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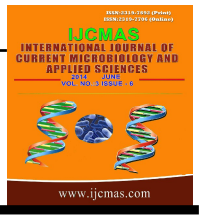


**Original Research Article**

# Biodiversity of Phytoplankton using RAPD molecular marker

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**Keywords**

Genetic diversity; Desmides RAPD; Strains; geographical region

**A B S T R A C T**

Algae collected from the Bangalore rural wetland ecosystem, by using a RAPD method. In this method, the Expressed Sequence database was used as the original genomic information for identification of polymorphic sites. Primers OPW-01 RAPD is designed and the amplified PCR products are then directly sequenced. 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 10 species of Desmids 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 Desmids species complex. The physico-chemical parameters like phosphate and nitrate exhibit a positive correlation with the dynamics of Desmids The results showed that 46 RAPD bands are useful for genetic diversity studies of Desmids as they showed geographical discrimination.

## Introduction

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 [5]. 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 Desmids species unicellular green algae 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 [1-4]. Morphological traits observed through the light microscope have been traditionally used to determine the species and the diversity of Desmid species, which has a complex life cycle with different morphological stages affected by environmental conditions. Molecular and genetic characters are affected less than the morphological characters by environmental conditions, hence they are more stable [6]. In addition to the necessity of the morphological study, there is a need for the molecular study of organisms in order to differentiate them geographically [7]. The combination of molecular and morphology provide a robust way to determine the organisms with lower mistakes. Biotechnological methods and molecular markers are great promising tools for improvement and enhancement of biomass production, and tolerance to stress in Desmids. Most of the molecular marker tools are valuable methods to investigate population genetic and diversity which were developed quickly over the three past decades [8]. There have been some studies on algae using Random Amplified Polymorphic DNA (RAPD) molecular markers [9-12], however, there have been none thus far on Desmids.

Therefore, this study was conducted for the first time with the aim of remedying this situation. RAPD 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 [13]. And RAPD do not require DNA sequence data and in terms of reproducibility, 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 [15]. The ISSR technique is a dominant marker too but its reproducibility is higher. Molecular and genetic study of any organism needs pure and cultures whereas the growth of Desmids *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 Desmids with desirable properties from various parts of the world. The objective of this study was to find out the genetic diversity of the green unicellular alga, Desmids 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 Iran in order to examine their diversity and to uncover their differences with CCAP (Culture Collection of Algae and Protozoa) strains, using molecular markers. If useful the new strains isolated from Iran water bodies could be deposited in culture collections in order to enrich the gene reserves.

## Materials and Methods

The 10 Desmides species of Algae such as *Closterium acutum* (Lyngb) *Breb, C. gracile, C. ehrenbergii menegh, C. parvulum Nag., Closterium acerosum* (schrank) *Ehreno, C. lunula* (Mull.) Nitzsch, C. *remarginatum, C. subtrigosum, Cosmarium quadrum* Lund. V. *minus* Nordst, C. *Protruberans* which were collected from the different region of Gubbi maintained at the Department of Botany, Siddaganga first Grade College of arts, science and commerce, B.H.Road, Tumkur

### DNA Extraction

The 10 Desmids species of Algae were collected and immediately kept in ice to reduce the nuclease activity. It was brought to the laboratory, weighed (2 gms each), and frozen in liquid nitrogen and stored at -70ºC till further use. The DNA was extracted using the CTAB method (Porebski *et. al*., 1997) with certain modifications. 2gms of Algae material was ground into a fine powder using liquid nitrogen. The powder was then transferred to sterile centrifuge tubes and 12ml of extraction buffer was added, mixed thoroughly and incubated 65ºC in a water bath for one hour with intermittent shaking. The tubes were brought to room temperature and centrifuged at 8000 rpm for 10 min at 4ºC.

