(05-1-16)

Construction of DNA chip for *Xanthomonas oryzae* pv. *oyzae* (KACC10331)

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Objectives

This study was to construct DNA chip of *Xanthomonas oryzae* pv. *oyzae* (KACC10331) and to establish experimental techniques using the DNA chip.

Materials and Methods

1. Materials: DNA chip-50 oligomer, Total RNA extraction-RNeasy kit (Quiagen), Labelling of RNA: Cy3/Cy5-labeled dCTP,
2. Methods: Concentrations of 50-oligonucleotides were normalized to 50 uM and spotted on CMT-GAP aminosilane-coated glass slides and hybridized with Cy3/Cy5-labeled cDNA from total RNA.

Results and Discussion

Whole genome sequence of *Xanthomonas oryzae* pv. *oryzae* (KACC10331) that incites bacterial blight disease on rice was determined. DNA chip technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously. In this study, 50-oligonucleotides were designed from 4,789 ORFs in Xoo genome to have optimal specificity for target genes. Optimized 50-oligonucleotides were designed from 3,057 ORFs of Xoo, while remnantORFs were unsuitable for it because they include repeatitive sequences such as transposons. Optimal conditions for interactive reaction between 50-oligonucleotides spotted on glass and fluorescence probes were investigated. Lysozyme concentration and bacterial cell growth rate revealed as important factors to extract high quality total RNA. DNA microarray that includes 50-oligonucleotides targeting 102 genes associated with pathogenesis of Xoo wastested to detect hybridization signal with Cy3/Cy5-labelled cDNA. Furthermore, the hybridized microarrays were scanned by a high-resolution fluorescence scannet, and the fluorescent image obtained was analyzed by specific software packages.

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