Genes, Genomes and Genomics ©2010 Global Science Books

Assessment of Genetic Polymorphism among Green Microalgae *Botryococcus* of Distinct Origin by RAPD

Dayananda Chandrappa • Venkatachalam Lakshmanan • Bhagyalakshmi Neelwarne • Ravishankar Gokare Aswathanarayana*

Department of Plant Cell Biotechnology, Central Food Technological Research Institute (Constituent laboratory of CSIR), Mysore- 570 020, India

Corresponding author: * **pcbt@cftri.res.in**

ABSTRACT

Genetic variation and relationships among 7 strains of *Botryococcus* sp. belonging to different geographical locations and different chemical races were evaluated using 35 decamer RAPD primers. Several RAPD primers were selected after preliminary screening for their ability to produce clear and multiple bands. The analyses resulted in the amplification of a total of 407 bands of 100-3000 bp, 380 of which were polymorphic, corresponding to 93.3% genetic diversity. The ability to distinguish genotypes and the Resolving power (Rp) of the primer showed a linear relationship. From these data, a genetic similarity matrix and dendrogram were obtained by using the unweighted pair group method with arithmetic means (UPGMA). The RAPD analysis produced genetic similarity coefficients ranging from 0.3312 to 0.7388. The study resulted in the identification of genetic relationship among various strains of *Botryococcus* sp. belonging to different climatic zones and origins. The study also revealed clear genetic distances between A race strains of *B. braunii* and B race strains of the same species.

Keywords: *Botryococcus* sp., genetic diversity, polymorphism
Abbreviations: PCR, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA; **Rp**, Resolving power; **SEM**, scanning electron microscopy; **UPGMA**, unweighted pair group method with arithmetic mean

INTRODUCTION

Botryococcus is a colonial unicellular green microalgae recognized as a potential source of lipids and liquid hydrocarbons. *Botryococcus* sp. were found to be present in geographical regions belonging to different climatic zones like continental, temperate, tropical and alpine, that indicates their cosmopolitan nature (Wolf *et al*. 1985; Metzger *et al*. 1985; Okada *et al*. 2000). In addition, fossil studies revealed that *Botryococcus* sp. are the major hydrocarbon sources in a variety of oil-rich deposits across the world and are dated from Ordovician period to the present (Cane 1977). *Botryococcus* sp. were also reported to produce bioactive carotenoids like β-carotene, lutein, violaxanthin, echinenone, botryoxanthin-A, botryoxanthin-B, and α-botryoxanthin-A and many other such bioactive molecules of importance in pharmaceutical and nutraceutical applications. The algae *B. braunii* is also known to produce large amounts (5-42% on dry weight) of lipids (Metzger and Largeau 2005), hydrocarbons (2-76%, w/w) and considerable amounts of exopolysaccharides (0.25-5.5 $kg.m^{-3}$) (Casadevall *et al*. 1985; Metzger *et al*. 1985; Fernandes *et al*. 1989; Allard and Casadevall 1990; Sawayama *et al*. 1994; Metzger and Largeau 2005; Dayananda *et al*. 2007; Ranga Rao *et al*. 2007; Eroglu and Melis 2010).

Taking these facts into consideration, the organism *Botryococcus* is gaining importance as a potential source from food to biofuel. There are more than 13 species of the genus *Botryococcus* reported to date, and among them *B. braunii* is being characterized and worked out in detail. This is may be because of their ability to produce large amounts of lipids and liquid hydrocarbons. *B. braunii* is classified into A, B and L races based on the type of hydrocarbons produced (Metzger and Largeau 2005). Race – A strains produce C_{21} to C_{33} odd numbered *n*-alkadienes, mono-, tri-, tetra-, and pentaenes and they are derived from fatty acids (Metzger and Largeau 2005). The L race strains produce a single tetraterpene hydrocarbon known as lycopadiene (C_{40}-C_{78}). The B race strains produce two types of triterpenes called botryococcenes of C_{30}-C_{37} of general formula C_nH_{2n-10} as major hydrocarbons and small amounts of methyl branched squalene (Achitouv *et al*. 2004). Therefore there is an increasing quest to isolate newer species and strains of the genus *Botryococcus* which are capable to produce lipids and other chemicals of industrial importance. Morphological heterogeneity and the chemical nature of hydrocarbons, and the degree of lipid and polysaccharide production were found to exist within the species and among the other species of the genus *Botryococcus*.