The supernatant was transferred to new tubes, 6 ml of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly. The tubes were centrifuged at 8000 rpm for 10min at 4ºC. The supernatant was transferred to new tubes and repeated the same steps twice. The DNA was then precipitated by adding half volume of 5M NaCl, an equal volume of chilled propanol and incubated at 4ºC over night. DNA was pelleted by centrifuging at 20,000 rpm for 12 min at 4ºC. The pellets were dried after adding 70% ethanol and 1ml of TE buffer was added to which 20 µl of RNase was added. This was incubated at 37ºC for one hour and added 300 µl of saturated phenol. It was mixed, centrifuged at 8000rpm for 10 min at 4ºC. The supernatant was transferred to another tube and repeated the same process by adding phenol: chloroform and chloroform respectively. The supernatant was treated with equal volume of isopropanol and incubated at 4ºC for overnight. The DNA was pelleted by centrifuging at 12000 rpm for 20min. The pellet was washed with 70% ethanol and dried. Around 300 µl of TE buffer was added to dissolve the pellet and stored at -20ºC for further use.

### Data Analysis

DNA binding patterns generated by RAPD were scored as ' 1 ' for the presence of band and '0' for its absence. All RAPD assays were performed twice and only the reproducible bands were scored. A similarity matrix was generated using a dendrogram was constructed based on distance matrix data sets by applying Wards method for cluster analysis using 'STATISTICA' 5.0

**Table.1.**Sequence information of RAPD oligonculeotide primers used for amplification and polymorphism study in 10 Desmides species of Algae

| OPW1-10 |
|---|
| CTCAGTGTCC |
| GTGAGGCGTC |
| GGGGGTCTTT |
| CCGCATCTAC |
| GATGACCGCC |
| GAACGGACTC |
| GTCCCGACGA |
| TGGACCGGTG |
| CTCACCGTCC |
| TGTCTGGGTG |

## Results and Discussion

The genomic of 10 Desmides species of Algae such as *Closterium acutum* (Lyngb) *Breb, C. gracile, C. ehrenbergii menegh, C. parvulum Nag., Closterium acerosum* (schrank) *Ehreno, C. lunula* (Mull.) Nitzsch, C. *remarginatum, C. subtrigosum, Cosmarium quadrum* Lund. V. *minus* Nordst, C. *Protruberans* were subjected to RAPD analysis using Primer OPW– 01 The genomic DNA of 10 Desmids species of Algae was amplified with decamer oligonucleotide primers such as OPW– 01 and as shown in Fig 1. The distinct and abundant RAPD fragments were recorded. The total numbers of bands were generated 46 RAPD gel profiles.

The sizes of the RAPD bands were placed in between 300 – 5000bp in length. The primer produced distinct polymorphic banding pattern in all the plant medicinal plant species, the number of RAPD bands per primer were 4.6 as expected IN Algae. The RAPD bands distributed in the Algae is important to know the value of breeding patterns in Algae. The number of RAPD bands was produced to reveals Mendelian inherited characters, and number scoring revealed characters. The banding patterns are important and distinct in Algae. The polymorphism was very high and RAPD values were useful to distinguish between the Algal species, apparently diverse elements species character.

The identification of RAPD is very unique in Algae, because chemical value revealed heterozygous character. In the present data the Algae like 1 showed 6 bands and remaing was reveled 10 to 2 RAPD bands respectively. Further, 1 and 2 showed 6 and 10 RAPD bands due to amplification of primer with the genomic DNA of these Algae species. However, it was observed that some of the Algae viz., 9 and 10 were recorded 2 and 0 bands indicating diverse character compared to other Algae. Polymorphic distribution as far as gene flow is concerned revealed high or low speciation. This has been used for various other calculations of Algae breeding programs. Therefore, amplification of genomic DNA of these Algae revealed moderate diversity among them

The results presented in Fig. 1 indicate the polymorphisms among the studied Desmides strains. It should be noted that the molecular weights of the bands ranged from This table depicts the total number of bands produced, their polymorphism, sequence and annealing temperature for 0PW-01 primers. Most of the primers produced bands that were 100% polymorphic and their annealing temperature varied from 45 °C to 67 °C. Maximum bands were obtained by more polymorphic bands of RAPD and it can be more powerful tool to investigate biodiversity. The higher annealing temperatures will provide the higher reproducibility and specific attachment of the primers to the template. Depicted that the maximum annealing temperature RAPD primer was 45 °C and67 °C respectively

The value of the average Shannon's indices for RAPD was 0.509 with a standard deviation of 0.144. The AMOVA tests for the bands generated by the RAPD primers were 74% within the populations and 26% among the populations ($p < 0.01$). On the other word based on the common loci investigated only 26% of the total variation in the *Desmides species* may be attributed to variation among the strains.

