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SUBJECT: Transmittal of AID/SCI Progress Report(s)

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AID/SCI Progress Report No. CS-344
PR 1986-1988

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AID - CDR C5-344

Progress Report 1986-1988

Title: Genetic improvement of Gracilaria,

an agar producing seaweed

Israel Levy & Michael Friedlander

IOLR Report No. H5/88

AID - CDR

Progress Report 1986 - 1988

Title: Genetic improvement of *Gracilaria*, an agar producing seaweed

Israel Levy and Michael Friedlander

No. DPE-5544-G-SS-6045-00

with the participation of

Ms. C. Maxim from I.O.L.R and Mr. I. Gozlan from I.M.I.

INTRODUCTION

Successful establishment of *Gracilaria*'s mariculture demands the use of proper strains in order to get higher and better agar yields. Many factors can influence the content and quality of the agar during the growth of the seaweeds: polysaccharides content and distribution, growth rate, growth conditions, resistance to epiphytes, resistance to bacterial degradation and others. Strain selection procedure for specific feasible characters, out of the wild type (W.T.) strains, can yield improved *Gracilaria* agar crop. The general genetics of the genus *Gracilaria* is known better than that of many other commercially importent seaweeds. Thus, some classical genetic manipulations with the thallus level are likely to enhance the growth of the seaweeds throughout the year and improve the agar quality and quantity.

OBJECTIVES

A. The establishment of a large stock of different strains and clones of *Gracilaria conferta* and other related species.

B. Characterization of the *Gracilaria* strains regarding morphology, physiology, growth and carbohydrates.

C. Introduction of selected laboratory strains to outdoor conditions.

D. Control of the life history of the local *Gracilaria* strains.

E. Enlargement of the natural variation of physiological and morphological characteristics by mutagenesis techniques

F. Establishment of strain selection procedures.

G. Definition of specific *Gracilaria* properties which can influance the agar quality and quantity, and be subjected to efficient strain selection procedures.

MATERIALS AND METHODS

ALGAL STOCKS: Different *Gracilaria conferta* strains isolated from an Israeli wild population were grown in the growth room under a controlled temperature of 25°C and a light regime of 8D:16L hours. The algal fronds were cut and cleaned

(using brushes) to leave cleaner tips. The procedure was repeated several times in order to remove algal contamination, and to avoid any damage to the *Gracilaria* strains. After 4 weeks during which no contaminating algae were observed, the algal clones were considered clean, and transfered to 100 ml Ehrlenmeyers with enriched and sterilized sea-water. The clean uni-algal strains were kept in very low growth conditions (25°C, 20 $\mu E/m^2/s$, no aeration, medium renewal once a month).

PIGMENT DETERMINATIONS: Chlorophyll-*a* was extracted directly from the fresh algal material using DMF (dimethylformamide), spectrophotometrically determined and calculated according to Moran's formula (Moran, 1982). Phycobillins were extracted using distilled water or phosphate buffer 0.01M, pH-6.8. After homogenisation and centrifugation, the different phycobillins (phycoerythrin and phycocyanine) were determined and estimated using the formulae of Kursor et al. (1983).

CARBOHYDRATES: Traditional carbohydrate determinations were done using the phenol-sulfuric and Resorcinol methods (Dubois et al., 1956; Yaphe & Arsenault, 1965). A more sophisticated method of gas chromatography (GC) was used to receive more detailed qualitative and quantitative information about the carbohydrate characteristics of the different algal strains (Holligan & Drew, 1971). The fresh or dried algal material was homogenized and hydrolysed with 2N TFA (trifluoroacetic acid), and boiled for 1 hour. The solution was completely dried with N_2 bubbling. The derivatisation of the monosaccharides was done using 20% TMSI (trimethylsilylimidazole) reagent in pyridine. After 40 min of the derivatisation reaction, the samples were ready to be injected into the GC. The gas-chromatograph (H.P. 5890A) was equipped with a fused silica capillary column (H.P-5, 0.5 FT; 0.2 ID; 25 m), and an FID detector connected to an integrator (H.P. 3392A). The running method was as follows: Initial temp. 120°C; initial time 1 min.;

temp. gradient 5°C/min; final temp. 240°C; final time 30 min. The samples (usually 0.5-1.0 µl) were injected into the capillary injector, and the carrier gas was usually helium. Using different known relevant standard compounds (glucose anomers, galactose, 6-O-galactose, xylose and agarose), most of the peaks (80%) could be identified on the chromatograms. Agar fractionation on DEAE Sephadex A-50 was discribed and discussed by Duckworth and Yaphe (1971). Diluted agar solution (in distilled water) was mounted on a DEAE Sephadex A-50 column and the agar fractions were collected, then NaCl 0.5 M solution was added and the second fraction was collected. The third fraction was obtained with 2M NaCl solution. The fractions were collected and analyzed by gas chromatographic and phenol-sulfuric techniques.

