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Research Paper

Phytochemical variation and genetic diversity of *G. corticata* and *K. alvarezii* from different environment

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Abstract

Due to wide range of secondary metabolites and its therapeutic properties, seaweeds have become natural alternative to the chemical based compounds. This project focuses on phytochemical evaluation and genetic diversity within seaweeds collected from different environment. Seaweeds samples of *Gracilaria corticata* and *Kappaphycus alvarezii* were collected from the Rameshwaram and Kanyakumari. These samples were subjected to phytochemical screening and found positive for anthraquinones, flavinoids, lignin and saponins. DNA was isolated by modified cetyltrimethyl ammonium bromide (CTAB) method and analyzed by Nanodrop spectrophotometer and agarose gel electrophoresis. Ten primers OPU 6- OPU15 were used for RAPD-PCR, out of which four primers generated highly reproducible and clear polymorphic bands. These four primers generated a total of 94 bands. The size of amplified products ranged from 100 bp to 2000 bp. As expected, individuals from same genus were clustered together in the dendrogram. The present study elaborates the bioactive content of seaweeds from different environment and demonstrates the effective application of RAPD technique for analyzing the genetic differentiation.

Keywords: Genetic diversity*, G. corticata, K. alvarezii*, Phytochemicals, RAPD.

Introduction

Due to its therapeutic properties, seaweeds have become natural alternative to the chemical based compounds. According to Newman, half of the new molecules introduced into the market were isolated from natural sources, between the years 1981 and 2002 [1]. Seaweeds possess phytochemical, which include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol and phycocyanins [2]. These compounds probably have diverse functions and can act as allelopathic, antimicrobial, antifouling, and herbivore deterrents, or as ultraviolet-screening agents [3]. Seaweeds are also known to be a healthy food with fiber, mineral, protein, vitamins, and trace elements of wide range of secondary metabolites [4,5].

Gracilaria is the third largest genus with more than 150 species reported across the world and consists of many commercially important agarophytes [6]. Kappaphycus alvarezii is one of the main seaweed cultivated in Philippines, Indonesia and Tanzania [7]. It is commercially used as a food additive, acting as a gelling, emulsifying, thickening and stabilizing agent in both pharmaceutical and nutraceutical products [8]. Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. The Random Amplified Polymorphic DNA (RAPD) markers are one of the tools to analyze genetic relationships and genetic diversity. The Major advantages of the RAPD technique are suitability for work on anonymous genomes, requirement of low DNA quantity, efficiency, ability to develop a large

number of DNA markers in a short time and low expense. As a result, RAPD markers have been extremely useful to analyze population genetics, in addition to parentage analysis, species delimitation and germplasm identification in seaweeds [9-12]. In this study our aim is to use RAPD technique to analyze seaweeds to determine their taxonomic identity and assess kinship relationships.

Materials and Methods

Sample collection

Seaweeds samples of *Gracilaria corticata* and *Kappaphycus alvarezii* were collected from the Rameshwaram and Kanyakumari.

Phytochemical Analysis

Phytochemical analysis was performed according to the standard protocol described [13]. All the prepared seaweed extracts were subjected to preliminary phytochemical screening for the presence of anthraquinones, flavinoids, glycosides, lignin, proteins, saponins, tannins and terpenoids.

DNA Isolation

DNA was isolated by modified cetyltrimethyl ammonium bromide (CTAB) method as described earlier with following modification [14]. The samples were incubated in CTAB buffer at 65 ^{0}C for 1 hr, and extracted twice with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 8000 g for 10 min. DNA was precipitated with chilled isopropanol with incubation -20 ^{0}C for 20 min. The DNA pellet was colleted after centrifugation at 10000 g for 10 min. The purity of the extracted DNA was checked by Nanodrop spectrophotometer and agarose gel electrophoresis.

