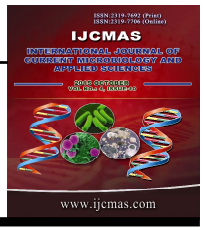
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 273-281

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 273-281**
http://www.ijcmas.com



Original Research Article

Study on the Biochemical Characterization of Marine Seaweeds of Mandabam Sea Coast

S. Mumtaj*

Dept of Zoology, Queen Mary's college, Chennai-600 004, Tamil Nadu, India
**Corresponding author*

A B S T R A C T

K e y w o r d s

Gracilaria folifera, Hypenea masiforms (Red) and *Sargassum longifolia, Turbinaria gonaidae* (Brown) and *Bryopsis, Caulerpa peldata* (Green), DNA, Protein, Amino acid

The aim of this study is to characterize the molecular concentration of biochemical substances in Marine sea weeds. Seaweeds are considered an important source of bioactive molecules. The large brown and red algae are used as organic fertilizers; these are rich in potassium but poor in nitrogen and phosphorus than farm manure. The algal species *Gracilaria folifera, Hypenea masiforms (*Red*) and Sargassum longifolia, Turbinaria gonaidae* (Brown) *and Bryopsis, Caulerpa peldata* (Green) was colleted at Rameswaram from the intertidal region at a depth of 1 m during March 2005 in India and molecular characterization was studied exclusively. Estimation of Biochemical constituents such as protein, amino acids and DNA was studied for their presence in concentration among sea weeds. *Turpanaria gonaidae* contains high DNA content as 8.10 ± 0.3 and it will be followed by *Caulerpa peldata* as 8.01 ± 0.6 and *Bryopsis* contains 7.61 ± 1.8 DNA concentration. Higher concentration of protein has been observed in *Turpanaria gonaidae* as 7.79 ± 0.04 and *Gracilaria folifera* as 6.54 ± 0.2 and *Bryopsis* as 6.44 ± 0.1. Amino acid concentration of different species of seaweeds are *Turpanaria gonaidae* 5.78 ± 0.2 and *Hypenea masiforms* 4.32 ± 0.07 and *Bryopsis* 4.30 ± 0.1. Marine seaweeds are always having rich source of biochemical constituents and enzymes.

Introduction

The main objective of the research work is to calculate the presence of DNA, Protein and Amino acids present among seaweeds.

By estimation of their presence in seaweeds we could able to use the seaweeds for different applications towards mankind and also to the environment.

Marine algae are primitive non-flowering plants without true roots, stems and leaves. The study of seaweed is known as phycology. The major seaweed growing areas include Gulf of Mannar, Gulf of Kutch, Palk Bay, Lakshadweep and Andaman and Nicobar islands.

They are one of the commercially important marine living renewable resources. Most sea weeds are fixed algae that grow attached to

firm substrates such as rocks, pilings and shells. They are confined to the fringes of continents and islands and to the tops of submarine banks (or) mountains where they can find attachment and enough light for photosynthesis.

Sea weeds naturally grow in the intertidal or sub tidal area up to 180m depth and also in estuaries and backwaters.

They get energy needed for growth from sun light by chlorophyll green pigment. They are responsible for the coloration of green, red, blue, brown (or) golden and leads to beautiful coloration.

Sea weeds play very important ecological roles in many marine communities. They are food source for marine animals such as sea urchins and fishes, and are the nutritional base of some food webs. They provide shelter and home for numerous fishes, invertebrates, birds and mammals.

Materials and Methods

Collection of seaweed

The algal species *Gracilaria folifera, Hypenea masiforms* (Red) and *Sargassum longifolia, Turbinaria gonaidae* (Brown) and *Bryopsis, Caulerpa peldata* (Green) was colleted at Rameswaram from the intertidal region at a depth of 1 m during March 2005 in India.

Immediately after collection, the algal species was washed in sea water; associated organisms and other extraneous matters were removed and subsequently washed in freshwater.

Then the algal species were sun dried. The dried material was powdered to fineness used for the biochemical analysis.