GROWTH: Growth experiments of the different strains were done mainly on the growth gradient table (light and temperature) which gave 25 different combinations of light and temperature (10-31°C, 40-280 µE/m^2/s). Fronds of different clones (up to 4 clones) were grown in deep covered petri dishes filled with enriched sea-water medium on the gradient-table. This device constantly rotated in order to maintain constant movement of the medium inside the dishes. The different fronds were cleaned and weighed each week, and growth rate was calculated at least for 4 weeks. Selected strains have been grown in outdoor tanks in order to evaluate their actual growth characteristics under the expected mariculture conditions, and to compare them with the mixed population grown in the ponds. Four strains of *Gracilaria* were placed in rectangular tanks (245 liters). The initial fresh weight of all strains was 1 kg, the water temperature was 20-22°C, and the light intensity in the tanks was 300-400 µE/m^2/s (in half depth). Regular running seawater with weekly pulse feeding of 2 mM ammonium and 0.2 mM phosphate in the water was the growth medium. Water movement in the tanks was caused by constant aeration.

AGAR: The agar content and quality was measured by actual extraction of the agar from the algal samples. Agar was extracted after an alkali treatment of the dried algae according to a modified procedure of Craigie & Leigh (1978), as follows. Five grams of dried ground *Gracilaria* were transferred into 250 ml beakers with 60 ml of 1.8% sodium borohydride ($NaBH_4$) for 18 hours. Next morning 40 ml of 3M sodium hydroxide were added to the beaker. The covered beaker was treated in a 90°C water bath for 1 hour. The treated algae were washed three times with 250 ml of distilled water over a plankton net. Then the algae were immersed in 100 ml of 0.1M phosphate buffer solution (pH 6.0) during the night. Next morning the pH of the suspention was brought to 6.5-7.0. The beaker was treated in an autoclave (110°C) for one hour, mixed with 2 g Celite and filtered through GF/A inside a hot metal cylinder. The filtrate, which gelled in room temperature, was cut into eight pieces and transferred into a freezer overnight. Next morning the frozen filtrate was thawed and filtered through a plankton net on a Buchner funnel. The precipitate was suspended in 100 ml iso-propylalcohol for 15 min, and filtered through a plankton net. The filtrate was frozen and dried in a lyophilizer (Virtis).

SPORE RELEASE AND SPORELINGS' GROWTH: After sporogenesis induction by temperature changes and/or light regime changes, accompanied most of the time by starvation treatment, the sporogeneous thalli (tetrasporophytes or cystocarps) were cleaned and placed in petri-dishes under low light conditions in a growth room (25°C, 8D:16L hours). The dishes were not moved or shaken for 3-4 days in order to let the spores (tetraspores or carpospores) be released and adhere to the bottom surface. Once the spores were fixed to the bottom, they started to germinate. After germination had started, the dishes were kept as clean as possible (mainly from bacterial contamination), and the medium was replaced every two days until small sporlings were observed. The sporelings were grown in medium light intensity (50 $\mu E/m^2/s$); they were also cleaned using very delicate painting brushes until they

were 2-3 mm length. Then the sporelings could be disconnected from the bottom of the dish and transfered to new flasks, especially if there were contaminating algae in the dishes.

MUTAGENESIS: The chemical mutagen EMS (ethyl methan sulfunate) was used in order to increase the normal rate of mutagenesis during the miosis which occurs during the tetrasporogenesis. In that manner it was possible to detect recessive as well as dominant mutations (the tetraspores which were subjected to the mutagen were haploid, and gametophitic plants developed out of them). The method was described by van der Meer (1979).

Tetrasporophytic thalli which were sporogeneous were immersed in 0.2 M EMS solution for 1-10 min, and the thalli were transferred to petri-dishes for spore release. After sufficient spores were released (thousands), the thalli were removed and the spores were left to germinate (as was previously described). Within one to two weeks after the germination, small discs of red cells (the first developmental stage of the *Gracilaria* sporelings) adhered to the bottom of the flasks. The effectiveness of the mutagenesis process could be estimated by the appearance of pigmentation mutants, which could be observed as soon as the disc stage had been formed.