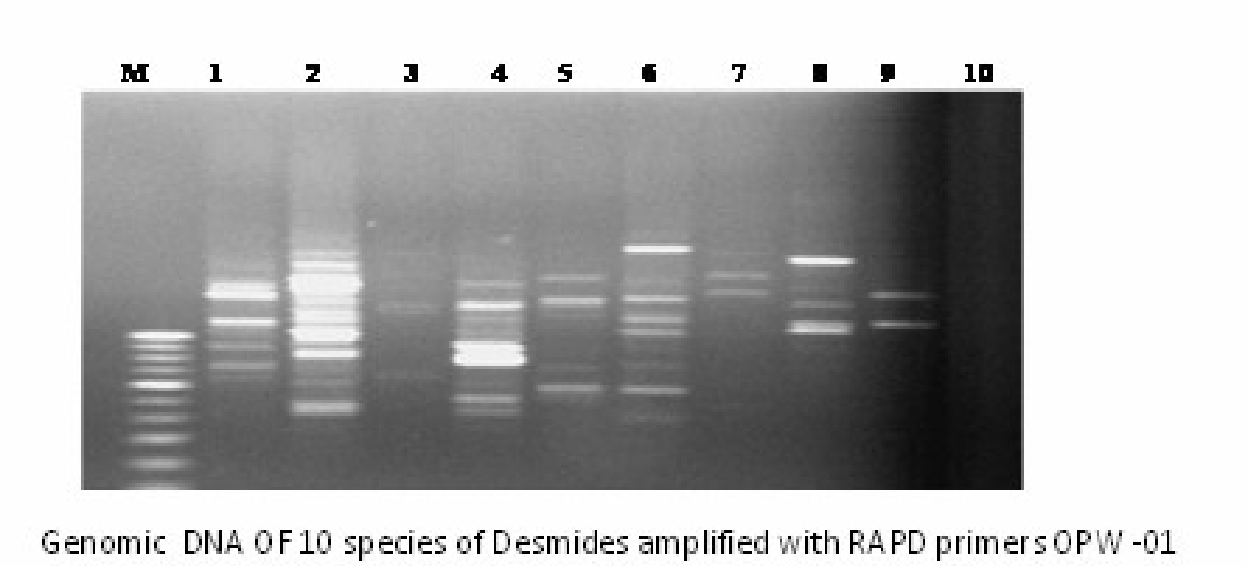
The algal strains belonging to the different geographical populations clustered

separately.The dendrogram constructed using the UPGMA method based on Jacard's similarity matrix showed the genetic variation among the ten *Desmides* strains studied. The species 2 showed high genetic diversity using the RAPD technique, even between strains from same bloom [16]. RAPD is a quick, produce sufficient polymorphism and reliable fingerprint method to distinguish potato cultivars [17]. The regression and association between the genetic diversity and geographical locations were visible in many cases [18]. The dendrogram distinctly separated the algae into three clusters based on the 0.74 reference line, with the strains from Iran making up two clusters, Finland one cluster, Switzerland one cluster and USA another clusters of Algae belongs to other group , therefore the dendrogram successfully grouped all the *Desmides species* based on their geographical origins (Figure.1). The results of the UPGMA and Wards methods dendrograms whether based on the RAPD marker data separately (data not shown) or the combined (pooled) RAPD markers were generally similar and supported one another with minor differences. For example, the RAPD dendrograms distinguished clearly between the ten species however in the RAPD data based dendrogram, the Finland1, 2 were clustered with the Iran major group. This small difference could be due to the different primer attachment sites of the two marker systems in the algal genome.