Therefore the present study focused on finding genetic polymorphism in different species of the genus *Botryococcus* as well as within the species belonging to different chemical races of *B. brauni* using RAPD (random amplified polymorphic DNA) technique. RAPD technique is being used widely as an efficient technique in detecting genetic variations (Williams *et al*. 1990) even in closely related organisms such as two near isogenic lines (NIL). At present, RAPD markers have been successfully applied to detect the genetic similarities or dissimilarities in various plants, algae, fungi and bacteria, etc. (Carvalho *et al*. 2004; Comeau *et al*. 2004; Martins *et al*. 2004; Ramage *et al*. 2004; Modgil *et al*. 2005; Martinez *et al.* 2006; Touzet *et al.* 2007; Zhao *et al.* 2007, 2008; Olmos *et al.* 2009; Small *et al.* 2009; Tilman *et al.* 2009). RAPD only requires a small amount of DNA and is simpler, cheaper and faster. Therefore, the study was employed here to differentiate the genetic variations among the different chemical races of *B. braunii* and between the indigenous strains of *Botryococcus*.

Received: 24 March, 2010. Accepted: 14 May, 2010.

Original Research Paper

MATERIALS AND METHODS

Algal strains

Botryococcus braunii (LB 572) was procured from the University of Texas, U.S.A. *Botryococcus braunii* (SAG 30.81) from the Sammlung von AlgenKulturen, pflanzenphysiologisches Institut, Universitat Gottingen, Germany. *Botryococcus braunii* (Strain 1) and *Botryococcus braunii* (Strain 2) from university of Berkeley, UK. *Botryococcus* sp. (DB-8) was obtained from the University of Pune, Pune, India and *Botryococcus* sp. (MCRC) from Murugappa Chattiar Research Centre, Chennai, India. *Botryococcus* sp. (CFTRI) was also used in this study. Stock cultures were maintained routinely on both liquid and agar slants of modified Chu 13 medium (Largeau *et al.* 1980) by regular sub culturing at two-week intervals. Cultures were maintained at 25 ± 1°C with 1.2 ± 0.2 klux and a 16: 8 light: dark cycle.

Scanning electron microscopy

The algal cells were observed under scanning electron microscopy (SEM) according to the method of Fowke *et al.* for cellular details (Fowke *et al*. 1994). The samples were fixed in 2% glutaraldehyde in 0.2 M phosphate buffer (pH 6.8) for 12 hrs, dried in alcohol series up to 100%, sputter coated with gold and examined in a LEO Scanning Electron Microscope 435 VP (Leo Electron Microscopy Ltd. Cambridge UK).

Preparation of template DNA

The genomic DNA from the two weeks grown cultures was extracted by using the GenElute^{TM} Plant Genomic DNA Mini prep kit supplied by Sigma (USA). RNA contamination in all the samples were removed using RNase-A by following the manufacturers protocol (100 µg mL^{-1}; Bangalore Genei, India) for 30 min at 37°C. The quality and quantity of DNA were analyzed by standard spectrophotometry and the samples were diluted to 25 ng μL^{-1}.

DNA amplification

RAPD amplifications were performed using PCR mixture (25 µl) having 1 µl of genomic DNA as template, 1X PCR buffer, 200 µM dNTPs, 1 unit (U) of *Taq* DNA polymerase and 1 µM of each primer (**Table 2**) with an initial denaturation at 93ºC for 4 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 36°C and 2 min extension at 72°C with a terminal extension of 72°C for 10 min using a thermal cycler (Eppendorf Thermal cycler). The amplified fragments were eletrophoretically separated in 2% agarose gels in TAE and stained in ethidium bromide (0.001%) and documented in a gel documentation system (Hero-Lab GMBH, Germany). The size of the amplification products was estimated from a 10-kb DNA ladder (Fermentas GMBH, Germany).