STRAIN SELECTION: Growth-rate was the first feasible characteristic for which sporelings were selected. After the mutagenesis procedure had been performed, the sporelings were incubated for growth up to 1 cm length. Then they were cut from the bottom and transfered to 1 liter Ehrlenmeyer flasks with enriched seawater medium, constant aeration, and medium light intensity (80-100 $\mu E/m^2/s$). Identical good growth conditions were established for the sporelings, so that the individual growth characteristics could be pronounced. After one month of such growth, 100 of the largest sporelings were selected to be grown in the next selection step. The sporelings selected were brought to the same length by cutting the larger

ones, and fixed into rectangular pieces of fly-net being easily identified. The fly net piece (10-15 cm length) was put into a 1 liter Ehrlenmeyer flask with the regular growth medium and growth conditions previously mentioned. The growth of the sporelings was weekly monitored by weighing them back and cutting them to the initial length, to get reliable growth rate values. After 3-4 weeks, it was possible to select 50 sporelings that showed the higest growth rates. The procedure was repeated with 50 sporelings for another month of growth measurments but with double initial weight. At the end of this stage, the 10 best sporelings could be selected. These 10 sporelings were grown separately during another 4 weeks for a final growth evaluation. In this time more parameters of growth, such as branching index (number of branches in 1 g of fresh material) and contamination resistance, were evaluated.

STATISTICS: In some cases when it was important to demonstrate the significance of change, the T-test was employed using the general formule of

$$\frac{\bar{x}_2 + \bar{x}_1}{\sqrt{\frac{Sd_2^2 + Sd_1^2}{n}}}$$

df=(n-1)2; x_1, x_2, - average numbers; Sd_1, Sd_2 - Standard deviations; n - number of values.

RESULTS AND DISCUSSION

ALGAL STOCK

Local wild-type (W.T.) strains of the agarophyte *Gracilaria conferta* were cleaned from other epiphytes and were grown clonally as uni-algal cultures in a growth room. Other species and related genera of red algae, such as *G. tikvahiae, G. verrucosa* (from Fort Pierce, Florida), and the local carrageenanophytes *Hypnea cornuta, H. musciformis, Gelidiopsis* sp. and *Sarconema* sp. have also been growing under the same conditions. In addition, some mutant strains of the different

Gracilaria species, showing morphological pigmentation and other growth markers, are also included in a stock of 20 uni-algal clones. Other strains are in the process of cleaning. Recently, numerous *Gracilaria* species such as *G. debilis*, *G. domingensis*, *G. cervicornis*, *G. mammillaris*, and more unidentified *Gracilaria* species were brought to the I.O.L.R. lab from the Jamaica coasts by our collaborator Dr. Devi Prasad. Another species, possibly *G. verrucosa*, was brought from Argentina, Puerto Madrin. All these *Gracilaria* species are in the process of acclimatization to our local environment.

LIFE HISTORY

In addition to the phenological characterization of the stock strains (Table 1), and in order to perform controlled crosses between them, different generations have been obtained and isolated. They were grown clonally in the lab, and the conditions inducing sexual and asexual breeding were investigated. Different generations have been produced by collecting tetraspores and carpospores for germination of the more promising strains including the foreign ones.

Gracilaria species brought from Jamaica (*G. domingensis*, *G. cervicornis*, *G. mammillaris*, *Gracilaria* sp.) and from Argentina (*G. verrucosa*), have been successfully grown in the laboratory. Gametophytic (male and female) and tetrasporophytic clones of the local strain GSC-14 were obtained. Spore release, germination and development of sporelings in petri dishes have been successfully demonstrated.

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISATION

Six of the local *Gracilaria* W.T. (*G. conferta*) clones (SGSC-11, SGYC-3, SGSC-6, GSC-14, SGY-2 and P), and 3 foreign strains (GFL-VR, GFL-TK, GFL-TKG) have been grown in larger quantities in order to evaluate their growth and agar, as well as to characterize their morphology. Some of these strains showed different pigmentation (Table 1): GSC-14 green-brown, GFL-TKG green,

GFL-TK brownish. Others showed much higher branching values (SGY-2, GFL-TK), and bushy growth pattern (SGYC-3). Preliminary agar evaluations (Table 2) showed a promising gel strength of 780 and 990 gr/cm^2 in the GSC-14 and SGY-2 strains, respectively, and about 18% of agar in D.W. There were also marked differences in epiphyte contamination of the various strains (Table 2): the strain GFL-VR showed an amazing epiphyte-free culture (less then 1%), and SGY-2 was also considerably cleaner than the other strains.

