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Biochemical constituents of *Gracilaria edulis* cultured from spores

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ABSTRACT

Gracilaria edulis was cultivated in west coast of India off Narakkal by reproductive method using the carpospores. The culture period was from November to March with regular sampling of growing plants for the estimation of biochemical constituents, quantitative and qualitative estimation of agar. The biochemical constituents like protein and carbohydrate content increased corresponding to the age of the plant whereas the lipid content declined. Harvesting of the crop can be determined from the qualitative and the quantitative estimation of agar along with other biochemical constituents. It was found out that crop harvested after 121 days of culture period had better quality of agar and also high protein content.

Introduction

Gracilaria edulis is an important agarophyte of India harvested regularly from the south east coast of India for the extraction of agar. Over exploitation led to the depletion of the natural stock in this area (Sakthivel,1999). Restoration techniques by cultivation can pave the way to meet the demand of the industry. *Gracilaria edulis*, being a fast growing species and having high regerative capacity, is considered as one of the candidate species for cultivation. The present work deals with the cultivation of *Gracilaria edulis* from carpospores and their biochemical constituents in different growth stages during the cultivation period.

Materials and Methods

The cystocarpic plants of *G. edulis* were collected from Thonithurai (9°17'N and 70°11'E), situated in the southeast coast of Tamilnadu in between Palk Pay and Gulf of Mannar, India during low tide in the morning and transported to the laboratory in plastic bags. Healthy plants were selected, brushed off epiphytes and washed several times in filtered seawater. The plants were transported to Cochin in enriched seawater by train and kept in the marine hatchery complex for a day or two to avoid transportation stress before using the plants for spore output. The plants were kept in shade to induce dehydration and used for spore output in 200 l perspex tank. The plants were spread uniformly on a nylon net just dipped in water. A difference of water column of 30-40cm was maintained between the substrata and the mother plants to ensure proper settlement of spores on the substrata. Old nylon fishing nets were used as substrata for spore attachment, which were inserted on a 6mm nylon rope (1.5 m) at a distance of 50

cm. A few glass slides were also put in the bottom of the tank for spore attachment. The tank was provided with mild aeration. After 24h, the mother plants were removed from the spore output tank. The spores were allowed to settle on the substrata, which was monitored by observing the glass slides under microscope. After the spores get attached to the substrata, they were transferred to the rearing tank filled with filtered and enriched seawater. Regular observations were made on the growth of the spores and the water was changed at weekly interval.

The germlings were transplanted to the natural environment in the open sea off Narakkal near Kochi in west coast of India after 17-20 days of nursery rearing. The substrata with attached germlings were tied to three floating rafts during the month of November. Regular observations were made on growth and the biochemical constituents of *Gracilaria edulis* by removing three sampling ropes from each raft at every sampling period. The fresh samples were washed thoroughly and dried in a preheated oven at 90^0C for 24 hrs. The dried plants were powdered and sieved. Total protein was estimated by the modified method of Lowry *et al.*, 1951 (Hatree, 1972). Total carbohydrate was estimated by phenol sulfuric method (Dubois *et al*., 1956) and the total lipid was extracted by the method of Folch *et al*, (1957) and estimated by phospho vanillin method (Barnes and Blackstock, 1973). All the values were expressed in percentage of dry weight.

Extraction of agar, their quantification and qualitative analysis were carried out by the method of Thivy (1960). The total galactose from agar was analysed by phenol sulphuric method (Dubois *et al*, 1956) and the sulphate content was analysed by the method of Jackson and Mc Candles, (1978). The data were analysed statistically by SPSS software.

Results

The lipid content of *G.edulis* was found to be least and did not show wide variation throughout the culture period except bimodal peaks on 86 and 107 days (Fig.1). The

