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Laboratory culture of *Gracilaria spp.* and *Ulva lactuca* in seawater enriched media

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ABSTRACT

Thallus bits of *Gracilaria edulis, G. corticata, G.crassa* and *Ulva lactuca* were maintained in three enriched seawater growth media under laboratory conditions. Weekly observation on growth rate of these algae showed that Walne's medium enhanced Daily Growth Rate (DGR) of *Ulva lactuca* (54.42 ± 3.82 mg/d), *Gracilaria corticata* (58.71 ± 3.35 mg/d) and *G. crassa* (72.42 ± 2.07 mg/d). *Gracilaria edulis* registered higher growth rate in PES medium (50.42 ± 3.82 mg/d) than in Walne's and Gamborg media. However, addition of 2% garden soil extract to Walne's medium promoted the DGR in all the four species considerably (62.28 mg/d for *Ulva*, 66.71 mg/d for *Gracilaria corticata*, 77.71 mg/d for *G. crassa* and 56.29 mg/d for *G. edulis*). The results are compared with the growth rate of seaweeds achieved elsewhere in *in situ* sea farming being carried out without addition of any nutrients extraneously.

Introduction

In the laboratory, economically important seaweeds are maintained in enriched seawater mainly for spore production and reproductive method of seaweed cultivation (Rao, 1974; Kale and Krishnamurthy, 1967; Oza and Supriya Gorasia, 2001). Vegetative thalli of seaweeds have been cultured in enriched seawater media in the laboratory mainly to study the growth rate, ultra structure, yield of polysaccharides, effect of growth regulators, physical conditions and nitrate concentrations (Vijayaraghavan *et al.*, 1995; Hemalatha and Rengasamy, 1999; Srinivasa Rao and Umamaheswara Rao, 1999, Sivakumar and Rengasamy, 2000) Seaweeds are maintained in laboratory in synthetic seawater medium for 30 days and the growth rate is compared with that of natural seawater medium (Kaladharan, 2000). In the present communication, daily growth rate (DGR) of thallus bits of *Gracilaria edulis, G. corticata, G. crassa* and *Ulva lactuca* cultured in laboratory condition were reported and the DGR was compared with that reported for *in vivo* mariculture results.

Materials and Methods

Fresh seaweed samples were collected from Pamban and Mandapam and they were brought to the laboratory at

CMFR Institute, Cochin using a portable aerator, after removing the epiphytes and epifauna. After acclimatizing them for five days in the laboratory at 32 ppt filtered seawater, the cleaned, preweighed (W_o) thallus bits of *Gracilaria edulis, G. corticata, G. crassa* and *Ulva lactuca* were cut into small bits of 2-3 cm size and transferred to conical flasks (500 ml) containing 200 ml of sterilized liquid media (Table 1). These explants were incubated under diffused light from two fluorescent tubes from a distance of 50 cm at 22 ± 2°C in a 14 hr day / 10 hr dark regime. Growth rate was recorded weekly by measuring the weight gained (W_t) and the cultures were reincubated with fresh medium. Five replicates were maintained for each sample and each medium. The DGR was estimated from the following formula, DGR (mg/day) = $W_t - W_o/T$, Where, W_o = Initial wet weight of sample incubated, W_t = Final wet weight of sample after culture, T = Duration of culture in days.

Results and Discussion

Daily Growth rate (DGR) of seaweeds maintained in different media are presented in Table 2. Walne's medium was observed better than PES and Gamborg medium for *Ulva lactuca, Gracilaria corticata* and *G. crassa*. However, PES was found more suitable than other media for the DGR of *G. edulis*. Addition of 2% garden soil extract to Walne's medium improved the DGR of *Ulva lactuca* and all the species of *Gracilaria* studied (Table 2). Soil extract (2%) increased the DGR of *Ulva lactuca* to 15%, *Gracilaria edulis* to 12 %, *G.corticata* to 18% and *G. crassa* to 7 % more than that of media without soil extract.