GROWTH

The influence of different combinations of light and temperature values on the growth and carbohydrate characteristics as well as chlorophyll-*a* content has been measured in 5 *Gracilaria* strains. These experiments were performed on the gradient table. The influence of temperature changes (10-30°C) on chlorophyll-*a* content was not clear. Only in one strain (GFL-VR) there seemed to be some kind of pattern where the highest chlorophyll value in the lowest PFD was in 20°C, whereas the highest pigment content in higher PFD was found in 25°C. More obvious was the influence of PFD. The highest chlorophyll content was in the lowest PFD values in all the strains (Table 3). The strains SGY-2 and GFL-VR demonstrated higher chlorophyll content than the other strains. The growth rate (Fig. 1) was mainly influenced by the temperature changes, and to a lesser extent by PFD changes. It seems that 24-25°C was the optimal temperature for growth activity, and 180 μE/m^2/s was the optimal PFD in all the strains. All the *Gracilaria* strains showed a significant decrease of growth rate in temperatures higher than 25°C. This growth inhibition was more effective in the highest PFD (280 μE/m^2/s). The strain SGY-2 showed the highest growth performance, and reached under optimal conditions (24°C; 180 μE/m^2/s) a 125% weekly growth rate.

The strains SGY-2, GSC-14 and GFL-VR have been introduced recently to outdoor growth conditions in order to determine their growth performances in less

controlled conditions which are closer to outdoor mariculture. Specifically, it is of great importance to verify the outdoor growth rate of the different strains, and their epiphyte contamination in larger cultures (1 kg F.W.), and to compare them with the mixed population which has been used in the outdoor mariculture. Preliminary results showed that the GFL-VR and SGY-2 strains exhibited weekly growth rates (WGR) of 23-24%, the GSC-14 strain showed a WGR of 11%, while the mixed WT population demonstrated a WGR of 14% under the same conditions.

CARBOHYDRATES

Carbohydrate characteristics, as opposed to growth rate, were influenced by PFD changes more than by temperature changes (Table 6). In most of the strains, high values of total carbohydrates and glucose content were found under high PFD (180-280 $\mu E/m^2/s$) conditions. There were complementary relationships between glucose and galactose which seemed to be more than 95% of the carbohydrates in *Gracilaria* strains. The GAL/GLU ratio decreased with the increase of PFD values. Agar quality markers (6-O-MG, 0.92 and 1.013 RRT) did not show any clear pattern either with temperature or with PFD changes (Table 6). It was possible to see significant changes in carbohydrate characteristics between the different strains (Table 5). The content of the carbohydrate fractions at 0.92 and 1.013 RRT values was significantly higher in SGY-2 than in the other two strains ($P < 0.01$). From these results (see also the GC section), it was expected that the agar quality of SGY-2 regarding gel strength would be the best. This was, indeed, confirmed by the direct gel strength measurements of the different *Gracilaria* strains (Table 2). Highest GAL/GLU ratio, highest content of T.GAL, and αGAL, and lowest content of T.GLU (average values) were obtained by the strain SGY-2 (Table 4).

Galactose yield of the different strains was calculated using growth rate and carbohydrate characteristic results. GAL yield = Initial weight (gr. fw) x GR (%) x DW (% of fw) x CRBH content (% of dw) x GAL content (% of CRBH content). Since

relevent data about growth under different temperatures and PFD of the different strains were obtained (Table7), it was possible to calculate their galactose yield. The initial fresh weight was 1 gr, and galactose yield was calculated for the end of the first and fourth week of growth. The results are presented as gr. galactose/week or month/1gr. (initial fresh weight). The results show that the growth rate (GR) was the most effective character in the determination of the galactose yield, since the highest monthly galactose yield was obtained under the same conditions as the highest growth rate for the three strains, namely 24°C and 180 $\mu E/m^2/s$ (Tables 7, 8). The galactose yield was less influenced by total carbohydrate and galactose content, since the optimal conditions for growth rate were different from those of other characteristics (Table 7). In this way, the superiority of the SGY-2 strain was demonstrated again in spite of its lower DW and T.CRBH (Tables 6, 7) as compared to the other strains.