RAPD-PCR

The RAPD-PCR was carried out in 25 µl, containing 50 ng of template DNA, 3 U of Taq, 2.5 mM MgCl, 2.5 mM each dNTPs and 100 pmol of primers. The DNA amplification was performed as complete denaturation (94°C for 5 min), 40 cycles of amplification (94°C for 30 sec, 32/34°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 1.5% Agarose gel.

Dendrogram Analysis

Band scoring was performed for each observed band as (1) for presence and (0) for absence of band. Jaccard coefficient method was used for generation of dendrogram. The similarity matrix was calculated and transformed the similarity coefficients into distances and made a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

Results and Discussion

This project focuses on evaluation of genetic diversity within seaweeds and its relationship with their bioactive compound. The detailed results of all tests for phytochemical screening of each species are summarized in table 1.

Seaweeds are also rich in polysaccharides such as alginates, fucans, and laminarans which have been reported to possess potential medicinal values [15]. The seaweed industry in India is mainly based on the algin yielding and agaryielding seaweeds, such as *Gracilaria.* Agar yielding seaweeds are collected throughout the year while algin yielding seaweed is collected seasonally from August to January on Southern coast of India [16].

The chemical and nutritional composition of seaweeds depends on many factors, including species, geographical origin or area of cultivation, seasonal, environmental, and physiological variations, time of harvest, water temperature, and processing methods [17-19]. Seaweed content of proteins, carbohydrates, lipids, fibre, metabolites, etc. can be influenced by their growing parameters. For this reason seaweeds can be considered as natural bioreactors, able to provide different types of compounds at different quantities.

Table 1: Phytochemical Analysis results. G1- *G. corticata* from Rameshwaram, G2- *G. corticata* from Kanyakumari, K1- *K. alvarezii* from Rameshwaram, K2- *K. alvarezii* from Kanyakumari

Test	G1	G2	K1	K2
Glycosides	-	-	-	-
Anthraquinones	+	+	+	+
Terpenoids	-	-	-	-
Flavonoids	+	+	+	+
Tannnin	-	-	-	-
Lignin	+	+	+	+
Saponin	+	+	+	+

The genomic DNA was extracted from the selected seaweed samples and quantified at 260 nm in a Thermo Scientific Nanodrop 1000 spectrophotometer. The 260/280 ratio of DNA was observed between 1.8 and 2.0 which represented pure DNA. The quality of the DNA was analyzed in 0.8% agarose gel. Ten primers OPU 6- OPU15 were used for RAPD-PCR. Out of 10 primers used four primers generated highly reproducible, clear polymorphic bands under optimized conditions (Figure 1).

