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Research Article

Assessment of Genetic Diversity and Relationship in Algae Using Rapd Marker

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Abstract

19 Algae collected from the wetland ecosystem of Tumkur and subjected to Random amplified polymorphic DNA RAPD method. In this method, the Expressed Sequence database was used as the original genomic information for identification of polymorphic sites. Primers OPW-06 RAPD is designed and the amplified PCR products are then directly sequenced. All sequences obtained are aligned to search possible RAPD altering the restriction enzymes recognition sites. At last, the method is used to genotype of these RAPD markers in large population samples of the chlorococcales [1-4] species complex. The physico-chemical parameters like phosphate and nitrate exhibit a positive correlation with the dynamics of chlorococcales. The results showed that 73 RAPD bands with PrimerOPW-0 6, as they showed geographical discrimination. Random Amplified Polymorphic DNA (RAPD) is the most abundant molecular markers in plants and animals and can be more relevant than other neutral markers mentioned above due to the high occurrence of RAPD in their functional genomic regions. Because of the potential for higher genotyping efficiency, data quality, genome-wide coverage, and analytical simplicity, RAPD have been used as molecular markers in evolutionary and ecological studies of a wide range of organisms in the current study, we report the characterization of 19 species of chlorococcales.

Keywords: Algal Strains, Chlorococcales, Genetic Diversity, Geographical Region, RAPD

Introduction

Algae are one of the most useful natural resources that can be used to produce different bioactive compounds such as vitamins, proteins, unsaturated fatty acids, antioxidants and carotenoids, including astaxanthin. During the past two decades, scientists have discovered that *Haematococcus,* a unicellular green alga, is the best source of organisms that produce astaxanthin, the most powerful naturally occurring antioxidant. Astaxanthin can be used as a preventive medicine, by being able to slow down degenerative diseases and cardiovascular problems, having anti-cancer and anti-immunological disease properties and finally, its ability to stimulate the proliferation of neural progenitor cells to recover stem cell function. Morphological traits observed through the light microscope have been traditionally used to determine the species and the diversity of Algae [5-8] which has a complex life cycle with different morphological stages affected by environmental conditions. The morphology alone is not able to recognize strains which have various shapes in diverse environmental conditions and the cryptic species (due to recent speciation) with similar morphological traits however they are different genetically. Molecular and genetic characters are affected less than the morphological characters by environmental conditions, hence they are more stable. In addition to the necessity of the morphological study, there is a need to the molecular study of organisms in order to differentiate them geographically. The combination of molecular and morphology provide a robust way to determine organisms with lower mistakes. Biotechnological methods and molecular markers are great promising tools for improvement and enhancement of biomass production, astaxanthin production and tolerance to stresses in Algae. Most of the molecular marker tools are valuable methods to investigate population genetic and diversity which were developed quickly over the three past decades. There have been some studies on algae using Inter Simple Sequence Repeat (ISSR) and Random Amplified Polymorphic DNA (RAPD) molecular markers; however, there have been none thus far on *H. pluvialis.* Therefore, this study was conducted for the first time with the aim of remedying this situation. ISSR markers are reliable, highly polymorphic, low

cost and less laborious, need only a small amount of DNA and are very fast when compared to most other molecular markers sequence data and in terms of reproducibility, ISSR is comparable to SSR. The RAPD technique has wide applications in breeding, genetic evolution, gene mapping and population genetics and is able to produce many markers with low cost and high speed. Although the reproducibility of RAPD technique is low and is dominant, it is one of the important molecular markers. The SNP technique is a dominant marker too but its reproducibility is higher than RAPD. Molecular and genetic study of any organism needs pure and axenic cultures whereas the growth of Algae is very difficult due to their sensitivity to contamination. The pH of medium is neutral and other algal species, bacteria or fungi easily can dominate and make a culture fail. The molecular markers are able to distinguish other strains of Algae with desirable properties from various parts of the world [9,10,1].

The advent of recombinant DNA technology heralded a morphologically completely approach to define potentially polymorphic DNA sequences. This new technology promises to revolutionize some areas of plant genetics and plant breeding. The markers based on DNA sequences have introduced a new dimension to the development of genetic maps and mapping of and physiologically important characters.

The objective of this study was to find out the genetic diversity and relationships of the different green unicellular algae, by using RAPD markers. There is a tendency to depend on the culture collection institutes that represents a limitation for scientists. This dependency on culture collections can deprive researchers from access to new species and strains with diverse characteristics and various bioactive compounds which can be found in other habitats. Four new strains were isolated from different cities of Tumkur in order to examine their diversity and to uncover their differences with CCAP (Culture Collection of Algae) strains, using molecular markers. If useful the new strains isolated from Tumkur water bodies could be deposited in culture collections in order to enrich the gene reserves.

In India there are thousands of collections of Algae whose genotypic status is not known. Besides an in-depth molecular marker genetic analysis of Algae has not been carried out so far. By making use of the molecular marker technology based on PCR approach, the diverse Algal species will be analyzed. The present investigation was carried out with the following objective isolation of genomic DNA from collecting samples of different phytoplankton.