Seaweeds collected for research work

Red algae

1. Gracilaria folifera (Plate 1)
2. Hypnea masiforms (Plate 2)

Brown algae

1. Sargassum longifolia (Plate 3)
2. Turbinaria gonaidae (Plate 4)

Green algae

1. Bryopsis (Plate 5)
2. *Caulerpa peldata* (Plate 6)

Estimation of DNA

Extraction of DNA

Extraction of high molecular weight DNA free from protein and RNA is essential for all molecular biological investigations. The cell walls must be broken or digested away in order to release cellular constituents which are usually done by grinding tissue in dry ice (or) liquid N_2. Cell membrane must be disrupted which is done by a detergent usually SDS (Sodium Dodecyl Sulphate) (or) CTAB (Cetyl Trimethyl Ammonium Bromide). DNA must be protected from the endogenous nucleases. The detergent used for this purpose is EDTA (Ethylene Diamine Tetra Acetic Acid). EDTA is a chelating agent which binds magnesium ions (Mg^{++}), generally considered as a co-factor for most nucleases. The tissue mixture is emulsified with either chloroform or phenol to denature proteins from DNA. Shearing of DNA should be minimized by quickly drawing through a small orifice. Time between thawing of frozen pulverized tissue and exposure to extraction buffer should be minimized to avoid nucleolytic degradation of DNA. The crop species where excess

polysaccharides are present must be eliminated by CsCl density gradient or by using CTAB method. The resulting DNA would be about 50–400 μg. The DNA extraction protocols described here are reproducible and are applicable for diverse crop species.

Materials required

Bench top centrifuge, mortar and pestle, liquid nitrogen, glass beads, brush, glass rod (solid), 50 ml polyethylene tube, water bath, cheese cloth, long bent Pasteur pipettes, beaker 15 ml polypropylene tube, soft tissue paper

Reagents used

SDS, urea, Tris-base (enzyme grade) EDTA, Sarkosyl, phenol, chloroform, isoamyl alcohol, isopropanol, 70% ethanol, TE, DNase-free RNase, PVP (polyvinyl pyrrolidone) sodium acetate.

Procedure

Leaf tissue was grinded with liquid nitrogen and transferred to 150 ml chilled nuclear extraction buffer in a sterile beaker. Then homogenized to a slurry using magnetic stirrer; squeezed through cheese cloth and distributed evenly to tubes kept on ice. To pellet nuclei it was centrifuged at 700 g for 15 minutes at 4°C. Supernatant was discarded which contains plastids and mitochondria. The pellets were resuspended in 10 ml of nuclear lysis buffer preheated to 65°C + 2 ml of 5% Sarkosyl and incubated at 65°C water bath for 90 minutes. Equal volume of chloroform isoamyl alcohol was added and mixed for 5 minutes. This was centrifuged at 5000 rpm for 15 minutes and supernatant was collected. This step was repeated twice. Supernatant was collected then equal volume of isopropanol was added and mixed gently by inverting the tubes several times. The precipitated DNA fibres were hooked out using a glass hook and then dried and dissolved in TE and purify.

Reagents

1. Nuclear extraction buffer

Sorbitol	-	56 g
1 M Tris HCl (pH 8.0)	-	50 ml
0.5 M EDTA (pH 8.0)	-	10 ml

Made up to 1000 ml and autoclaved.

2. Nuclear lysis buffer

CTAB :20 g; 1 M Tris-HCl (pH 8.0):200 ml; 0.5 M EDTA (pH 8.0):400 ml; 5 M NaCl:400 ml; pH adjusted to 7.5 with concentrated HCl. Made up to 1000 ml and autoclaved.

Estimation of biochemical constituents

Estimation of protein

Method

Protein reacts with Folin-Ciocalteu's reagent to give a colored complex. The color so formed is due to the reaction of the alkaline copper with the protein at the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the color depends on the amount of these aromatic acids present and will thus vary for different proteins.

Reagents

1. Alkaline sodium carbonate (Na_2CO_3) solution:
2 g of Na_2CO_3 dissolved in 0.1 N NaOH.

2. Copper sulphate ($CuSO_4$) (5%):
5 g of $CuSO_4$ dissolved in 100 ml of distilled water.

3. Sodium potassium tartarate:
This was prepared by dissolving 10 g in 100 ml distilled water.

4. Copper sulphate – Sodium potassium tartarate solution:
One part of $CuSO_4$ solution was mixed with one part of sodium potassium tartarate and light parts of distilled H_2O.

5. Alkaline reagent:
Prepared on the day of use by mixing 50 ml of alkaline sodium carbonate solution and 1 ml of copper sulphate – sodium potassium tartarate solution (reagent 4).

