

## Detection of genetic variation in four *Ulva* species based on RAPD technique

Umashankar prasad<sup>1</sup>, Geetanjali Deshmukhe\*<sup>2</sup>, Alkesh Dwivedi<sup>2</sup> & S. D. Singh<sup>2</sup>

<sup>1</sup>RVC Kanke, Ranchi, Jharkhand

<sup>2</sup>Central Institute of Fisheries Education, Yari Road Campus, Versova, Mumbai 400 061, India  
[Email: dgeetanjali@gmail.com]

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DNA yield was obtained by using 2 protocols, CTAB (Cetyltrimethylammonium bromide) and modified Wattier *et al*; out of which the latter yielded more quantity of DNA (0.85 µg/g). Out of total amplified product 53% bands were shown monomorphic and remaining of the bands were polymorphic. The high frequency of polymorphic bands suggests that the isolates of *Ulva* represented in our collection have sufficient genetic diversity for conducting a valid heterosis experiment. The intra species genetic similarity (GS) value was found highest for *U. lobata* and lowest for *U. fasciata*. The interspecies GS value was found highest between *U. fasciata/U. lactuca* and lowest between *U. lobata/U. reticulata*. The large number of differences among isolates revealed by the RAPD technique indicate that it would be possible to establish a unique “fingerprint” for individual plants based on the combined results generated from a small collection of primers.

**[Key words:** seaweed, *Ulva*, DNA isolation, genetic variation, RAPD]

### Introduction

*Ulva*, commonly known as sea lettuce is reportedly good source of protein and vitamins and is usually used in Japan and other East Asian countries for human consumption. Since the demand for seaweed is constantly on rise, their identification is of primary importance. Lot of taxonomic work has been done in early 60-70s based on the morphological and anatomical features. Characterization based on morphometric characters created confusion for exact identification of the same. Hence, the need arises to identify these species precisely based on molecular tools.

The genetic pattern of a given species or stock may be disturbed or totally lost even by small and localized disturbance of the environment due to man induced activities like over exploitation, environmental degradation etc. Individual with high level of genetic variation have greater prospect in aquaculture in terms of higher growth rate, developmental stability, viability and resistance to environmental stress and disease<sup>1</sup>. Genetic variation at species level helps to identify the taxonomic units and to determine the species distinctiveness that provides essential information for conservation, systematic, ecological and evolutionary studies<sup>2</sup>.

The genetic variability within population is extremely useful together with the information on individual identity. Such studies are highly informative for the management of small group in *ex-situ* collection, culture identity, and breeding or clonal identification for paternity testing. RAPD-PCR amplifies the genomic region using short oligonucleotide primers resulting in DNA banding profiles. RAPD profiles are strongly influenced by the different components of the reaction, such as template, primer, Mg<sup>2+</sup>, dNTP and Taq Polymerase concentrations and are modulated by thermocycling conditions such as annealing temperature and duration of each phase of PCR. In view of above facts the present studies has been undertaken to evaluate genetic variation within and among four species of *Ulva* based on RAPD profiles.

### Material and Methods

Four species of *Ulva* were collected from Maharashtra coast, India (Dahanu, Mumbai, Shriwardhan, Murud, Ratnagiri and Malvan) and identified Samples were washed in distilled water to remove salt, air-dried and stored at -20°C. DNA was isolated from of *Ulva* spp. Using different methodologies<sup>3,4</sup> with slight modifications.

DNA samples were quantified based on both spectrophotometric measurements as well as by intensity of fluorescence emitted by the ethidium

\*Corresponding author

bromide<sup>5</sup>. Four primers (two 20-mer and two 10-mer) screened<sup>6,7</sup> were used for the genetic characterization of individuals in the population and among the populations from different geographical locations of *Ulva* spp. The RAPD nuclear DNA polymorphisms were examined for the individuals from all the six populations using a modification of the procedure described<sup>8,9</sup>. Primer used for the studies were 1. OPB05-TGCGCCCTTC, 2. OPB03-CATCCCCCTG. The primers were finally diluted in TE buffer (pH 7) to a working solution of 10 pmol/ $\mu$ l as per the direction given by the supplier (Qiagen Operon, Germany).