Table 1 *Botryococcus* strains used for the study.

Algal strains
Botryococcus brauni [Strain 1]
Botryococcus brauni [Strain 2]
Botryococcus brauni [LB-572]
Botryococcus brauni [SAG 30.81]
Botryococcus sp. [DB-8]
Botryococcus sp. [MCRC]
Botryococcus sp.[CFTRI]

Table 2 List of RAPD primers selected from Operon Technologies.

Primer	Primer sequence (5'–3')	TNB	NPB	%PB	Rp	AFS
OPA 03	AGTCAGCCAC	15	15	100	7.71	250-3000
OPA 04	AATCGGGCTG	14	11	78.57	5.14	200-3000
OPA 09	GGGTAACGCC	16	14	87.50	7.14	250-2200
OPA 11	CAATCGCCGT	13	12	92.31	7.14	200-2500
OPC 06	GAACGGACTC	12	11	91.66	6.85	200-1500
OPC 08	TGGACCGGTG	11	11	100	6.28	200-1500
OPC 13	AAGCCTCGTC	11	11	100	5.43	350-1500
OPD 07	TTGGCACGGG	15	15	100	8.86	250-2000
OPD 08	GTGTGCCCCA	12	11	91.66	7.71	250-1800
OPD 11	AGCGCCATTG	13	9	69.23	5.14	250-3000
OPD 16	AGGGCGTAAG	11	10	90.91	6.29	400-2500
OPB 07	GGTGACGCGA	12	12	100	7.71	100-1500
OPC 03	GGGGGTCTTT	14	9	64.28	6.28	300-3000
OPF 12	ACGGTACCAG	14	11	78.57	6.00	300-3000
OPM 20	AGGTCTTGGG	11	11	100	6.57	400-2000
OPA 20	GTTGCGATCC	11	11	100	7.14	400-2500
OPJ 10	AAGCCCGAGG	13	13	100	6.86	200-2500
OPN 09	TGCCGGCTTG	12	11	91.67	7.14	200- 2000
OPN 10	ACAACTGGGG	10	9	90	5.71	350-1500
OPN 14	TCGTGCGGGT	5	5	100	3.43	700-3500
OPC 01	TTCGAGCCAG	11	11	100	7.43	200-2500
OPC 05	GATGACCGCC	10	10	100	5.71	350-1500
OPJ 18	TGGTCGCAGA	9	9	100	5.14	400-2000
OPJ 20	AAGCGGCCTC	10	10	100	6.00	250-1500
OPJ 09	TGAGCCTCAC	9	9	100	6.00	400-2000
OPJ 01	CCCGGCATAA	11	9	81.81	6.57	300-1300
OPJ 11	GTCCCGTGGT	13	13	100	8.29	250-2000
OPJ 19	GGACACCACT	11	10	90.91	5.71	200-1500
OPJ 13	CCACACTACC	10	10	100	6.00	200-2500
OPC 02	GTGAGGCGTC	11	11	100	6.57	200-2500
OPN 06	GAGACGCACA	12	12	100	4.29	400-2500
OPC 9	CTCACCGTCC	9	8	88.88	3.71	200-1200
OPJ 11	ACTCCTGCGA	14	14	100	8.29	400- 2500
OPN 4	GAGACGCACA	10	10	100	4.85	400-1500
OPC 7	GTCCCGACGA	12	12	100	8.85	300-3000
Total		407	380	93.3	223.94	

Total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (%PB), resolving power (Rp) and amplified fragment size (AFS) obtained per RAPD primer

Resolving power (Rp)

Resolving power (Rp) for each primer was calculated following the method of Prevost and Wilkinson's (1999) for selecting primers that can distinguish a maximal number of accessions. Resolving power (Rp) of a primer is = Ib where Ib (band informativeness) takes the value of: Ib = 1 - [2 x |0.5 - p|], p being the proportion of the 7 genotypes (algal strains analyzed) containing the bands.