Srinivasa Rao and Umamaheswara Rao (1999) conducted culture experiments to determine the growth rates of *Sargassum polycystum* in relation to temperature, photon flux and salinity in laboratory and found maximum growth rate at 25°C temperature, 30 µE m^2 S^1 photonflux density and 25 ppt salinity. ASP-6 medium is proved to be the best for the *in vitro* culture of *Callithamnion sp.* and photoperiod of 16 h light and 8 h dark and temperature regime of 10-15 °C are found optimal for the growth and differentiation of carpogonial branches (Vijayaraghavan *et al.*, 1995). Pretreating the explants of *Gracilaria edulis* in auxin, gibberellin and kinetin have remarkable effect on increasing the yield and the agar content (Hemalatha and Rengasamy, 1999). Culture of *Hypnea musciformis* and *H. valentiae* in PES-2 medium with increasing concentrations of nitrates upto 0.3 mM concentration enhances growth but reduces the *k*- carrageenan content (Sivakumar and Rengasamy, 2000).

Perusal of DGR of seaweeds maintained in the three seawater-enriched media (Table 1) reveals the biomass production to be very low. The DGR of these seaweeds under *in vitro* mariculture experiments are many times higher (Table 3) than that of the present *in vitro* culture (Table 3). Hence, laboratory culture of thallus in seawater-enriched medium can not be recommended for augmenting resources. However, the techniques of thallus culture of seaweeds in laboratory can be of much help to i) conserve the germplasm of seaweeds that are facing threat of extinction, ii) for the introduction of exotic species, iii) genetic improvement of stock through somatic hybridisation and iv) *in vitro* production of phycocolloids.

Table 1. *Composition of seawater enriched media tried*

Ingredients (mg/l)	Walne's medium	Walne's + 2% soil extract	Provasoli Enriched Seawater medium (PES)	Gamborg medium
$(NH_4)_2SO_4$			70	
$MgSO_4.7H_2O$				134
$CaCl_2.2H_2O$				500
KNO_3	100	100		150
$NaH_2PO_4.H_2O$				300
Na_2HPO_4	20	20		150
EDTA	45	45		
$MnSO_4.4H_2O$			0.41	
$ZnSO_4.7H_2O$			0.055	
$CuSO_4.5H_2O$	4	4		
$CoCl_2.6H_2O$	4	4		
Fe (sequestrence)				28
H_3BO_3	36.4	36.4	2.85	3
$Na_2MoO_4.7H_2O$				0.25
$FeCl_3$	1.3	1.3	0.1225	
$MnCl_2$	0.36	0.36		
$ZnCl_2$	4.2	4.2		
$(NH_4)_2MoO_4$	1.8	1.8		
Sodium glycerophosphate			10	
Fe EDTA			0.5	
Tris Buffer			10	
Na_2 EDTA			2.5	
$CoSO_4$	0.2	0.2	0.012	
Cyanocobalamine	0.05	0.05	0.1	10
Thiamine HCl			0.005	
Biotin			0.05	100
Myo-Inositol				1.0
Soil extract (2%)		10 ml		

Table 2. *Daily Growth Rate (mg [wet]/day)of seaweeds grown in seawater enriched media*

Species	Walne's medium	PES medium	Gamborg medium	Walne's medium + 2% Soil extract
Ulva lactuca	54.00 ± 3.46	35.43 ± 1.90	24.00 ± 1.73	62.28 ± 1.70
Gracilaria edulis	42.00 ± 2.08	50.42 ± 3.82	26.85 ± 1.68	56.28 ± 4.02
Gracilaria corticata	56.71 ± 3.35	48.00 ± 1.91	32.43 ± 2.22	66.71 ± 2.83
Gracilaria crassa	72.42 ± 2.07	63.71 ± 1.38	42.85 ± 2.19	77.71 ± 2.29

(DGR= mean ± s.d; n=5)

Table 3. *Daily Growth Rate of seaweeds cultivated in in vivo and in vitro conditions (mg (wet)/day).*

Seaweeds	In Vivo	In Vito (present study)
Ulva lactuca	4200	62.28 ± 1.70
Gracilaria edulis	3400	56.28 ± 4.02
Gracilaria corticata	3500	66.71 ± 2.83
Gracilaria crassa	3800	77.71 ± 2.29

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