Figure 1 and showed the analysis where strains of *Desmides* from different Bangalore rural wetland ecosystem were grouped in four separate places and confirm the results of dendrogram. The Switzerland strain was grouped in the major branch of USA and it is more similar to the USA strain. The diversity in different strains of *Desmides* was high, based on RAPD molecular markers. Bhau [19] reported that RAPD markers were more informative than ISSR markers whilst others have reported that RAPD [13,20,22].

Similar investigation of Noroozi Mostafa Hishamuddin Omar Soon Guan Tan and Suhaimi Napis According to this study, RAPD markers for *Haematococcus pluvialis* strains were more diverse as they produced more polymorphic bands with higher annealing temperature however its results were the same as the RAPD results confirming each other with small differences. It is important to find different species of Desmides to discover the source of useful genes and desired characteristics such as a thin cell wall or high growth rate species living a common area or near to each other have genetic relationships and genetic exchanges during sexual reproduction, which explains the Wards results illustrated by the dendrogram clusters of strains according to their geographical location.

The massive genetic diversity of the organisms provides scientists with good opportunities to find new bioactive compounds. Although morphological characteristics are useful for the detection the species and genetic diversity, they depend on environmental conditions and vary under diverse conditions and they are not precise enough to detect the strains and populations, hence the use of the RAPD molecular marker have proven to be more powerful methods to distinguish species geographically.




Genomic DNA OF 10 species of Desmides amplified with RAPD primers OPW -01

## References


1. Ahmad, I., Akhar, K., Hussain, T., 2008. Hughes, D.A. Effects of dietary antioxidants on the immune function of middle-aged adults. *Proc. Nutr. Soc*. 1999, *58*, 79–84.
2. Grossa, G.J.; Lockwood, S.F. Cardioprotection and myocardial salvage by a disodium disuccinate astaxanthin derivative (CardaxTM). *Life Sci.* 2004, *75*, 215–224. *Molecules* 2011, *16* 2607
3. Samuel, F.L.; Marc, S.P.; Stanley, L.; Hazen, Z.B.; Ferenc, Z. The effects of oral Cardax™ (disodium disuccinate astaxanthin) on multiple independent oxidative stress markers in a mouse peritoneal inflammation model: influence on 5-lipoxygenase *in vitro* and *in vivo. Life Sci.* 2006,*79*, 162–174.
4. Kim, J.; Nam, S.; Kim, B.; Choi, W.; Lee, J.; Kim, W.; Choi, Y. Astaxanthin Improves Stem Cell Potency via an Increase in the Proliferation of Neural Progenitor Cells. *Int. J. Mol. Sci.* 2010, *11*,5109–5119.
5. Cianciola, E.; Popolizio, T.; Schneider, C.; Lane, C. Using Molecular-Assisted Alpha Taxonomy to Better Understand Red Algal Biodiversity in Bermuda. *Diversity* 2010, *2*, 946–958.
6. Park, Y.; Lee, J.K.; Kim, N. Simple Sequence Repeat Polymorphisms (SSRPs) for Evaluation of Molecular Diversity and Germplasm Classification of Minor Crops. *Molecules* 2009, *14*, 4546–4569.
7. Bittencourt-Oliveira, M.C.; Massola, J.N.S.; Hernandez-Marine, M.; Romo, S.; Moura, A.N. Taxonomic investigation using DNA fingerprinting in *Geitlerinema* species (Oscillatoriales, Cyanobacteria). *Phycol. Res.* 2007, *55*, 214–221.
8. Arif, I.; Bakir, M.; Khan, H.; Al Farhan, A.; Al Homaidan, A.; Bahkali, A.; Al Sadoon, M.; Shobrak, M. A Brief Review of Molecular Techniques to Assess Plant Diversity. *Int. J. Mol. Sci.* 2010, *11*, 2079–2096.
9. Hall, M.M.; Vis, M.L. Genetic variation in *Batrachospermum helminthosum* Batrachospermales, Rhodophyta) among and within stream reaches using intersimple sequence repeat molecular markers. *Phycol. Res.* 2002, *50*, 155–162.