Data analysis

The well-resolved RAPD fragments ranging from 100 to 3000 bp were scored as present (1) or absent (0) for each primer analysis. Bands with the same migration distance were considered homologous. The data was computed and analyzed with NTSYS pc (Rohlf 1998) version 2.02 using the simple matching coefficient (Sokal and Michener 1958). Cluster analyses were obtained based on similarity matrices, using the unweighted pair group method with arithmetic mean (UPGMA), and relationships between accessions were visualized as dendrograms.

RESULTS AND DISCUSSION

Selection of primers and RAPD analysis

Three indigenous strains of *Botryococcus* and four strains, two each of race-A and race-B were subjected for genetic analysis using RAPD markers (**Table 1**). Several decamer primers were screened for their ability to amplify DNA fragments. Based on the results of their ability to produce good number of distinct bands, 35 RAPD primers were selected. DNA samples from all the 7 strains of *Botryococcus* were amplified using the decamers listed in **Table 2**, where all the primers produced polymorphic bands. The majority of band positions varied between the strains. The polymorphisms were scored visually based on the presence or absence of amplified fragments. All the primers used in the study produced large numbers of polymorphic bands (**Table 2**). The total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (%PB), Resolving power (Rp), amplified fragment size (AFS) obtained for each primer are detailed in **Table 2**. The total amplified products of 35 RAPD primers was 407 (average of 11.62 bands per primer) ranging from 100 to 3000 bp, of which 380 were polymorphic (93.3%). The number of bands for each RAPD primer varied from 5 (OPN 14) to 16 (OPA-09). The resolving power of the 35 RAPD primers ranged from 3.43 for primer OPN-14 to 8.86 for primer OPD-07 and the samples of RAPD analysis are visualized in **Fig. 1**.

Molecular analysis and fingerprinting of *Botryococcus* strains

The genetic similarity coefficients for 7 strains of *Botryococcus* species were obtained with RAPD markers ranged from 0.3312 to 0.7388 between the strains evaluated. The unweighted pair group method with arithmetic mean (UPGMA) analysis made it possible to discriminate all the genotypes of *Botryococcus* sp. used in this study. The dendrogram obtained based on RAPD data showed a clear distinction into two major clusters (**Fig. 2**). The dendrogram obtained using percent disagreement coefficient (**Table 3**) showed the presence of 2 main clusters (Cluster 1 and 2, having 2 and 5 strains in each, respectively). The cluster 1 had two strains (Strains 1 and 2) which belong to B race, while the A race strains LB -572 and SAG 30.81 formed a sub-cluster A1 in cluster 2 and the other 3 indigenous strains (CFTRI, MCRC and DB-8)formed an another sub cluster A2 of cluster 2.

The similarity coefficient matrix (**Table 3**) and the dendrogram data have shown clear distinction amomg all the strains of *Botryococcus* species studied and have also revealed a genetic relationship between the race A and race B strains. All the indigenous strains have shown their gene-

