Microarray-Based Analysis of 16s rRNAs for the Detection of Food-Borne Pathogens

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Abstract

Surface plasmon resonance imaging (SPRI) have been used for the microarray-based analysis of molecular interactions by fabricating biomolecule arrays on gold surfaces.[1)~3)] In the previous study, we developed a method to improve the sensitivity of SPRI-based immunoassays using a horseradish peroxidase (HRP)-catalyzed precipitation reaction.[4)] The precipitation reaction catalyzed by HRP bound to the SPR biosensor surface via a sandwich immunoassay induced a shift in the SPR angle. In the present study, the biocatalytic signal amplification method was applied to a high-sensitive detection 16s rRNA of food-born microorganisms such as *Escherichia coli* and *Listeria monocytogenes*. Biotinylated oligonucleotide probes were designed to be complementary to the 16s rRNA gene sequences of target bacteria. The biotin probes were spotted on a surface of streptavidin immobilized on a carboxyl-modified gold surface. Total RNA of the bacteria was partially purified, mixed with biotinylated detection probes and streptavidin-HRP conjugate, and hybridized to the oligonucleotide-arrayed chip. High sensitive and specific detection of food-born pathogens on the oligonucleotide microarray was achieved using the method described in the present study.

References

1. H.-S. Ro, S.O. Jung, B.H. Kho, H. P. Hong, J. S. Lee, Y.-B. Shin, M.-G. Kim, B. H. Chung, A surface plasmon resonance imaging (SPRI)-based protein array chip system for monitoring a

hexahistidine-tagged protein in its expression and purification processes(2005), Appl. Envir. Microbiol., 71(2), 1089-1092.

2. J-M. Jung, Y.-B. Shin, M.-G. Kim, H.-S. Ro, H.-T. Jung, and B.H. Chung, A fusion protein expression analysis using surface plasmon resonance imaging(2004), Anal. Biochem. 330(2), 251-256.
3. B. P. Nelson, M. R. Liles, K. B. Frederick, R M. Corn, R. M. Goodman, Label-free detection of 16s ribosomal RNA hybridization on reusable DNA arrays using surface plasmon resonance imaging(2002), Envir. Microbiol., 4(11), 735-743.
4. M.-G. Kim, Y.-B. Shin, J.-M. Jung, H.-S. Ro and B. H. Chung, Enhanced sensitivity of surface plasmon resonance (SPR) immunoassays using a peroxidase-catalyzed precipitation reaction and its application to a protein microarray , J. Immunol. Meth. In Press.