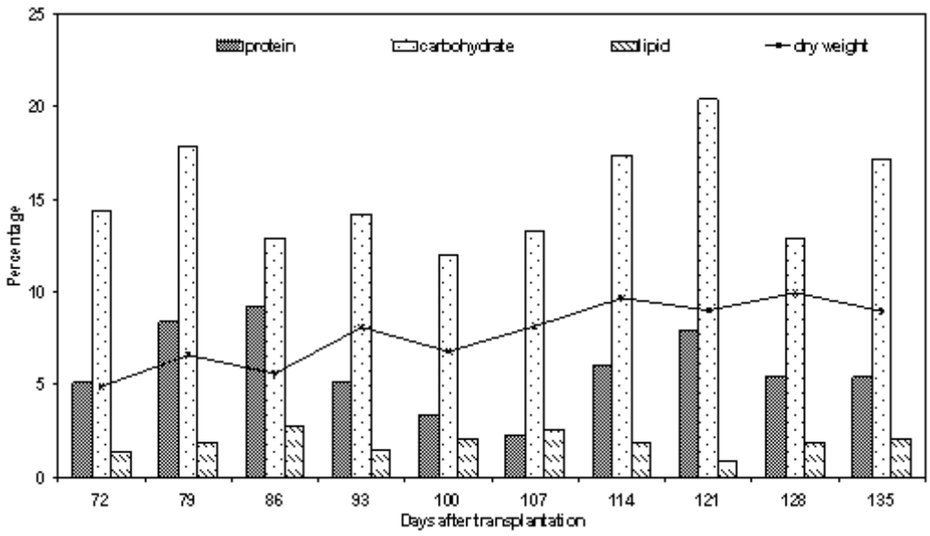

Fig. 1. *Biochemical constituents of* G. edulis *cultured from spores*

average yield of lipid was 1.91 + 0.55% (n=10). Carbohydrate content showed two distinct peaks on 79 and 121 days of culture period with an average yield of 15.29% + 2.79 % (n=10). Initially the carbohydrate content increased from 72 to 79 days and then declined on 86 days (Fig.1). Further, there was a gradual increase in carbohydrate till 121 days with a peak value of 20.4 %. The irregular pattern from 121 to 135 days and from 72 to 86 days may be due to influence of environmental parameters like rainfall and salinity. The carbohydrate content showed significant negative correlation with the lipid content (r= - 0.578*).

Protein content showed a different trend. It increased gradually from 72 to 86 days coinciding with the peak yield of lipid content and then declined till 107 days again coinciding with the second peak of lipid content (Fig.1). This did not show any significant correlation with lipid. Further, the protein content increased till 121 days coinciding with the second peak of carbohydrate content showing positive correlation (r= 0.538*) and then declined. From 128 to 135 days, it did not show any change. While comparing the dry weight with the biochemical constituents, it was observed that there was a gradual increase in the dry matter accumulation as the plants grow and influence positively the carbohydrate content but negatively the protein and lipid contents. The correlation was not significant in any of the case (Fig.1).

The qualitative and quantitative estimation of agar was carried out during the same period of growth. The yield of agar ranged between 25.5 to 29.06 % with an average yield of 26.29 + 1.94 % (n=10). The agar content showed two peaks, one on 93 days and the other on 128 days of transplantation (Fig. 2). While comparing the quality of agar, there was no marked variation in the gelling temperature throughout the culture period but the melting temperature was found to be more in 93 days after transplantation coinciding with high agar yield showing significant positive correlation (r=0.767**). The gel strength of agar showed gradual increase till harvest (Fig. 3). Marked

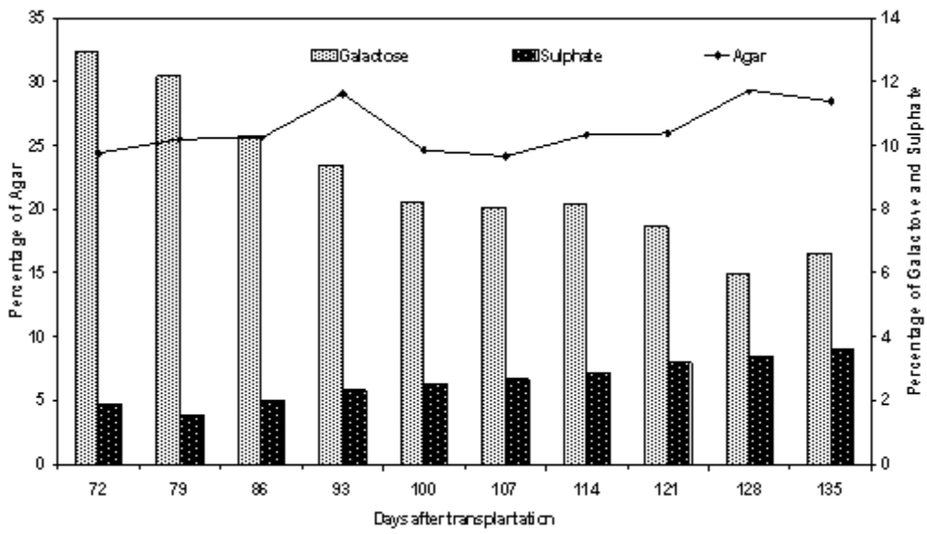

Fig. 2. *Quantitative estimation of agar, galactose and sulphate content in* G. edulis *cultured from spores*

increase of gel strength was observed from 121 to 135 days of transplantation showing positive correlation with agar (r=0.663*) and negative correlation with the galactose content (r=-0.684*). The galactose content showed gradual decline and sulphate noticed increase with respect to growth of the plant (r=-0.931**). Similarly the sulphate content showed positive correlation with the gel strength (r=0.767**). Accumulation of dry matter increased the sulphate content (r= 0.838**) and showed reduction in the galactose content (r= -0.854**).

