Abstract

Bacillus cereus is a gram-positive and anaerobic spore **.**This bacteria produces enterotoxin that causes diarrhea and vomiting. Due to its resistance to temperature changes, conventional methods of strilization and cooking are not enough to control it, hence rapid and precise detection of the bacteria is requierd in the food quality control centers. In this study to identify the *Bacillus cereus*, *nheB* and *hblD* genes were used to design primers. PCR results showed amplification of expected fragments of 842 and 396 bp corresponded to *nheB* and *hblD* genes, respectively. The sensitivity and specificity of the primers in *Bacillus cereus* and 10 negative controls were evaluated. According to the results, sensitivity for *hblD* and *nheB* genes were 0.01 and 0.5 ng/µl respectively, and specificity were 100% for both genes. After that *nheB* gene sequencing was performed and result was deposited to NCBI gene bank. After designing probes for binding to the target molecule and the gold nanoparticle, experiments on optimization of detection using gold nanoparticles were carried out. The results revealed specificity of 100% and sensitivy of 12 x 10^5 CFU/mL. Comparison of PCR and gold nanoparticles probes with biochemical methods to detect *Bacillus cereus* showed greater sensitivity and speed of these methods to biochemical methods. In addition hybridization of probes and DNA molecules at wavelengths between 400 and 700 nm was investigated, changing in color from red to blue related to the binding of probes for target DNA were observed.

Keywords: *Bacillus cereus*, *hblD* gene, *nheB* gene, PCR, Gold nanoparticles

University of Zabol
Faculty of Sciences
Graduate school
Department of Biology

The Thesis Submitted for the Degree of M.Sc (in the field of Genetics)

Detection of *Bacillus cereus* by PCR-based technique and Gold nanoparticles

Supervisers:

Dr. F. Haddadi

Dr. H. kamaladin

Advisers:

Dr. M. H. namaii

Dr. A. Rashki

By:

E. Amini

Janaury 2015