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BIODIVERSITY OF *CHLORELLA* SPECIES USING RANDOM AMPLIFICATION OF POLYMORPHIC DNA (RAPD) MOLECULAR MARKERS

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ABSTRACT

Microalgae including *Chlorella* spp. have been applied in various biotechnological areas such as biofuels production, food industry, wastewater treatment etc. In this study, Random amplification of polymorphic DNA analysis using the polymerase chain reaction(RAPD-PCR) involving 4decamer random primers was used to evaluate the genetic variation in *Chlorella* spp. These twenty-eight (28) *Chlorella* spp. tested, were collected from freshwater environment in Abakaliki, Nigeria and cultivated in Bold basal medium (BBM). RAPD markers were used for amplification of the microalgal DNA isolated through cetyl trimethyl ammonium bromide (CTAB) method. The results showed that OPB-01, OPB-11 and OPH-08 primers exhibited maximum intra-species variation (100% polymorphism) while OPH-07showed the least (13%) polymorphism at amplicon levels. Despite morphological resemblance, a great deal of genetic polymorphism was observed among the *Chlorella* spp. Overall, the results revealed that the 4 RAPD bands are useful for genetic diversity studies of microalgae as there was species discrimination.

Keywords: Microalgae, Polymorphism, Variation, RAPD markers, *Chlorella* spp.

INTRODUCTION

The morphological description alone is not adequate to define microalgal species which have different forms in various environmental conditions. Molecular approach is required to clearly identify the organisms to the species level [1].

Microalgae are one of the most valuable natural resources that could be applied in medicine, food industry, and clean energy supply and wastewater management. Morphological behaviors identified through the light microscope have been conventionally used to determine the species and the diversity of microalgal species, which has a complex structure with different morphological phases affected by environmental conditions. The environmental conditions have no or less influence on genetic make-up than the morphological constitution [2]. Therefore, there is need for molecular studies of organisms in addition to the morphological examination, in order to distinguish them to species level [3]. The combined morphology and molecular offer a robust approach to analyze the organisms with less errors.

Molecular markers are fascinating techniques which can improve and enhance biomass accumulation and confer resistance to stress in microalgae. Also, they are valuable tools to explore population genetic and diversity which were rapidly developed over 30 years ago [4]. There have been some studies on algae using Random Amplified Polymorphic DNA (RAPD) molecular markers [5]; [6]; [7]; [8].

Molecular markers (RAPD, ITS RFLP, SSR, ISSR, etc.) were developed and incorporated into the molecular techniques and presented for molecular identification of species [9]. DNA bands are applied as component characters and its presence or absence in the PCR products may be used to study genetic association and inter- specific and intra-specific genetic distinctions [10].

DNA markers have been used widely for evaluating genetic difference in intra-species to measure genetic diversity [11]. In this study, RAPD markers were used to evaluate the genetic diversity within *Chlorella* populations collected from different freshwater environments in Abakaliki of Ebonyi state, Nigeria. Genetic variation is important for the long-term being of species and it is a critical feature in conservation [12]. Therefore, tracing successfully improved variants at genetic level of *Chlorella* spp. *is* of instant requirement for their long-term conservation of these organisms. For resourceful preservation and application, the genetic structure of the species in different environments needs to be evaluated. Because of practical simplicity and speed, RAPD methodology has been used for diversity study in algae [13]. This study aimed at evaluating genetic diversity among the *Chlorella* spp. using RAPD

markers to provide genetic data and a hypothetical basis for protection of the species. Hence, effort was made to study genetic disparity among twenty-eight *Chlorella* spp. using RAPD markers. RAPD markers are built on the amplification of unknown DNA sequences using single, short, random oligonucleotide primers, therefore, RAPD polymorphism is the reflection of variation of the whole genomic DNA and would be a better parameter to study the pattern of genetic diversity in these novel organisms [14].