6. Folin-Ciocalteu's phenol reagent:
The reagent with equal amounts of distilled water was diluted.

7. Trichloro acetic acid (TCA):
10% w/v in distilled water.

8. Standard protein (BSA) bovine serum albumin (100 μg $ml^{-1)}$

Procedure

The homogenized cyanobacteria was centrifuged at 5,000 xg for 10 min. The pellet was washed twice in distilled water. To the pellet 5 ml of 10% TCA was added and left for half an hour in boiling water bath.

The contents were cooled and centrifuged at 5,000 xg for 5 min. The resulting pellet was dissolved in 1 ml of 1 N NaOH. From this 0.1 ml was taken and made up to 1 ml with distilled water.

To this 5 ml of alkaline reagent was added and incubated for 3 min.
Then 0.5 ml of Folin-Ciocalteu's reagent was added and mixed thoroughly and allowed to stand for 30 min.

The absorbancy at 750 nm was read in a spectrophotometer. Similarly this was done for the standard protein (Conc. 10 μg to 100 μg) and a standard graph was prepared.

Results and Discussion

Calculate the amount of protein from the standard curve and express as mg g^{-1} dry weight.

Estimation of amino acids

Method

The ninhydrin (triketohydrindene hydrate) is a powerful oxidizing agent. All amino acids in the presence of ninhydrin get decarboxylated and deaminated resulting in ammonia (NH_3), carbondioxide (CO_2), the corresponding aldehyde, and a reduced form of ninhydrin.

The liberated ammonia then reacts with an additional mole of ninhydrin and the reduced ninhydrin to yield a purple substance which has absorption maxima at 570 nm.

Reagents

Standard amino acid solution

10 mg of aspartic acid dissolved in small volume of (0.1 N) HCl and made up with distilled water to the 100 ml mark in the standard flask.

Citrate buffer – pH 5.5 (0.1 M): 0.1 M of citric acid (2.101 g in 100 ml distilled water) and 0.1 M of sodium citrate (2.940 g in 100 ml of distilled water) were prepared. Sodium citrate solution was added to citric acid solution until the pH reaches 5.5 and maintained the volume to 100 ml.

Ninhydrin reagent

0.16 g of stannous chloride ($SnCl_2$. $2H_2O$) was dissolved in 100 ml citrate buffer. This solution was added to 100 ml of methyl cellosolve containing 4 g of ninhydrin and mixed thoroughly (every time either prepared freshly (or) stored in the dark bottle under nitrogen atmosphere).

Methyl cellosolve (ethylene glycol monomethyl ether)

Diluent: Ethanol: H_2O (50% v/v).

Procedure

A known volume of amino acid solution to a test tube was pipetted out. The volume was made up to 2 ml with citrate buffer.

3 ml of buffered ninhydrin reagent was added and mixed well. The contents were heated in the boiling water bath for 15 min and cooled to room temperature. 3 ml of 50% ethanol was added and the absorption at 570 nm was read against ninhydrin citrate buffer blank. The standard was run following the same procedure (Conc. 1–20 μg).

Results and Discussion

Following are the most important findings of the present work pertaining to isolation and characterization of DNA, protein and amino acid of six different marine algal seaweeds.

DNA quantification of marine seaweeds

In the present investigations, among the different marine seaweeds *Turpanaria gonaidae* contains high DNA content as 8.10 ± 0.3 and it will be followed by *Caulerpa peldeata* as 8.01 ± 0.6 and *Bryopsis* contains 7.61 ± 1.8 DNA concentration. All these results are exhibited in table 1 and figure 1.

Protein concentration of marine algal seaweeds

At higher concentration of protein has been observed a *Turpanaria gonaidae* as 7.79 ± 0.04 and *Gracilaria folifera* as 6.54 ± 0.2 and *Bryopsis* as 6.44 ± 0.1 (Table 2 and Fig. 2).

Amino acid status in different marine algal seaweeds

Table 3 and figure 3 showing the amino acid concentration of different species of seaweed are *Turpanaria gonaidae* 5.78 ± 0.2 and *Hypenea masiforms* 4.32 ± 0.07 and *Bryopsis* 4.30 ± 0.1.

