

***In Vitro* Antioxidant Activities of Selected Green, Brown and Red Seaweeds from Southeast Coast of India**

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Total Phenolic Content (TPC) and Antioxidant Activity (AOA) of three selected seaweeds *Halimeda tuna*, *Turbinaria conoides* and *Gracilaria foliifera* in two different solvent extracts (Methanol and Diethyl ether) were studied. Total phenolic content was measured using Folin-Ciocalteu method, while Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Activity (TAA) were used to study their antioxidant activity. *Turbinaria conoides* was found to have the highest TPC and AOA of dried sample, followed by *Halimeda tuna* and *Gracilaria foliifera*. Thus reducing power of the samples was the following order Methanol > Diethyl ether. Hence these seaweeds could be considered as natural antioxidants and may be useful for curing diseases from oxidative deteriorations.

Studies on *Fucus spiralis* Subject to Increased Temperature and Acidity

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The present study follows a series of previous ones conducted at a shallow water hydrothermally active site where at low tide temperature increases about 10°C and pH values decrease about 2, relative to open seawater conditions. The main goal is to assess the physiological response under controlled temperature and acidity conditions in the laboratory. Tips of *F. spiralis* of about 1cm in length were collected at one open shore site and placed inside plastic vessels (15 tips per vessel) with 400ml of seawater. Water was collected at open ocean conditions (pH[~]8) and from the hydrothermally active site (pH[~]7), then filtered and subject to microwave sterilization. Six vessels were filled with water from the hydrothermally active site (3 kept at 25°C and 3 kept at 15°C), six vessels were filled with open ocean seawater (3 kept at 25°C and 3 kept at 15°C), and six vessels were filled with acidified (pH[~]7) open ocean seawater (3 kept at 25°C and 3 kept at 15°C). The experiment was kept during a period of two weeks with a 12h/12h light/dark periods, during which water was exchanged every three days, and acidity controlled four times

a day. Tips were scanned in the beginning, mid and end of this period and growth assessed through image analysis. The results indicate that *F. spiralis* presents higher growth rates at lower temperature and lower pH. Additionally, samples kept in water collected at the hydrothermally active site grew less than those kept in open ocean seawater, and with a decreasing rate over time, possibly indicating a tendency to die off. A longer experiment is planned to check the verified tendency in growth. Histological comparison of cellular structures, reproduction and photosynthesis studies under the same conditions are planned for the period August-October, to complement the results obtained until the present moment.

Genetic Engineering of Carotenoids in *Chlamydomonas reinhardtii*

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Carotenoids are C40 hydrocarbon pigments synthesised in the plastid organelle of plants and algae. Carotenoids provide two crucial functions in photosynthetic organisms: they serve as auxiliary pigments during the light harvesting process, and they act as protectants against photooxidative damage that can result from absorption of excessive light energy. These pigments, especially the oxygenated ketocarotenoid derivatives, are of considerable commercial interest because of their strong antioxidant properties and their use in the aquaculture, food and pharmaceutical industries. We are investigating whether the green unicellular alga *Chlamydomonas reinhardtii* can be engineered to produce high levels of ketocarotenoids. Our approach is to target foreign genes involved in carotenogenesis into the chloroplast genome such that the gene product is expressed at a high level in the organelle. A chloroplast expression vector (pASapI) was created in our lab such that the coding sequence for any foreign gene can be fused to a promoter, 5' untranslated sequence and 3' untranslated sequence derived from *C. reinhardtii* chloroplast genes to create a functional 'gene cassette'. Following transformation of the chloroplast with the plasmid, the gene cassette is inserted via homologous recombination into the genome at a neutral, intergenic site immediately downstream of the photosystem II gene, *psbH*. Furthermore, the chloroplast promoter element is active also in *E. coli*, allowing us to confirm the functionality of the gene cassette prior to chloroplast transformation. The coding sequences for several algal β -carotene ketolases (BKT), including a codon-optimised version of the *Muriella zofingiensis* enzyme, were cloned into the pASapI and their activity in *E. coli* was tested by HPLC by employing strains that have been previously engineered to synthesize the BKT substrates, β -carotene or zeaxanthin. *C. reinhardtii* has been transformed with the first of these constructs containing the synthetic gene. Molecular analysis of the transformant lines has confirmed that the gene cassette has integrated successfully into the chloroplast DNA and that this polyploid genome has been driven to a stable homoplasmic state in which all copies contain the transgene. The further analysis of this transformant and other lines will be presented.