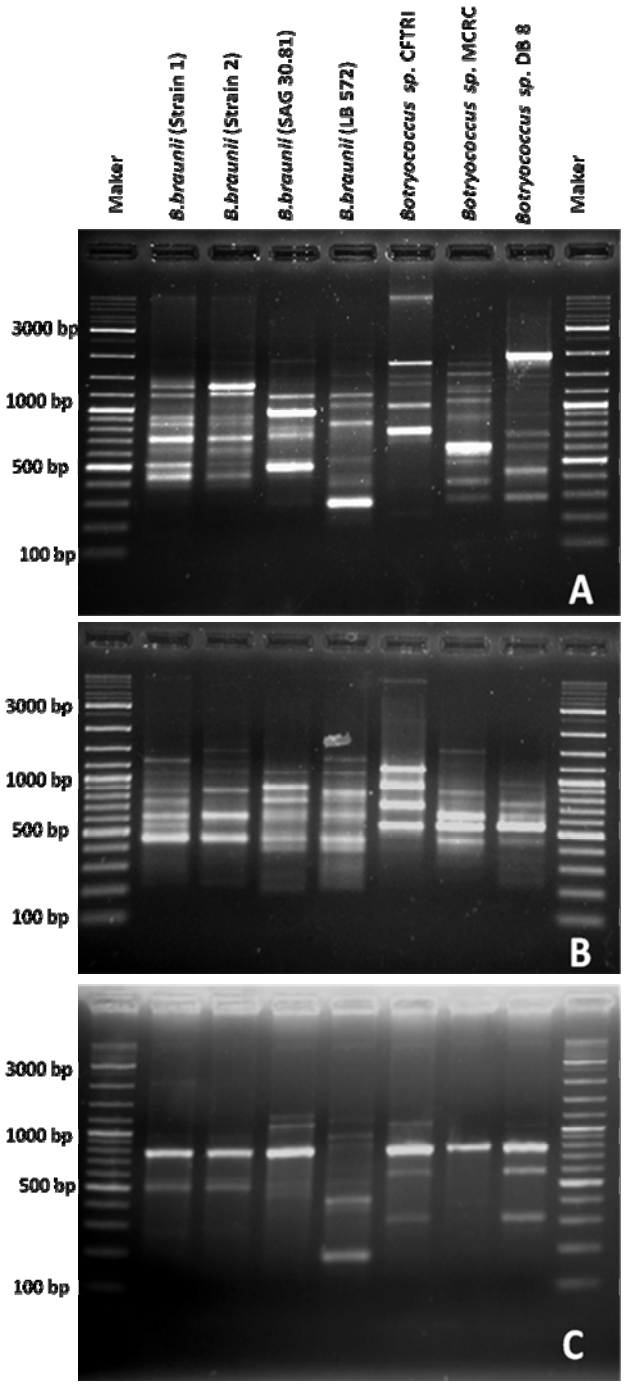

Fig. 1 RAPD profile of DNA from 7 strains *of Botryococcus*. Primers OPC-07 (**A**), OPC-09 (**B**) and OPN-04 (**C**). Lane marker represents 10 kbp GeneRuler DNA ladder.

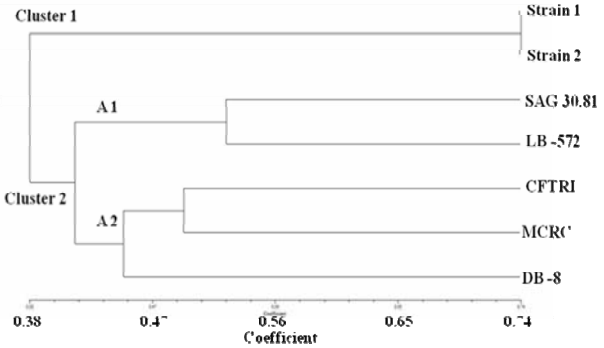

Fig. 2 Dendrogram displaying the genetic distances among *Botryococcus* strains obtained from cluster analysis of RAPD data.

Table 3 Genetic similarity coefficient among the 7 strains of *Botryococcus* species based on RAPD analysis.

Strains	Strain 1	Strain 2	SAG-0.81	LB-572	CFTRI	MCRC	DB-8
Strain 1	1						
Strain 2	**0.7388**	1					
SAG-30.81	0.3846	0.3806	1				
LB-572	0.4359	0.3918	0.5254	1			
CFTRI	0.3896	0.3766	0.4333	0.4481	1		
MCRC	0.3980	0.3940	0.4474	0.4430	0.4947	1	
DB-8	0.3485	**0.3312**	0.3595	0.3628	0.4256	0.4765	1