MATERIALS AND METHODS

Twenty-eight (28) *Chlorella* spp. were sampled from different sites in Abakaliki, Nigeria. Fresh samples were examined as wet mounts under light microscope before cultivation for biomass production.

DNA Extraction

Genomic DNA was extracted from the microalgae following the modified CTAB method described by Doyle and Doyle (1990). Quantification and purity of isolated DNA was checked through uv spectrophotometer and agarose gel electrophoresis, respectively. The whole double stranded DNA forming a thick single band established the good quality of genomic DNA.

DNA Amplification

The OPB-01, OPB-11, OPH-07 and OPH-08(RAPD) primers were amplified from the algal DNA by the polymerase chain reaction (PCR) using a thermocycler. Each PCR sample consisted of a 25 µL solution containing 1 µL of DNA template (20 ng), 2 µL of each primer (Invitrogen, USA), 12.5 µL of 2Taq Mix (Taq Mix, Japan) and 9.5 µL of sterile water. The PCR (Biometra, Germany) was performed with a PCR condition, which consist of an initial preheating at 94 ℃ for 6 min followed by 35 cycles comprising a denaturation at 94 ℃ for l min 10 s, annealing at 54 ℃ for 50 s, and an extension at 72 ℃ for l min 30 s, followed by a final extension step for 10 min at 72 ℃ and incubated at 4 ℃.The amplicons were separated by gel-electrophoresis in 1.5% agarose gels (stained with ethidium bromide) (M' Ribu and Hilu, 1994). The Amplified DNA were visualized by U.V transilluminator and size of RAPD-PCR products determined by comparing with the marker DNA ladder [15].

Data analysis

Individual polymorphic DNA band was considered to be a unit character and the populations were physically scored as binary data with the absence as "0"and presence as "1". Only clearly distinguishable DNA bands were used in the genetic analysis. The

molecular weight of the amplicon was deduced from a standard curve based on the known size of the DNA fragments of the ladder.

RESULTS

Morphological identification

The preliminary morphological identification of microalgal cultures by the microscopic analysis revealed 28 isolates belonging to the genus *Chlorella*. As indicated in Plate1*Chlorella* genus is a single-celled green alga, belonging to the family Chlorellaceae within the class Trebouxio-phyceae. The cells were solitary, 3–6µm in diameter and spherical, ellipsoidal or globular in shape. They lacked flagella, having a parietal and cup-shaped chloroplast with a single pyrenoid surrounded by a thin cellulose wall and no mucilaginous sheath was observed.

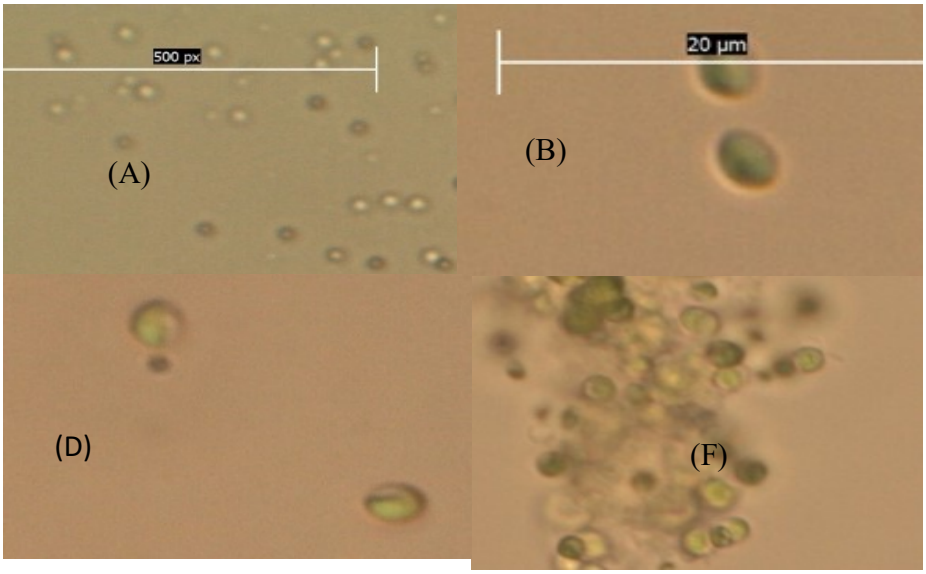

Plate 1: Four *Chlorella* spp.