Methodology

Collection and conservation of *Ankistrodesmus falcatus* (Corda) Ralfs, *Ankistrodesmus Spiralis* (Turner) Lemm. *V fasciculatus, Closteriopsis longissima Lemm, Coelastrum microsporum, Crucigenia crucifera, Gonatozygon kinahanii, Micractinium pussillum, Pediastrum duplex var. coronatum, P. duplex var. clathratum, P. duplex var. reticulatum, P. simplex, P. teras var. tetraedon, Scenedesumus accuminatus, S. arcuatus, S. bijiugatus var. bicellularis, S. dimorphous, S. platidiscus, S. protuberans, S. quadricauda var. maxima*

Genomic DNA Isolation: For RAPD

The 19 Algal samples were collected from different regions of Tumkur dist is subjected to DNA Extraction. Total DNA was extracted using Cetyl Trimethlyl Ammonium Bromide (CTAB) method described by 23 (1990) with minor modification. Quality and quantity of DNA will be analyzed by both gel electrophoresis and spectrometric assays using UV-visible double beam pc scanning spectrophotometer

DNA Amplification

Polymerase Chain Reaction (PCR) amplification was performed in a volume of 20 ul comprising 1.5 units of Taq DNA polymerase. 1x Taq assay buffer (10 mM Tris-Hcl, 1.5 mM Mgcl2, 50 mM Kcl add. 2.01 gel stained with PH 8.0) 200 um of primer, 200 um of dNTPs and 50 mM 50 DNA template. Amplification was performed in thermo cycler. The optimized PCR Condition for RAPD was determined. The amplified products are separated by electrophoresis on 1.25-1.5% (w/v) Agarose gels for 75-100 volts in 1x TBE buffer (Tris-Borate- EDTA buffer). After completion of electrophoresis, gels are stained with Ethidium bromide solution. The amplified products in gels are visualized and photographed using gel documentation bio profile image analysis system. The size of the amplification products are determined by comparisons to lambda DNA/EcoR-Hind lll double digest DNA ladder. PCR reaction was repeated at least twice to check the reproducibility of the banding. The PCR bands were then scored for analysis (Table 1).

Components	Range Used	Optimal Concentration
MgCl2	1.5mM, 2.5 mM, 3.5mM	1.5mM
Template DNA	10ng,15ng,20ng,25ng,30ng	25ng
dNTPs	100µM,200µM,300µM	200µM

Table 1: Details of the optimal concentrations of the PCR mix.

Results and Discussion

The genomic of were subjected to RAPD analysis using Primer OPW– 06. The genomic DNA of 19 *Ankistrodesmus falcatus (Corda) Ralfs, Ankistrodesmus Spiralis (Turner) Lemm. V fasciculatus, Closteriopsis longissima Lemm, Coelastrum microsporum, Crucigenia crucifera, Gonatozygon kinahanii, Micractinium pussillum,*

Pediastrum duplex var. coronatum, P. duplex var.clathratum, P. duplex var. reticulatum, P. simplex, P. teras var. tetraedon, Scenedesumus accuminatus, S. arcuatus, S. bijiugatus var. bicellularis, S. dimorphous, S. platidiscus, S. protuberans, S. quadricauda var. maxima. Rapd analysis of 19 species of chlorococcales algae amplified with primer, OPW-06 the genomic DNA of 19 species of chlorococcales was amplified with the oligonucleotide primer OPW-07 are shown in the figure. The distant and abundant RAPD fragments were recorded. The total number of bands are generated were found to be 73. The size of the RAPD band was placed in between 300 to 5000 bp length. The primer produced distinct banding patterns. The number of bands per primer is 3.8 as expected in Algae. The RAPD bands distributed in 19 species of chlorococcales [11] are known to important [12-14]. The number of RAPD bands produced to reveal Mundelein inherited character and number scoring revealed divers properties. The banding patterns are important and distinct in algae. The RAPD banding patterns showed high polymorphism, were useful in distinguishing algae. Although diverse elements and other characters revealed as many as RAPD bands. The identification of RAPD bands in 19 species of chlorococcales [15-19,2,3] is important because of differentially distributed. The marker distribution revealed both polymorphic and monomorphic character. Species [5-8] revealed 3 bands each where as other species showed more than 3-5 bands whereas variety 1 as not shown as shown bands as shown in figure 7. rapd analysis of 19 species of chlorococcales algae amplified with primer, opw-6 shown in the figure 1 as expected in algae. The RAPD bands distributed in 19 species of chlorococcales algae are known to important. The number of RAPD bands produced to reveal Mendelein inherited [20-22]

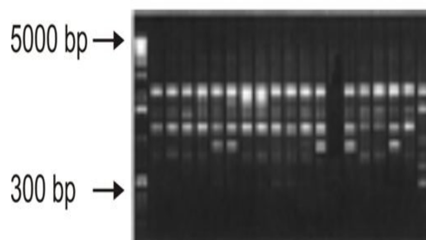

Figure 1: Gel profile of algae species amplified with primer Opw -6

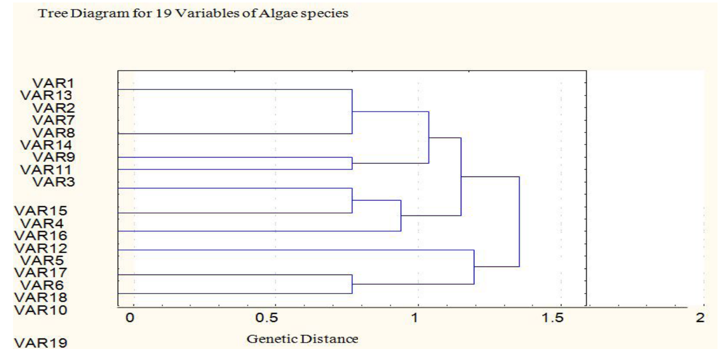

Figure 2: Genetic diversity of 19 varieties of algae.

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