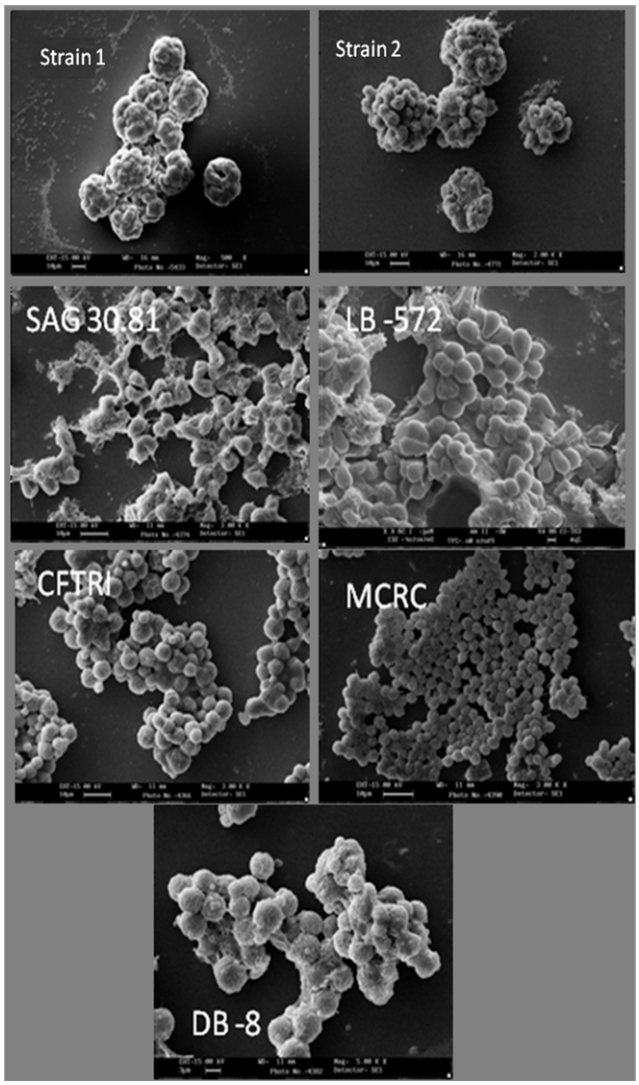

Fig. 3 Scanning electron microscopic images of different strains of *Botryococcus sp*. used for RAPD analysis.

tic relationship with A race strains and among the indigenous strains, *Botryococcus* sp. (DB-8) has shown its genetic diversity with that of the other two indigenous strains (MCRC and CFTRI). Morphological details of 7 strains of *Botryococcus* species (**Fig. 3**) shows that the features of A race strains, B race strains and indigenous strains were found to substantiate the observations of RAPD data as well. Gomez and Gonzalez (2004) have also used RAPD technique to see the genetic variations among the several strains of microalgae *Dunaliella salina.* The detection of genetic variations of strains with industrial prospective has a great relevance in applied phycology because it allows the differentiation of phenotypic variation into environmental and genetic components. In addition genetic diversity studies will also give the details of exotic genotypes. RAPD has been successfully used to identify the genetic variation in both micro- and macroalgae (Neilan 1995; Nishihara *et al*. 1997; Bolch *et al*. 1999a, 1999b; Gomez and Gonzalez 2004; Martínez *et al.* 2006; Touzet *et al.* 2007; Zhao *et al.* 2007, 2008; Olmos *et al.* 2009; Small *et al.* 2009; Tilman *et al.* 2009).

The genetic diversity among the different strains of *Botryococcus* is a clear demonstration of the genome participation in determining the attributes related to production of different nature of hydrocarbons and morphological features. The results of the study are very important since the existence of genetically characterized strains will reduce incorrect assumptions about the biotechnological important traits of different strains, which could be due to phenotypic flexibility.

In conclusion, RAPD markers having high resolution power appear to offer many advantages in establishing genetic distances among the microalgae. They were found to be effective for assessing genetic variation in different species and strains of *Botryococcus* belong to various geographic locations and climatic zones as well. This study has revealed a molecular classification of *Botryococcus* strains of different chemotypes and between two strains belonging to different climatic zones and different geographical locations. Thus diversity analysis by RAPD technique efficiently discriminate phenotypic and environmentally acquired characteristics. RAPD primers have rarely been applied for establishing diversity of algal forms.

