Abstract

Molecular techniques, such as polymerase chain reaction (PCR) are rapid and reliable diagnostic method for detection of microbial pathogens. In recent decades, following antibiotic treatment, *Pseudomonas aeruginosa* has been identified as one of the most important nosocomial pathogen that causes acute and chronic infections in patients admitted to the hospital. Many diagnostic PCR based methods for detection of *Pseudomonas aeruginosa* have been stablished, however many of these protocols detect only one target gene and do not provide perfect and reliable detection method to identify the bacteria, because *Pseudomonas aeruginosa* species show high genotypic diversity. To overcome these problems, several PCR methods have been used. Meanwhile due to genetic exchanges between *pseudomonas aeruginosa* and closely bacteria the specificity of this technique is often low, therefore there is a need to design highly specific technique based on specific probe and Multiplex PCR to detect *Pseudomonas aeruginosa*. In this study, two molecular methods used were Multiplex PCR and gold nanoparticles colorimetric assay and the sensitivity and specificity of the test was evaluated. Optimization of Multiplex PCR and its sensitivity and specificity was performed using four gene-specific *pseudomonas aeruginosa* (*oprL*, *oprI*, *toxA* and *16s rDNA*) and results showed 100% specificity. Sensitivity of this method to detect bacteria using colony counting was 80 CFU/mL; in addition the sensitivity based on genomic DNA concentration was 0.05ng/µL. Eventually confirmation of detection was investigated by gold nanoparticle probes. Probe attached to gold nanoparticles in the presence of the target DNA caused aggregation of gold nanoparticles in the form of connected network and resulted in colour exchange. The colour change indicates the presence of the target molecule in the sample and could be observed by naked eye. In this study thiolated probe was designed based on *16s rDNA* gene sequence and after hybridization of probes changes in wavelength between 400 and 600 nm was investigated. Results showed that the wavelength of gold nanoparticles was shifted from 524 to 558 nm. This method indicated 100% specificity and colorimetric detection sensitivity based on the concentration of genomic DNA achieved at 0.01ng / µL, which was about 5 times higher than PCR using *16s rDNA* gene.

Keywords: *Pseudomonas aeruginosa*, gold nanoparticle probes, colorimetric assay, Multiplex PCR, *oprL* ،*oprI* ،*toxA* and *16s rDNA* genes

University of Zabol
Graduate school
Faculty of Sciences

Department of Biology

The Thesis Submitted for the Degree of M.Sc

(in the field of Molecular Genetics)

Molecular detection of *Pseudomonas aeroginosa* by Gold nanoparticles probe

Supervisers

Dr. H. Kamaladini
Dr. F. Haddadi

Adviser

Dr. M.Y. Alikhani

By

Z. Mosivand

January 2015