Thesis Title

Molecular Aspects of the Carcinogenic and Anticarcinogenic Potential of Natural Compounds from Cyanobacteria

Abstract

Cyanobacteria (blur-green algae) are interest member of a group known as eubacteria, they are photosynthetic prokaryotes, that were originally classified as microalgae and distributed worldwide from polar to equatorial latitudes. They have very specialized cells (heterocyst, Akinetes) that are able to fix nitrogen and serve at high temperature or drought. Synthesis of toxin by many cyanobacterial bloom an also be viewed as giving a selective advantage to these groups and become an increasing worldwide problem in aquatic habitats. Species of cyanobacteria are sometimes difficult to identify mainly because of high phenotypic plasticity. Recently, molecular studies suggest that the taxonomic validity of numerous species and genera to identify and classify cyanobacterial strains rather than the morphological and physiological properties which are not appropriate markers for the study of cyanobacterial taxonomy.

The present study proposed a new technique as a model for studying of cyanobacterial strains called Whole Genome Amplification (WGA) that propagates substantially scarce quantities of DNA into thousand folds more of like DNA. WGA used to amplify the cyanobacterial DNA for further genotyping and sequencing.

Thus, the specific goals of the present study involving optimizing the use of WGA by different primers for genomic amplification and identifying the cyanobacteria species to evaluate phylogenetic diversity of species included Saudi Arabia strains. The identification based on the molecular techniques such as 16SrRNA, phycocyanin intergenic spacer (PC-IGS) and nitrogenase (nif*H*) amplification. In addition, the differentiation between all cyanobacterial strains applying short tandemly repeated repetitive (STRR), randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and sequencing the gene produced. Detection and quantitative analysis of the cyanobacterial toxins were done by molecular techniques such as Microcystin, Nodularin,

Cylindrospermopsin, Saxitoxin and plasmid gene amplification. Finally, Liquid Chromatography Mass Spectrometry (LC-MS) used for toxin analysis.

The present study used four foreign cyanobacterial strains; *Microcystis aeruginosa flos-aquae*, *Anabaena circinalis*, *Nodularia spumigena* and *Cylindrospermopsis raciborskii* and two other domestic one; *Microcystis sp.* an *Anabaena sp.*

16SrRNA-PCR amplification has been performed for all strains resulting 780bp band which determine the identity of the genomes as prokaryotes. Amplification by PCR of the phycocyanine gene of intergenic spacer region (PC-IGS) observed that is highly conserved within genus, but differs significantly between genera. Nitrogenase (nif*H*) gene amplification have been highly conserved in the genera. Thus, the conclusions from these comparisons with WGA and without WGA helped to distinguish the genetic abundance profiles.

Detection and genetic characterization of cyanobacterial strains have been developed in this study based on DNA amplification and gene sequence.

DNA amplification applied by Short tandemly repeated repetitive (STRR), Randomly amplified polymorphic DNA (RAPD) and Restriction fragment length polymorphism (RFLP) and sequencing of identified and toxicity genes. Different primers corresponding to each technique used by PCR amplification. The above applications resulted in generating specific fingerprints for each individual isolated. The results showed a genetic heterogeneity among isolates from different geographic regions such as STRR technique. Also DNA profile from all strains by RAPD-PCR made it possible to discriminate among all toxigenic cyanobacteria PCR product for cyanobacteria identification (16SrRNA, phycocyanin intergenic spacer (PC-IGS) and nitrogenase (nif*H*)) and toxicity genes have been sequenced and the data was analysed by seqMan and MaClad4 software. Similar conclusions with bacteria showed that 16SrRNA sequence similarity of 96± 97% and DNA± DNA hybridization value of 70% relative binding (RB) represent the lower boundary of a species and that a genus may be defined by species with 95% or greater sequence similarity. The relation between relative binding (RB) and sequence similarity varies even within the same genera. It therefore seems that 16SrRNA sequence similarity does not always adequately reflected total genomic relationships.

The phylogenetic tree were conducted from the use of distant outgroups, long branch attraction, heterogeneous base composition and site-specific rate variation using MaClade4 software.

Special attention was given to detect the presence of toxic genes from the six strains by amplifying gene fragment with specific primers; Microcystin (755bp), Nodularin (150bp), Cylindrospermopsin (650bp) and Saxitoxin (300bp and 202bp). PKEF1/PKER1 primers used to amplify and sequence 755bp PCR product of mcy*E* gene which gave a result with four of the strains; *Microcystis aeruginosa flos-aquae*, *Nodularia spumigena*, *Cylindrospermopsis raciborskii* and domestic *Microcystis sp.*

The identification of the nodularin biosynthetic genes cluster and evolution of hapatotoxicity using NSOR10 genome-specific primer was repeated in this study, which will be valuable for future studies on toxic cyanobacterial bloom formation. Phylogenetic studies have been shown that toxicity is restricted to the bloom-forming strain of *N. spumigena* isolated from blooms. The PKS gene identified in cylindrospermopsin producing cyanobacteria. Amplification of 153 F and 219F clones for detection of saxitoxin producers among the different strains appeared that no strain but Anabaena circinalis involved in the toxin production.

Real-Time PCR was applied to detect plasmids A, B, C, pM1, pM2 and pM025 with positive control strain. No plasmid detected in the six strain by plasmid A and B while plasmid showed with plasmid C, pM1, pM2 AND pM025, respectively.

Quantitative analysis of toxins using liquid chromatography mas spectrometric (LC-MS) was performed. Microcystin was detected in the commercial solution of LR, RR and YR microcystin. Nodularin has been detected in both extracted growth culture of *Nodularia spumigena* and in the solution standard. Also cylindrospermopsin was detected in *Cylindrospermopsis racborscii* extracted growth culture and in standard solution. Finally, saxitoxin has been detected in *Anabaena circinalis* extracted growth culture and in a commercial standard.