University of Zabol
Graduate School
Faculty of Sciences
Department of Biology

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

Title

Molecular identification of *chlorella vulgaris* micro algae by multiplexPCR and gold nanoparticles-based colorimetric assay

Supervisors
Dr. H. Kamaladdini
Dr. F. Haddadi

Advisor
Dr. S. Ganjali

By
S. Gholamrezaei

January2022

Abstract:

Algae, especially microalgae, are of great importance in food, pharmaceutical, etc. industries. Algae have very high antioxidant and antimicrobial activities. These properties can be used for medical, therapeutic, and pharmaceutical purposes. The present study aims to identify Chlorella vulgaris microalgae at Molecular Level using two methods of multiplex PCR and colorimetric assay with a gold nanoparticle attached probe.For this, first, the algea specimen was prepared and cultured, then the microalgae was identified by its Morphligic and cellular characteristics using light microscopy. In addition, genetic studies Were performed by amplifying two genes tuf A and Cp071 using multiplex PCR. The results of molecular analysis these genes indicated the amplification of two bands bp246 and bp540. Using multiplex PCR, the specificity and sensitivity of this algal species were estimated to be 99% and 81.7×10^{-3} of genomic DNA, respectively. Next, using a probe designed for tuf A gene, it was examined the diagnosis of this microalgae using a gold nanoparticle probe. Changes in color and wavelength were observed in the presence of the target gene. This method showed 99% specificity and sensitivity of 81.7×10^{-6} of genomic DNA. Both methods were specific and sensitive enough to diagnose Chlorella vulgaris microalgae. However, due to its higher sensitivity and specificity and use of less time, the gold nanoparticle probe is a better option for diagnosing Chlorella vulgaris microalgae than multiplex PCR.

Keywords: Microalgae, Chlorella vulgaris, Multiplex PCR, *Cp071* gene, *tuf A* gene, Colorimetric assay, Gold nanoparticle probe