Molecular polymorphism

Twenty-eight *Chlorella* spp. revealed 78% of polymorphism with 4 RAPD primers. A total of 266 bands were scored of which 210 are polymorphic bands. The number of bands ranging from 57 to 79 per primer corresponds to an average of about 66 bands (Table 1). The maximum number of polymorphic bands (79) was produced from primer OPB-01 and minimum (9) from OPH-07 (Figure 1). However, other primers OPB-11, OPH-08 yielded 57 and 65 bands, respectively (Table 1). The results further revealed 100% polymorphism with primers OPB-01, OPB-11 and OPH-08 while OPH- 07 showed the least polymorphism (13%) (Figure 1).

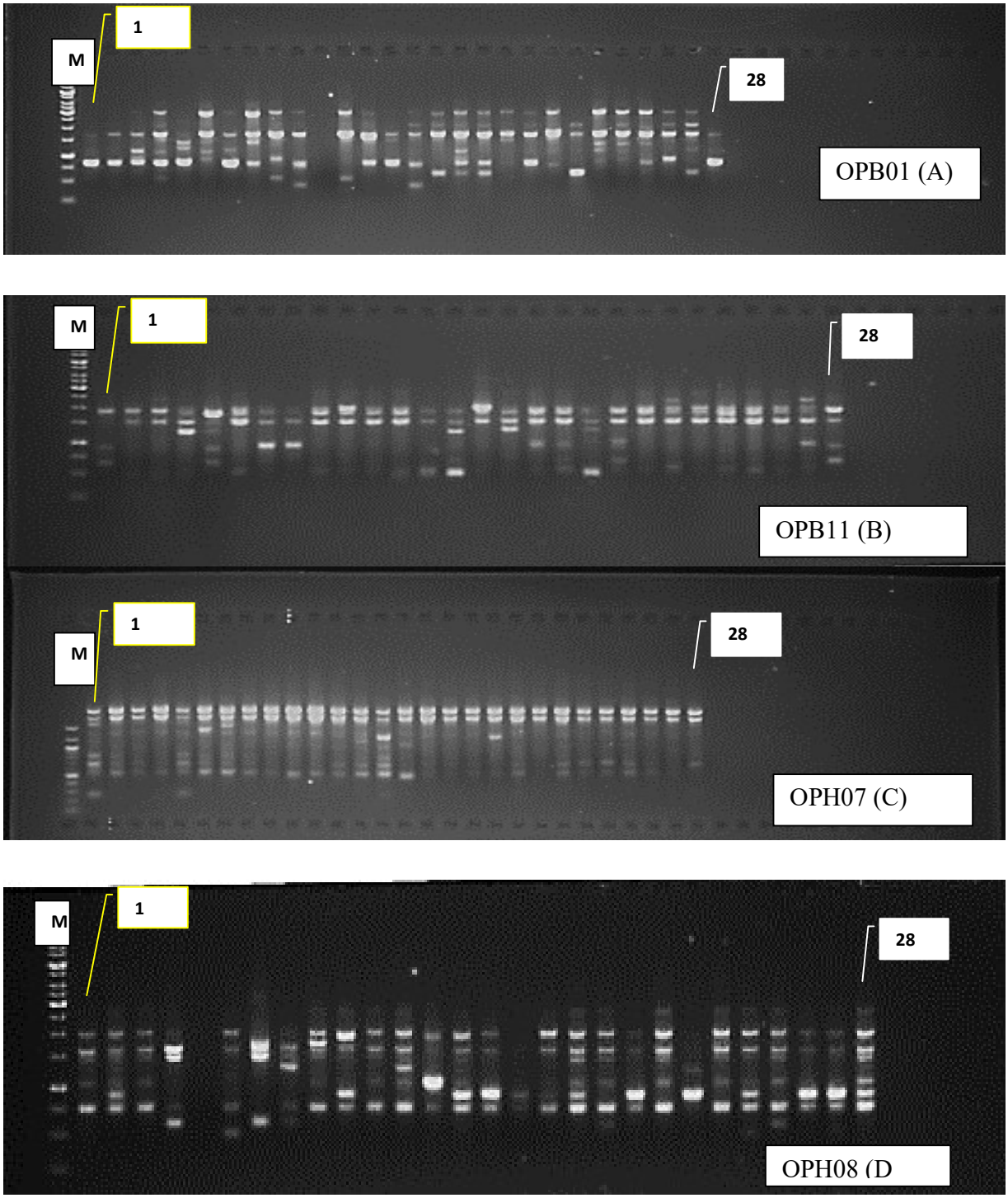

Figure 1: PCR amplified RAPD profile of *Chlorella* spp. with OPB01 (A), OPB11 (B)OPH07 (C) and OPH08 (D) primers

Table 1: Summary of the Results obtained from bands Amplified by the 4 RAPD primers.

Primer Name	Total No of Bands	Size Range(bp)	Polymorphic Bands	Percentage (%) Polymorphism
OPB01	79	100-1000	79	100%
OPB11	57	100-600	57	100%
OPH07	65	500-1500	9	13%
OPH08	65	300-900	65	100%
TOTAL	266		210	78%

DISCUSSION

RAPD markers represent efficient and inexpensive techniques to produce molecular data and thus, have been used successfully in various taxonomies (Iruela *et al*., 2002; Nebauer *et al*., 2000; Jayaram and Prasad, 2008).

High level of genetic similarity is likely among *Chlorella* spp. found Abakaliki due to similar environmental conditions. But in contrast, this investigation showed wide genetic base indicating earlier introduction of this species and afterward, leading to accumulation of variation. The genetic variation of the 28 *Chlorella* spp. could broadly be described as a result of environmental factors, hence, the percentage polymorphism (78%) among *Chlorella* spp. is relatively much higher than other organisms. This undoubtedly indicates high level of genetic diversity within *Chlorella* spp.

RAPD fingerprinting can be applied successful to differentiate closely related species. This technique is suitable for fast and accurate species differentiation and is an alternative and complementary method to the traditional approaches for studying microalgal systematics (Rabin and Chikkaswamy, 2014).