Genomic DNA was amplified in sterile 0.2 ml PCR tube. All the chemicals used for PCR were supplied by Bangalore Genei Pvt. Ltd., Bangalore, India. The reaction volume of 25  $\mu$ l using following components were constituted on ice. Template DNA 1  $\mu$ l (100 ng/  $\mu$ l), dNTP (mix)(2.5mM) 2  $\mu$ l (200  $\mu$ M to final volume, 10 X DNA polymerase buffer 2.5  $\mu$ l (1X to final volume), MgCl<sub>2</sub> 1.5  $\mu$ l (2.5 mM to final volume), Primer (Taq Polymerase (3U) 0.5  $\mu$ l (1.5 U) and d H<sub>2</sub>O 14.5  $\mu$ l. Amplification was carried out in the PCR Express Thermocycler (HBPX220, Thermohyaid U.K.) The reaction was programmed for 10-mer primers for 45 cycles with following temperature set-up: a) T= 95° for 4 minute, b)T= 94° for 1 minute, c) T= 37° for 1 minute, and d) T= 72° for 2 minutes. The b, c, d was repeated for 45 cycles, T= 72° for 10 minutes and finally hold at 4°C. The reaction was programmed for 20-mer Primer for 45 cycles with following temperature set-up: a) T= 95° for 4 minutes, b) T= 94° for 1 minute, c) T= 55° for 1 minute, and d) T= 72° for 2 minutes. The b, c, d was repeated for 45 cycles, T= 72° for 10 minutes and finally hold at 4°C and 12  $\mu$ l of reaction products were separated by electrophoresis through 1.8 % agarose gel and stained with Ethidiumbromide. Agarose gel was photographed with a MP- 4 Polaroid camera over a UV transilluminator (312 nm) using Polaroid 665 and 667-instant packfilm.

The similarity index between individuals was calculated following the method<sup>10</sup>. Genetic similarity between individuals A and B ( $S_{AB}$ ) was calculated using the formula:

$$S_{AB} = 2 N_{AB} / (N_A + N_B)$$

Where,  $N_{AB}$  is the RAPD bands (DNA fragments) shared in common between individual A and Band  $N_A$

and  $N_B$  are the total number of band scored for individual A and B respectively.

### Result and discussion

Out of the two methods used to extract DNA, CTAB method yielded comparatively less yield at A260/180, The average yield given by CTAB was 0.56  $\mu$ g/g whereas the modified Wattier method gave DNA yield up to 0.85  $\mu$ g/g of thalli. Primer concentration kept constant at 0.2 mM showed that G+C- rich random primers give more positive amplification in seaweeds as compared to A+T rich primers<sup>11, 12</sup>. Both the primers used for the present study were G+C rich (70%) giving the amplification. The primer-template ratio is reported to be crucial. However, a variation in DNA concentration up to 100 ng did not generate variation in the RAPD profiles. DNA concentration above 100 ng resulted in smearing of PCR product. Increasing concentration of Taq Polymerase above 1.5 units per reaction resulted in smearing. Concentration of Mg<sup>2+</sup> was found to be 2.5mM instead of normally used 1.5 mM Mg<sup>2+</sup> concentration. Typically, in case of Mg<sup>2+</sup> ions, a decrease in the concentration lower than 2.5 mM resulted in few or no bands due to the increase in stringency of the reactions. Optimal program parameter used for this study was same as described<sup>6</sup>.

It has been reported that in the absence of template DNA, same primer gave rise artifact<sup>13</sup>, which were completely abolished when template DNA was included in the reaction mixture<sup>8,14</sup>. Despite the ability of PCR based technology to tolerate low quality DNA, there are pitfalls associated with the low quality DNA. Artifact such as non-reproducible and faint bands may be generated due to Chimeric PCR products. These need to be kept in mind any future work and precautions should be taken to conduct the reaction under absolutely identical conditions. Minor bands produced by RAPD analysis were much more variable, so only reproducible one were considered in the conclusion of similarity coefficient. Since some of the minor bands may be product of nonspecific priming, the number of such bands may be reduced by using more stringent reaction condition and/or through the use of formamide<sup>15</sup>. Minor bands can also be reduced somewhat by maintaining an appropriate ratio of template DNA to Taq Polymerase, so that enzyme is present in limiting amounts and thus less likely to prime loci that are not perfectly complementary to the primer.

