Institute of Molecular Biology, Member of the Centre of Excellence for Molecular Medicine, Slovak Academy of Sciences, SK-84551 Bratislava 45, Slovakia; phone: +421 2 59307418, fax: +421 2 59307416, e-mail: imrich.barak@savba.sk

Abstract: Broad range of conventional methods as PCR, immunodetection, cultivation, reverse line blot or microscopy is available for identification of bacterial pathogens. Although these methods are used in clinical laboratories for years, they do not allow detection of large spectrum of pathogenic bacteria in a single assay. Availability of DNA microarray technology using chips with size of the microscopic slide containing thousands of detection probes makes DNA chips attractive tool for simultaneous detection of microorganisms in biological samples. A number of DNA microarray-based methods have been developed for detection of bacterial pathogens. In this review we summarize actual microarray-based detection strategies, different approaches to microarray-based detection of pathogenic bacteria and consider benefits and limitations of these approaches.

Key words: DNA chips, detection, pathogens.

Abbreviations: CCD – charge-coupled device, MPID – multi-pathogen identification, RDP – Ribosomal Database Project, RST – restriction site tagged, SNP – single nucleotide polymorphism.

Introduction

Fast and robust diagnostic methods are demanded to minimize threat of pathogenic bacteria to humans, animals and plants. Diagnostic methods used in clinical laboratories are usually able to detect limited spectrum of bacteria in one step and detection of different pathogens by conventional methods as PCR, immunodetection, cultivation, reverse line blot or microscopy is laborious and time-consuming. Invention of DNA microarrays with potential to carry thousands of probes allows to manufacture chips containing redundant number of probes targeting sequence(s) of genome unique for particular pathogens.

DNA microarrays consist of nucleic acid probes attached to a substrate in a two-dimensional pattern. Probes are hybridized to labeled "target" nucleic acid, which can be genomic DNA, RNA, PCR products, or oligonucleotides. Labeled target hybridizes to complementary probes and creates a target-probe complex that can be detected by scanner, sensitive to a reporter label. Fluorescent molecules hybridized to probes are excited, emitted light is collected and digital images of the fluorescent signal are generated. Probe-target complexes appear as bright spots at image produced by the scanner.

In this review we describe design of specific microarrays, different microarray-based approaches to detection of pathogens and selected technologies possessing improved parameters compared to traditional chips. We also describe high density probe chips with sensitivity of detection up to classical DNA sequencing, electronic detection platforms with elevated discrimination power up to point mutations recognition, integrated chips performing isolation of target DNA, its amplification and hybridization in one monolithic device, powerful bead based detection systems and gel pad based chips.

Design of specific DNA microarray

Conventional microarray experiment for detection of pathogens consists of steps shown in Figure 1. DNA isolated from a given sample serves as a template for amplification and labeling of target. Labeled target is hybridized to DNA microarrays carrying specific probes, targeting different pathogens. After hybridization that may last for several hours, excess of unhybridized target is washed away from surface of microarray by buffer with desired stringency and microarray is then scanned and image of hybridization signals is obtained. The crucial steps of microarray experiment are described in detail below.

* Corresponding author