ACKNOWLEDGEMENTS

The author DC thanks Council of Scientific and Industrial Research, New Delhi, for Senior Research fellowship. The authors thank Prof. Joseph Chappel, University of Kentucky and Dr. Amy Lau, University of Berkeley., Director of MCRC, Chennai, Dr. Aditi Pant, Emeritus professor, Department of Botany, University of Pune for providing *Botryococcus* cultures. We express our sincere thanks to Prof. Jiri Komarek, Institute of Botany, Trebon, Czech Republic for providing scientific literature.

REFERENCES

Achitouv E, Metzger P, Rager MN, Largeau C (2004) C_{31}–C_{34} methylated squalenes from a Bolivian strain of *Botryococcus braunii*. *Phytochemistry* **65**, 3159-3165

Allard B, Casadevall E (1990) Carbohydrate composition and characterization of sugars from green microalga *Botryococcus braunii*. *Phytochemistry* **29**, 1875-1878

Bolch CJS, Blackburn SJ, Hallegraeff GM, Vaillancourt RE (1999b) Genetic variation among strains of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Journal of Phycology* **35**, 356-367

Bolch CJS, Orr P, Jones GJ, Blackburn SJ (1999a) Genetic, morphological and toxicological variation among globally distributed strains of *Nodularia* (Cyanobacteria). *Journal of Phycology* **35**, 339-355

Cane RF (1977) Coorongite, balkashite and related substances- an annotated bibliography. *Transactions of the Royal Society of South Australia* **101**, 153

Carvalho LC, Goulão L, Oliveira C, Gonçalves JC, Amâncio S (2004) RAPD assessment for identification of clonal identity and genetic stability of *in vitro* propagated chestnut hybrids. *Plant Cell, Tissue and Organ Culture* **77**, 23-27

Casadevall E, Dif D, Largeau C, Gudin C, Chaumont D, Desanti O (1985) Studies on batch and continuous cultures of *Botryococcus braunii*: hydrocarbon production in relation to physiological state, cell structure, and phosphate nutrition. *Biotechnology and Bioengineering* **27**, 286-295

Comeau AM, Short S, Suttle CA (2004) The use of degenerate-primed random amplification of polymorphic DNA (DP-RAPD) for strain-typing and inferring the genetic similarity among closely related viruses. *Journal of Virological Methods* **118**, 95-100

Dayananda C, Sarada R, Usha Rani M, Shamala TR, Ravishankar GA (2007) Autotrophic cultivation of *Botryococcus braunii* for the production of hydrocarbons and exopolysaccharides in various media. *Biomass and Bioenergy* **31**, 87-93

Eroglu E, Melis A (2010) Extracellular terpenoid hydrocarbon extraction and quantitation from the green microalgae *Botryococcus braunii* var. Showa. *Bioresource Technology* **101**, 2359-2366

Fernandes HL, Tome MM, Lupi F, Filho AM, Sá-Correia I, Novais JM (1989) Biosynthesis of high concentrations of an exopolysaccharides during the cultivation of the microalga *Botryococcus braunii*. *Biotechnology Letters* **11**, 433-436

Fowke LC, Stephen MA, Pat JR (1994) Scanning electron microscopy of hydrated and desiccated mature somatic embryos and zygotic embryos of white spruce (*Picea glauca* [Moench] Voss). *Plant Cell Reports* **13**, 612-618

Gomez PI, Gonzalez MA (2004) Genetic variation among seven strains of *Dunaliella salina* (Chlorophyta) with industrial potential, based on RAPD banding patterns and on nuclear ITS rDNA sequences. *Aquaculture* **233**, 149-162

Largeau C, Caradevall E, Berkaloff C, Dhamliencourt P (1980) Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. *Phytochemistry* **19**, 1043-1051