Regarding the protein concentration *Turpanaria gonaidae* ranking first and it will be followed *Gracilaria folifera* and *Bryopsis*. The previous researcher has critically analyzed the protein picture in the marine seaweed *Ulva lactuca* and it is more or less the related to our present observation. In the present observation *Turpanaria gonaidae* reflecting more concentration of amino acid and it will be followed by *Gracilaria folifera* and *Bryopsis*. The present investigation can be conveniently compared with the findings of Chennubhotla *et al.* (1990). The present study has critically assayed the DNA quantification is three different marine seaweeds such as the brown algae *Turpanaria gonaidae*, red algae *Gracilaria folifera* and the green algae *Bryopsis*.

Table.1 Strategy of DNA content in different marine algal seaweed species

S.No.	Algal species	DNA content mg/g
1.	*Turpanaria gonaidae*	8.10 ± 0.3
2.	*Caulerpa peldata*	8.01 ± 0.6
3.	*Bryopsis*	7.61 ± 1.8
4.	*Sargassum longifolia*	7.31 ± 1.4
5.	*Gracilaria folifera*	6.33 ± 0.2
6.	*Hypenea masiforms*	5.81 ± 0.4

Table.2 Strategy of protein content in different marine seaweed species

S.No.	Algal species	Protein content mg/g
1.	*Turpanaria gonaidae*	7.79 ± 0.04
2.	*Gracilaria folifera*	6.54 ± 0.2
3.	*Bryopsis*	6.44 ± 0.1
4.	*Caulerpa peldata*	5.71 ± 2.6
5.	*Sargassum longifolia*	5.21 ± 2.1
6.	*Hypenea masiforms*	4.21 ± 1.8

Table.3 Strategy of amino acid content in different marine seaweed species

S.No.	Algal species	Amino acid mg/g
1.	*Turpanaria gonaidae*	5.78 ± 0.2
2.	*Hypenea masiforms*	4.32 ± 0.07
3.	*Bryopsis*	4.30 ± 0.1
4.	*Caulerpa peldata*	3.9 ± 1.3
5.	*Sargassum longifolia*	3.85 ± 0.06
6.	*Gracilaria folifera*	3.13 ± 0.08

Fig. 1. DNA content in Marine seaweeds

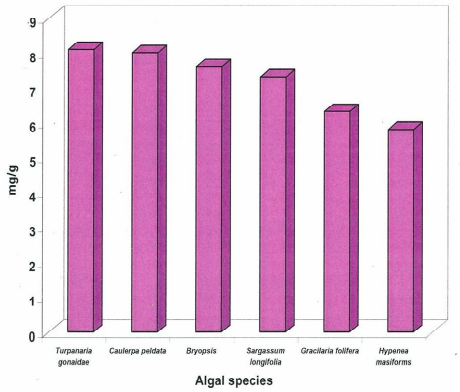


Fig.2. Protein content in Marine seaweeds

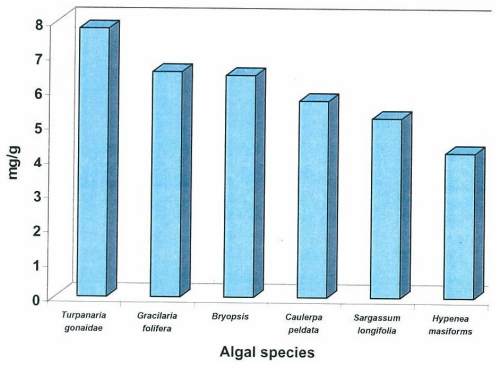


Fig.3. Amino acid content in Marine seaweeds

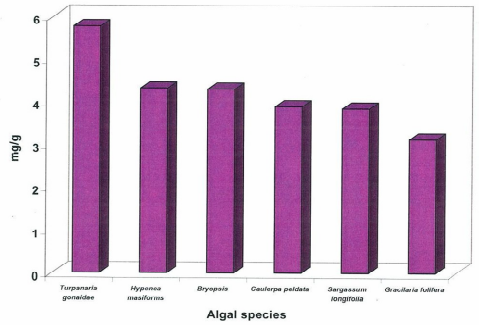


PLATE – I

Red Algae
Gracilaria folifera

PLATE – II

Red
Hypenea masiforms

PLATE – III

Brown
Sargassum langifolia

PLATE – IV

Brown
Turpanaria gonaidae

PLATE – V

Green
Bryopsis

In our observation *Turpanaria gonaidae* showing more quantity of DNA than *Gracilaria folifera* and *Bryopsis*. Our findings are in good agreement with the earlier contribution of Colemann *et al.* (1996). Further variation in DNA concentration in the above seaweed species proved their molecular philogenic relationship among their species.