10.Bornet, B.; Antoine, E.; Francoise, S.; Marcaillou, Le.; Baut, C. Development of sequence characterized amplified region markers from inter simple sequence repeat fingerprints for the molecular detection of toxic phytoplankton *Alexanndrium catenella* (Dinophyceae) and *pseudo - Nitzschia Pseudodelicatissiam* (Bacillariophyceae) from French coastline waters. *J. Phycol.* 2005,*41*, 704–711.
11.Dayananda, C.; Sarada, R.; Kumar, V.; Aswathanarayana, R.G. Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electron. J. Biotechnol.* 2007, *10*, 78–91.
12.House D.L.; Sherwood, R.A.; Vis, L.M. Comparison of three organelle markers for phylogeographic inference in *Batrachospermum helminthosum* (Batrachospermales, Rhodophyta) from North America. *Phycol. Res.* 2008, *56*, 69–75.
13.Zietkiewicz, E.; Rafalski, A.; Labuda, D. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genetics* 1994, *20*, 176–183.
14.Bornet, B.; Branchard, M. Nonanchored Inter Simple Sequence Repeat (ISSR) Markers: Reproducible and Specific Tools for Genome Fingerprinting. *Plant Mol. Biol. Rep.* 2001, *19*, 209–215.
15.Bardakci F. Random Amplified Polymorphic DNA (RAPD) Markers. *Turk. J. Biol.* 2001, *25*, 185–196.
16.Martı´nez, R.; An˜ı´barro, C.; Ferna´ndez, S. Genetic variability among *Alexandrium tamarense* and *Alexandrium minutum* strains studied by RAPD banding pattern analysis. *Harmful Algae* 2006, *5*, 599–607.
17.Prevost, A.; Wilkinson, M.J. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 1999, *98*, 107–112.
18. Singh, R.; Zaki, N.M.; Ting, N.; Rosli, R.; Soon-Guan, T.; Leslie, L.E.; Ithnin, M.; Cheah, S. Exploiting an oil palm EST database for the development of gene-derived SSR markers and their exploitation for assessment of genetic diversity. *Biologia* 2008, *63*, 227–235.
19. Bhau, B.S.; Medhi, K.; Sarkar, T.; Saikia, S.P. PCR based molecular characterization of *Nepenthes khasiana* Hook Pitcher. *Plant Genet. Resour. Crop. Evol.* 2009, *56*, 1183–1193.
20. Ferna´ndez, M.; Figueiras, A.; Benito, C. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet.* 2002, *104*, 845–851.
21. Zhao, K.G.; Zhou, M.Q.; Chen, L.Q. Genetic diversity and discrimination of *Chimonanthus praecox* (L.) Link germplasm using ISSR and RAPD markers. *HortScience* 2007, *42*, 1144–1148.
22. Rahimmalek, M.; Bahreininejad, B.; Khorrami, M.; Tabatabaei, B.E.S. Genetic Variability and Geographic Differentiation in *Thymus daenensis* subsp. *daenensis*, an Endangered Medicinal Plant, as Revealed by Inter Simple Sequence Repeat (ISSR) Markers. *Biochem. Genet.* 2009, *47*, 831–842.
23. Dellaporta, S.L.; Wood, J.; Hicks, J.B. A plant DNA mini-preparation

Version II. *Plant Mol. Biol.Reptr*. 1983, *1*, 19–21.
24. Bornet, B.; Antoine, E.; Bardouil, M.; Marcaillou-Le, Baut, C. ISSR as new markers for genetic characterization and evaluation of relationships among phytoplankton. *J. Appl. Phycol.* 2004, *16*, 285–290.
25.Rohlf, F. *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System*; Exeter Software: New York, NY, USA, 2001.
26. Huangfu, C.; Song, X.; Qiang, S. ISSR variation within and among wild *Brassica juncea* populations: implication for herbicide resistance evolution. *Genet. Resour. Crop. Evol.* 2009, *56*, 913–924.
27. Sokal, R.R.; Sneath, P.H.A. *Principles of Numerical Taxonomy*; W. H. Freeman: San Francisco, CA, USA, 1963. *Sample Availability:* Samples of the ten of *H. pluvialis* strains are available from the authors. © 2011 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).