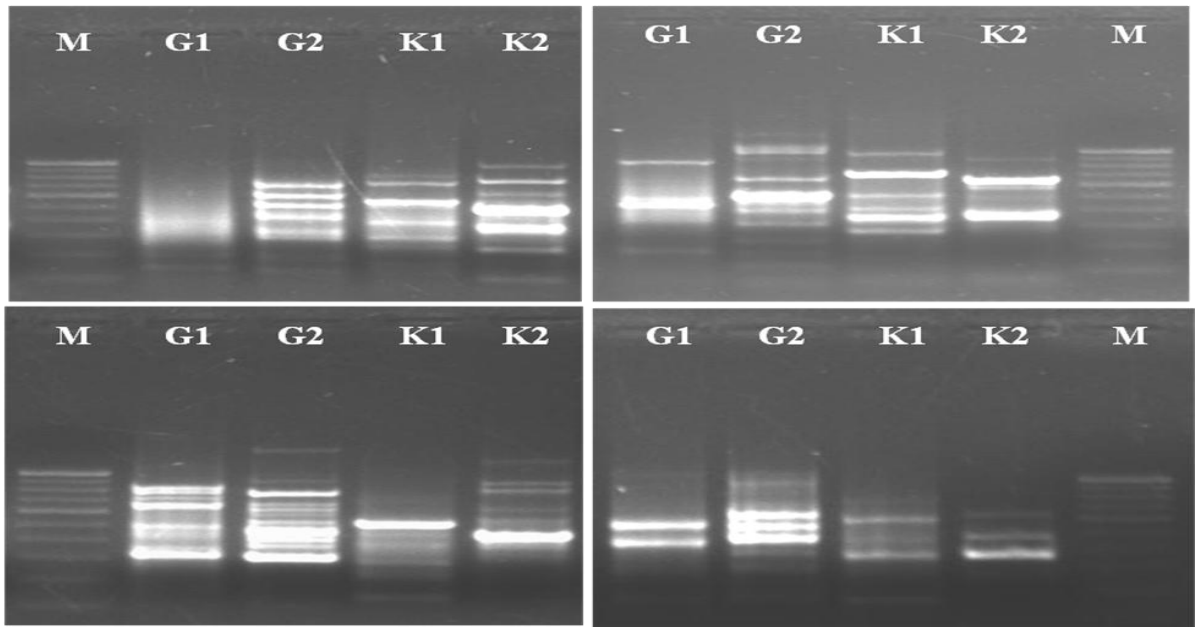
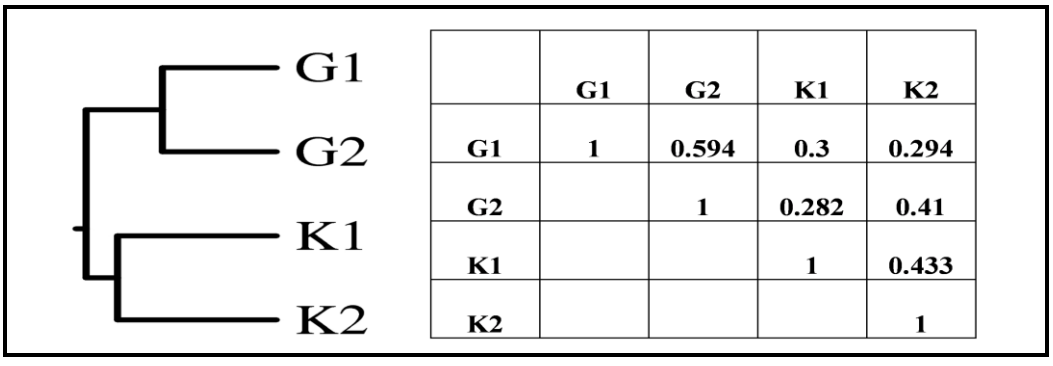


Figure 1: DNA fingerprint generated by primer OPU 8, OPU 10, OPU 12 and OPU 13 on 1.5 % agarose gel. M- 100 bp DNA ladder, G1- *G. corticata* from Rameshwaram, G2- *G. corticata* from Kanyakumari, K1- *K. alvarezii* from Rameshwaram, K2- *K. alvarezii* from Kanyakumari



	G1	G2	K1	K2
G1	1	0.594	0.3	0.294
G2		1	0.282	0.41
K1			1	0.433
K2				1

Figure 2: Dendrogram and similarity matrix generated from the DNA fingerprint. G1- *G. corticata* from Rameshwaram, G2- *G. corticata* from Kanyakumari, K1- *K. alvarezii* from Rameshwaram, K2- *K. alvarezii* from Kanyakumari

Only data from intensely stained unambiguous, clear bands were used for analysis. The four primers generated a total of 94 bands. The size of amplified products ranged from 100 bp to 2000 bp. Most of the bands (more than 95%) were polymorphic among populations and the number of polymorphic bands varies in each population, from 25% to 75%. As expected, individuals from same genus were clustered together in the dendrogram (Figure 2). The genetic similarity coefficient between *G. corticata* was found to be 0.59 and between *K. alvarezii* was found to be 0.43.

Conclusion

The present study elaborates the bioactive content of seaweeds from different environment and demonstrates the effective application of RAPD technique for analyzing the genetic differentiation.

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