Generally, the RAPD markers used in this study displayed appreciable intra-population variation or molecular polymorphism, which is pre-existed in different collections (Jayaram and Prasad, 2008). In spite of their morphological similarity, substantial polymorphism was observed among the algal species under study (Priya and Maridass, 2008). This report revealed that, though the decamer primers are small in comparison to the large genome of *Chlorella* spp., they produced appreciable amplicons sufficient to demarcate all species collected from different locations. This study confirms the suitability of RAPD as a reliable, simple, easy to handle and powerful tool in molecular analysis of different species of microalgae available in Abakaliki. Currently, it is also a proof that, the entries that were found to be similar in taxonomical classification based on

morphological characteristics do have divergence at DNA level. The immense molecular diversity of the organisms offers researchers with good prospects to discover new metabolites. Even though morphological analyses are beneficial for the descriptions of the species and genetic diversity, they rely on environmental conditions and vary under varied conditions and they are not accurate enough to distinguish the strains and populations, hence the practice of the RAPD molecular markers which have shown to be more reliable tools to distinguish species.

CONCLUSION

The present study showed the diversity of microalgae based on genetic investigation. Random amplification of polymorphic DNA analysis using the polymerase chain reaction supplied a useful tool in the biodiversity study of this microalgal spp. The high diversity revealed by RAPD agrees with the conclusion that, out of the same genus *Chlorella* retains considerable variability. Also, variation within the species suggests that, this species has large effective population size.

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