Table 1—Summary of the number of total bands, common bands, polymorphic bands and size range of amplified product of four *Ulva* sp. generalized from two decamer primers of random sequences

S. No	Species	Primer No.	Total no. of bands	No. of common bands	No. of polymorphic bands	Mol. Wt. Range (Kbp)	% of polymorphic bands
1	<i>U. lactuca</i>	1	5	2	3	0.8 -2.5	40.00
		2	5	4	1	0.3-1.8	
2	<i>U. fasciata</i>	1	5	3	2	0.8-2.1	60.00
		2	5	1	4	0.4-1.7	
3	<i>U. lobata</i>	1	5	3	2	0.7-2.0	33.33
		2	7	5	2	0.5-1.4	
4	<i>U. reticulata</i>	1	6	2	4	0.7-2.4	54.54
		2	5	3	2	0.7-1.9	

The DNA samples of each species of *Ulva* were isolated from randomly collected specimens (total number of sample 20) for primer screening. Total of 4 primers, two 10-mer and two 20-mer plant universal primers were tested for amplification in 4 species of *Ulva* viz. *U. lactuca*, *U. fasciata*, *U. lobata*, *U. reticulata*. Of these, two 10 mer primers yielded strong amplification product whereas the remaining two did not generate amplification product. Therefore only these two primers were selected for the present investigation. These two primers' generated total 43 bands. Out of which 23 bands were monomorphic (53%) and remaining 16 bands were polymorphic (47%). The size of amplified product generated by two primers ranged from 300 bp to 2500 bp. [Table-1]

The genetic similarity (GS) matrix for intra-species variation was calculated manually by primer wise comparison of 5 individual of each species following the method<sup>9</sup>. The GS value of each species ranges from 0.750 - 0.941, 0.571 - 0.933, 0.842 - 0.952 and 0.705 - 0.947 for *U. lactuca*, *U. fasciata*, *U. lobata* and *U. reticulata* respectively. Mean intra species GS values were 0.844, 0.748, 0.904, and 0.833 for *U. lactuca*, *U. fasciata*, *U. lobata* and *U. reticulata* respectively. The highest GS values within population were obtained for *U. lobata* (0.904) followed by *U. lactuca* (0.844), *U. reticulata* (0.833) and *U. fasciata* (0.748).

The individuals from each of the four species were selected primer wise for inter-species genetic variability analysis and calculated manually based on similarity matrix<sup>10</sup> (Table 2). The inter-species GS values were found highest between *U. fasciata/U. lactuca* (84.2), followed by *U. reticulata/U. lactuca* (73.6), *U. reticulata/U. fasciata* (70.0) and *U. lobata/U. lactuca* (47.0). *U. lobata/U. fasciata* (44.0). *U. lobata/U. reticulata* (44.0).

Table 2—Similarity matrix among four species of *Ulva* based on polymorphic RAPD band

Species	<i>U. lactuca</i>	<i>U. fasciata</i>	<i>U. lobata</i>	<i>U. reticulata</i>
<i>U. lactuca</i>	1.000			
<i>U. fasciata</i>	0.842	1.000		
<i>U. lobata</i>	0.470	0.444	1.000	
<i>U. reticulata</i>	0.736	0.700	0.444	1.000

