Design of ITS and 23S rDNA-Targeted Probes and Its Usefulness for the Identification of Bacterial Pathogens

So Young Yoo[1,3]
syy007@mail.kaist.ac.kr

Kyung Hee Chang[2]
lynne@yumc.yonsei.ac.kr

Seung Min Yoo[3]
yshamo@mail.kaist.ac.kr

Sun Young Park[3]
psy1404@hanmail.net

Nae Choon Yoo[4]
ync0011@yumc.yonsei.ac.kr

Ki Chang Keum[5]
kckeum@yumc.yonsei.ac.kr

Won Min Yoo[6]
wnmnyoo@yumc.yonsei.ac.kr

June Myung Kim[2]
jmkim@yumc.yonsei.ac.kr

Sang Yup Lee[1]
leesy@mail.kaist.ac.kr

[1] Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Scienceand Technology, Daejeon 305-701, Republic of Korea
[2] Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[3] Medigenes Co., Ltd., Seoul, 135-090, Republic of Korea
[4] Department of Oncology, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[5] Department of Radiation Oncology, Yongdong Severance Hospital, Yonsei University College of Medicine, Seoul 135-720, Republic of Korea
[6] Department of Plastic and Reconstruction Surgery, Yondong Severance Hospital, Yonsei UniversityCollege of Medicine, Seoul, 135-720, Republic of Korea

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1 Introduction

Currently used bacterial identification methods include analysis of morphological, physiological, biochemical, and genetic data. Traditional method of diagnosis of bacterial pathogens is cultivation-based in several selection medias, which is time-consuming and not sensitive enough [1].

Here, we propose a new approach to identify and type bacteria based on ITS and 23S rDNA sequences. Especially the ribosomal internal transcribed spacer (ITS) region, a stretch of DNA that lies between the small (16S) and large (23S) ribosomal subunit genes, is highly variable among strains [2, 3]. This may provide its usefulness for the resolution of strains within several species. For this reason, we designed probes based on the ITS and 23S rDNA sequences which are applicable to microarray technology for the diagnosis of pathogenic bacteria. Hybridization results show that ITS and 23S rDNA targeted probes can be successfully used in the identification of bacterial strains. DNA chips containing specific probes for detecting various pathogenic bacteria and fungi were developed under the trade name PathoChip (Medigenes Co., Ltd., Korea).

Staphylococcus epidermidis is a common member of the normal florae of skin and mucous membranes. Its presence in large numbers and ubiquitous distribution make it one of the most commonly isolated organisms in the clinical laboratory. Even though the appearance of *S. epidermidis* in clinical material could be dismissed as a contamination at one point, it is now one of the most important pathogenic agents of hospital acquired infections. Immunosuppressed or neutropenic patients are particularly at risk, as are individuals with indwelling catheters or prosthetic devices. It can also cause endocarditis in individuals with previous heart valve damage. The hydrophobic nature of the organism's cell surface facilitates its adherence to synthetic devices as well as damaged heart valves. Following initial colonization, a copious amount of extracellular polysaccharide or slime is synthesized,

forming a protective biofilm around the colony. Because many isolates are multiply antibiotic resistant, these infections are very serious and can even be fatal.

In this study, clinical applications were performed with *Staphylococcus epidermidis* as an example.

2 Method and Results

2.1 Design of Bacterial Species-Specific Probe

The species-specific probes of 15-mer oligo-nucleotides were designed based on the ITS and 23S rDNA sequences. The ITS and 23S rRNA sequences of pathogenic bacteria were obtained by sequencing with ABI 3700 (PerkinElmer Inc., USA). The ribosomal sequences for those bacteria completely sequenced were obtained from GenBank (`http://ncbi.nlm.nih.gov/Genbank/`) and TIGR (`http://www.tigr.org/`). Multiple alignment analysis was performed using airBASE^{TM}(Bioinfomatix Inc., Korea) and Vector NTI (InforMax Inc., USA). The probes which do not show cross-reaction with other bacterial species were finally selected according to the hybridization results.

2.2 Hybridization

The probes were spotted on the aldehyde coated slide glass by using home-made microarrayer [4] at room temperature. The chip was blocked by sodium borohydride ($NaBH_4$) to minimize the fluorescent background. Each hybridization sample was prepared by asymmetric PCR amplification using 5' FITC-modified reverse primer. Samples were hybridized in a sealing chamber for 6 hours. After washing, the slides were air-dried and scanned using ScanArray5000 (GSI Lumonics Inc., USA) with Argon laser (488nm). As an example, clinical applications were performed for the diagnosis of *Staphylococcus epidermidis* (Fig. 1).

3 Discussion

The ITS and 23S rDNA targeted species-specific probes were successfully designed, applied to the DNA chip, and were used to demonstrate their usefulness for the identification of pathogenic bacteria. Reference strains confirmed our designed probes to be species-specific. Clinical applications were also performed with *Staphylococcus epidermidis* as an example. As demonstrated above, it clarified that our PathoChip system is able to accurately identify bacterial pathogens. The ITS and 23S rDNA targeted nucleic acid probes which are defined by our experiments should prove to be invaluable in diagnosing pathogenic bacteria and fungi, by improving by identification rate and offers prompt clinical treatment.

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