Discussion

In India, the agar extracted from *G.edulis* and used as food grade agar in the domestic market needs qualitative assessment of the crop to evaluate the nutritional value and food safety. In the structural composition of agar by ^{13}C-NMR spectra of *Gracilaria* spp, showed agar constitutes various types of agarobioses (Ji, 1988 and Ji *et al.*, 1990), which influence the quality. *G.asiatica* is mainly composed of methyl group and it improves the gel strength, whereas *G.textorii* contains sulphate group and hence it retards the gel strength. The accumulation of sulphate group in *G.edulis* might have influenced the gel strength making it unsuitable for pharmaceutical industry (Fig.3).

Agar is usually compatible with most other polysaccharides and with proteins in near neutral condition. As the plant is mostly used for the food industry, the other biochemical constituents also play a major role for nutritional evaluation. These biochemical constituents are influenced by environmental parameters as well as varied with the age of the plant. Cultivation of *G.edulis* from spores can yield the crop of same growth stage and harvest can be done when the nutritional value of the crop is high.

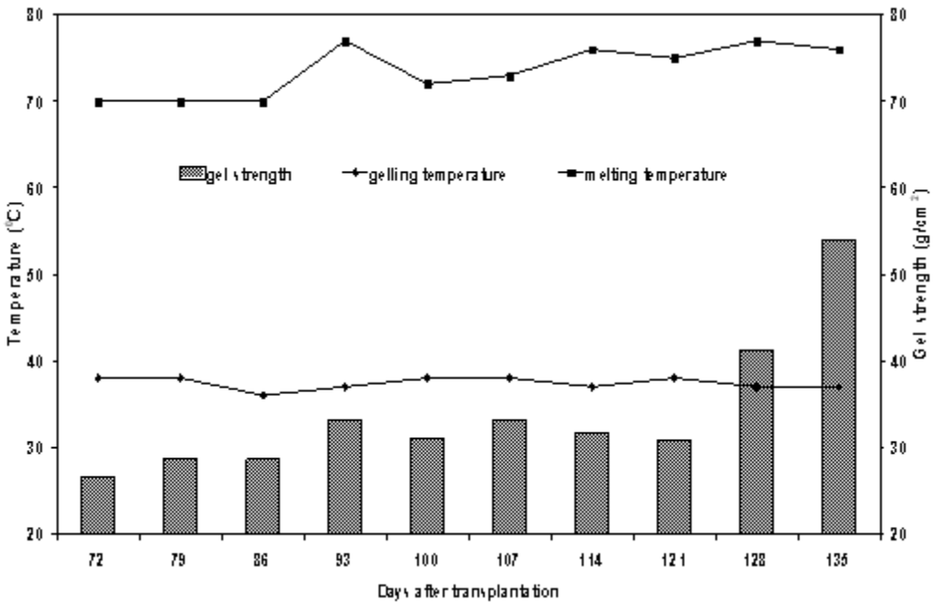

Fig. 3. *Estimation of agar quality*

In the present study, it was observed that the accumulation of agar and sulphate was high from 121 to 135 days after transplantation of the germlings corresponding to the age of the plant when the accumulation of dry matter is more. Formation of storage polysaccharides increased as the plant gets matured and improvement in gel strength was noticed after 121 days of culture period. Similarly, the galactose content declined. Thus dry matter accumulation showed positive correlation with agar and sulphate content but negative correlation with galactose content. The lipid content was found to be least showing a negative correlation with the carbohydrate content. This result is in conformity with Reeta and Kulandaivelu (1999). Although there is enormous literature on the effect of sulphate group for reducing gel strength (Lahaye, 2001; Montana *et al*.,1999), this statement will not hold good in this case. It may be presumed that the accumulation of storage polysaccharides and the sulphate content increased as the plant gets matured. During the initial stage of estimation (72 days of culture period), the galactose content was very high and might not have converted to the storage polysaccharides. As the plant gets matured, the polysaccharides content increased with respect to total galactose. While comparing the gel strength with other species like *Gelidiella*, the lower gel strength of *G.edulis* might have been contributed by the sulphate group with agar.

From this study it was observed that plants harvested from 121 days of culture period can yield higher protein, carbohydrate, low fat content and optimum yield of agar with good gel strength. In such case, the plant can be utilised during this period as a source of protein directly, source of agar for human use, as an additive to cattle feed or as a source of fertilizer (molasses) after extraction of agar.

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