RAPD have been used extensively in the identification and differentiation of many seaweed<sup>11,12,16</sup>. The banding pattern generated through RAPD technique in the present investigation were used to characterize the four species of *Ulva* viz. *U. lactuca*, *U. fasciata*, *U. reticulata* and *U. lobata*. The similarity matrix of RAPD profile best way to estimate genetic variation and nucleotide diversity<sup>17</sup>. The primer (1 and 2) used in the study generated distinguishable banding profile in all the species. The percentage of polymorphic bands were observed 47% (Fig. 1 & 2). The high in the percentage of polymorphic band is presumably because of the large amount of sequence deference between the genomes<sup>9</sup>. The use of large number of primer may proved more species- specific RAPD marker for the analysis<sup>18</sup>. The Intra-species genetic similarity values estimated in four *Ulva* species did not much differ from one another. A very high GS value was obtained in each species and in the order of *U. lobata* (0.904), *U. lactuca* (0.844), *U. reticulata* (0.833) and *U. fasciata* (0.784). A possible explanation to the above fact is that samples were collected from a narrow geographical range.

The interspecies genetic similarity among the 4 species of *Ulva*, was found highest between *U. fasciata/U. lactuca* (84.2) followed by *U. reticulata/U. lactuca* (73.6), *U. reticulata/U. fasciata* (70.0) and *U.*

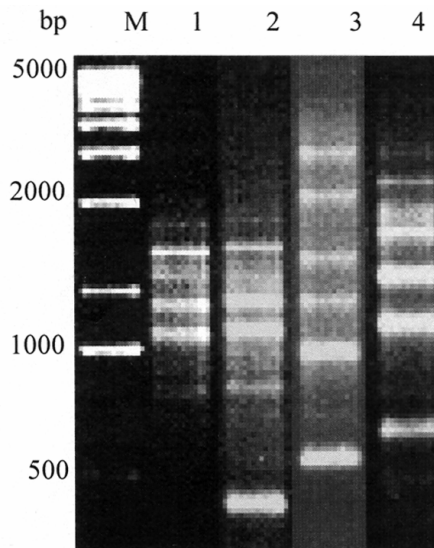


Fig. 1—RAPD profile generated by PCR using primer no. 1 of *Ulva* spp. obtained by 1.8 % agarose gel electrophoresis. Lane M is the molecular weight Marker (500 bp ladder). Lane 1 *U.lactuca*, Lane 2 *U.fasciata*; Lane 3 *U.lobata*; Lane 4 *U.reticulata*

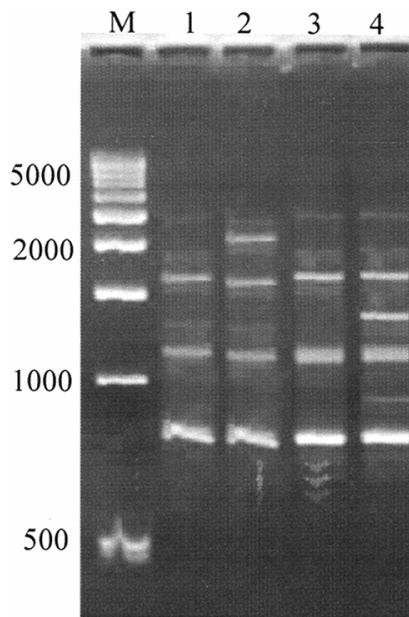


Fig. 2—RAPD profile generated by PCR using primer no. 2 of *Ulva* spp. obtained by 1.8 % agarose gel electrophoresis. Lane M is the molecular weight Marker (500 bp ladder). Lane 1 *U.lactuca*, Lane 2 *U.fasciata*; Lane 3 *U.lobata*; Lane 5 *U.reticulata*.

*lobata/U. lactuca* (47.0). *U. lobata/U. fasciata* (44.0). *U. lobata/U. reticulata* (44.0). The interspecies Genetic similarity values indicated genetic relatedness of the four species<sup>19</sup> supported the utility of RAPD analysis in the *Ulva* species. They suggested that RAPD marker is useful in discriminating *Ulva* and *Enteromorpha* and reported close relationship between *E. linza* and *Ulva*.

The present investigation suggests that RAPD-PCR is useful in discriminating individual samples within the genus *Ulva*. It may also be useful in determining relationship between species within a genus and in developing individual fingerprints for individual samples. The applicability of RAPD-PCR in assessing classification of wild population needs to be evaluated further.

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