Their protein contents differ according to the species and seasonal conditions. It is confirmed in the present study brown algae *Turpanaria gonaidae* showing maximum concentration of protein, DNA and amino acid and it will be followed by *Gracilaria folifera* and *Bryopsis*.

Researchers are looking to seaweed for proteins with health benefits for use as functional foods. Historically, edible seaweeds were consumed by coastal communities across the world and today seaweed is a habitual diet in many countries, particularly in Asia. Indeed, whole seaweeds have been successfully added to foods in recent times, ranging from sausages and cheese to pizza bases and frozen-meat products.

Seaweed packs super-high amounts of calcium—higher than broccoli—and in terms of *protein*; it's almost as rich as legumes.

As one of the major utilizable algal resources of the sea, they are known to contain carbohydrates, proteins, vitamins and minerals and micro nutrients (Chapman, 1980). Moreover, they are promising source of supplementary food, feed, fertilizer and renewable energy (Dave and Chauha, 1989). Chemical composition of green, brown seaweeds and protein contents of red seaweeds of Sourashtra coast were reported by Maru *et al.* (1975), Parekh *et al.*, 1977, Dave *et al.* (1977).

Marine algae or seaweeds the most accessible marine resources of the coastal zone, occupy potentially important place as a source of biochemical compounds (Rao *et al.*, 1986). Successful extraction of total DNA from brown algae, which are generally polysaccharide and polyphenol rich, is often problematic using current methods. A rapid and economical method of simultaneous extraction of DNA and RNA from seaweeds has been developed by the use of lithium chloride (Hong *et al.*, 1996).

The amounts of nuclear DNA in ten species of seaweeds belonging to the Rhodophyceae, Phaeophycease and

Chlorophyceae were determined by flow cystometric analysis of nuclei isolated from protoplasts.

Macro and micro algae are potentially important food resources for penacied shrimp inhabiting coastal nursery ground and aquaculture pond. However, there are few data on the nutritional contribution of different algae species to juvenile shrimp growth. In this study, changes in whole body weight, RNA and DNA concentration on appreciable feature.

Nuclear genome profiles were developed for representative species of the Gelidiales and Gracillariales using information from present and previous studies of cytogenetics, cytophotometry and DNA reassociation kinetics.

Application of molecular techniques to study marine macro algae is in its infancy and it is likely to be facilitated by the ability to routinely isolate high quality DNA from these plants.

Acknowledgements

I wholeheartedly thank almighty God for blessing me and giving this opportunity to do this project and seen me through its completion. I take pleasure in expressing my deep sense of gratitude to Dr. M.Ayyavoo M.Sc.,M.Phil.,Ph.D., for his help in collection of seaweeds. He helps a lot of my reference work. He has been my source of inspiration throughout the course of my dissertation work.

Reference

Chapman, D.J. (Ed.), 1980. Seaweed and their uses, 2nd edn. Chapman & Hall, New York, Pp. 334.

Chennubhotla, V.S.K., Najmuddin, M., Ramalingam, J.R., Kaliyaperumal, N. 1990. Biochemical composition of some marine algae from Mandapam coast, Tamil Nadu, *CMFRI Bull.*, 44: 442–446.

Colemann, A.W. 1996. DNA analysis methods for recognizing species invasion. *Hydrobiologia*, 326(327): 29–34.

Dave, M.J., Chauhan, V.D. 1989. Seaweed as a source of food, feed and fertilizer. *Invent. Intell.*, 24(3): 115–121.

Dave, M.J., Gany, S.K., Iyengar, R.R. 1977. Assessment of the possibility at seaweeds to be utilized as supplementary – animal feed. *CSMCRI, Bhavnagar Salt Res. Ind.*, 13(122): 33–40.

Hong, Y.K., Kim, Y.T., Kim, S.K. 1996. Molecular characterization of seaweeds using RAPD and differential display. *J. Korean Fish. Soc.*, 6: 770–778.

Rao, P.P.S., Rao, P.S., Karmarkar, S.M. 1986. Biological investigations on genus *Sargassum* I. Antifungal activity of fraction of different species of *Sargassum. Phykos.*, 25: 6–11.