Martínez R, Anibarro C, Fernández S (2006) Genetic variability among *Alexandrium tamarense* and *Alexandrium minutum* strains studied by RAPD banding pattern analysis. *Harmful Algae* **5**, 599-607

Martins M, Sarmento D, Oliveira MM (2004) Genetic stability of micropropagated almond plantlets as assessed by RAPD and ISSR markers. *Plant Cell Reports* **23**, 492-496

Metzger P, Berkaloff C, Caradevall E, Coute A (1985) Alkadiene and botryococcene producing races of wild strains of *Botryococcus braunii*. *Phytochemistry* **24**, 2305-2312

Metzger P, Largeau C (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Applied Microbiology and Biotechnology* **66**, 486-496

Modgil M, Mahajan K, Chakrabarti SK, Sharma DR, Sobti RC (2005) Molecular analysis of genetic stability in micropropagated apple rootstock MM106. *Scientia Horticulturae* **104**, 151-160

Neilan BA (1995) Identification and phylogenetic analysis of toxigenic cyanobacteria by multiplex randomly amplified polymorphic DNA PCR. *Applied and Environmental Microbiology* **61**, 2286-2291

Nishihara H, Mina H, Watanabe M, Nagashima M, Yagi O, Takamura Y (1997) Random amplified polymorphic DNA (RAPD) analysis for discriminating genotypes of *Microcystis* cyanobacteria. *Bioscience, Biotechnology and Biochemistry* **61**, 1067-1072

Okada S, Devarenne TP, Chapell J (2000) Molecular characterization of squalene synthase from the green microalga *Botryococcus braunii*, race B. *Archives of Biochemistry and Biophysics* **373**, 307-317

Olmos J, Ochoa L, Paniagua-Michel J, Contreras R (2009) DNA fingerprinting differentiation between β-carotene hyperproducer strains of *Dunaliella* from around the world. *Saline Systems* **5**, 5

Ramage CM, Borda AM, Hamill SD, Smith MK (2004) A simplified PCR test for early detection of dwarf off-types in micropropagated Cavendish banana (*Musa* spp. AAA). S*cientia Horticulturae* **103**, 145-151

Ranga Rao A, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresource Technology* **98**, 560-564

Rohlf FJ (1998) NTSYS-pc. Numerical taxonomy and multi-variate analysis system, Version 2.02, Exeter software. State University of New York at Stony Brook, New York

Sawayama S, Inoue S, Yokoyama S (1994) Continuous culture of hydrocarbon-rich microalga *Botryococcus braunii* in secondarily treated sewage. *Applied Microbiology and Biotechnology* **41**, 729-731

Small HJ, Shields JD, Haas LW, Vogelbein WK, Moss J, Reece KS (2009) Genetic variation among strains of *Pseudopfiesteria shumwayae* and *Pfiesteria piscicida* (Dinophyceae). *Journal of Phycology* **45**, 1315-1322

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. *The University of Kansas Science Bulletin* **38**, 1409-1438

Tilman JA, Urban T, Bank B, Allan DC, Uwe J (2009) Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate *Alexandrium tamarense* (Dinophyceae). *Journal of Phycology* **46**, 18-32

Touzet N, France JM, Raine R (2007) Characterization of nontoxic and toxin-producing strains of *Alexandrium minutum* (Dinophyceae) in Irish coastal waters. *Applied and Environmental Microbiology* **73**, 3333-3342

Wolf FR, Nanomura AM, Bassham JA (1985) Growth and branched hydrocarbon production in a strain of *Botryococcus braunii*. *Journal of Phycology* **21**, 388-396

Zhao F, Wang X, Liu J, Duan D (2007) Population genetic structure of *Sargassum thunbergii* (Fucales, Phaeophyta) detected by RAPD and ISSR markers. *Journal of Applied Phycology* **19**, 409-416

Zhao F, Liu F, Liu J, Ang Jr. PO, Duan D (2008) Genetic structure analysis of natural *Sargassum muticum* (Fucales, Phaeophyta) populations using RAPD and ISSR markers. *Journal of Applied Phycology* **20**, 191-198