MUTAGENESIS AND STRAIN SELECTION

Mutagenesis experiments on one of the *Gracilaria* species (*Gracilaria* sp.) using the mutagen E.M.S. (ethylmethansulphonate) showed a 100-fold increase in mutation rates of pigmentation and morphological markers. This technique was previously used by Dr. I. Levy and others (van der Meer, 1979). As a result of the mutagenesis procedure, some morphological (W.T.M. - increase in the branching index), pigmentation (MG-16,18,19 - green and brown pigmentations), and growth (W.T.S.) mutants were obtained.

Strain selection procedures have also been tested with one of the *Gracilaria* strains. Sporlings (gametophytes), which were grown attached to petri dishes, were grown until they reached sexual maturation (1-2 cm long). Female gametophytes were isolated and grown in a detached way for 1 month. All the fertile tissues on the female gametophytes were removed, and only vegetative tips were grown until they reached 2 cm length, with some branches. Large amounts of such sporelings (about

100) were subjected to a strain selection procedure. In that way, it was possible to isolate one sporeling (W.T.S.) which showed a remarkable increase of weekly growth rate of 200% as compared to 30-60% of the others. This procedure enabled us to monitor growth performances of many sporelings without having to mark individual sporelings.

GAS CHROMATOGRAPHIC MEASUREMENTS

In collaboration with a chemist, Mr. I. Gozlan, an effort has been made to identify some markers that can reflect quantitative and qualitative characteristics of the agar polymer in small quantities (a few milligrams of algal d.w.). Gas chromatographic techniques with M.S. processing have been used to identify the components appearing on the chromatograms. The advantage of this technique was the simplicity, the great sensitivity and the good resolution of the different monosaccharides in the solution. The separation of glucose and galactose is very important to evaluate the agar/starch ratio in the algal material. Other compounds, such as 6-0-methylgalactose, xylose, α and β galactose, and α and β glucose were already identified on the chromatograms (Fig. 2). Studies are currently performed to identify the anhydrogalactose component of the agar. It seems that because of the instability of that compound, one has to look for other compounds into which the anhydrogalactose was degraded during the hydrolysis. However, there are two peaks which increased dramatically in the agarose compound when compared to regular agar or algal material (Fig. 2, Table 5). These compounds are marked by their relative retention time as compared to α-glucose on the chromatogram (Fig. 2), namely 0.92 and 1.013 RRT (relative retention time). They are suspected of being related to the anhydrogalactose component. The anhydrogalactose content in the agar polymer is one of the most important markers which can reflect the quality of the agar.

Fractionation of the agar into three fractions by a gel filtration technique, using DEAE-Sephadex A-50, yieded three fractions: 1. a neutral fraction which was eluted with distilled water, 2. a slightly negatively charged fraction which was eluted with 0.5 M NaCl, and 3. a more negatively charged fraction eluted with 2 M NaCl. Gas chromatograph analysis showed that the carbohydrate patterns and distributions in all three fractions were identical. It is therefore suspected that the second and third fractions contain agar with more sulfate groups, which increase the negative charge of the agar polymer. The partition of the agars of different strains, between those quantitatively evaluated fractions, can estimate the quality of the different agars. The purpose is to produce agar with as little as possible charged fractions.

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Table 1: Morphological characterization of Gracilaria strains which have been growing in the experiments.

Strain	Species	Generation	Origin	Morphology description
SGY -2	G. conferta	Male	ISRAEL (Med.)	Red, short, cylindrical, soft and very flexible branches, very slender with no constrictions. Spermatangial loci are found on special small and short branches.
SGYC-3	G. conferta	Female	ISRAEL (Med.)	Red thallus, very bushy appearance short cylindrical branches, in three dimensions, without any constrictions, very flexible thallus.
SGSC-6	G. conferta	Female	ISRAEL (Med.)	Red cylindrical long branches, very flexible thallus.
SGSC-11	G. conferta	Female	ISRAEL (Med.)	Red cylindrical long branches.
GY - 12	G. conferta	Female	ISRAEL (Med.)	
GSC- 14	G. conferta	Female	ISRAEL (Med.)	Brownish green, long cylindrical and hard branches. Less flexible than the previous strains.
		Tetrasp.	ISRAEL (Med.)	Tetraspores were observed on special short lateral branches. The thallus pigmentation was pink.
	G. conferta	Female	ISRAEL (Med.)	Red cylindrical thallus.
GFL-VR	G. verrucosa	Tetrasp.	U.S.A. (FL.)	Red cylindrical very hard branches. Dichotomous branching, no constrictions.
GFL-TK	G. tikvahiae		U.S.A. (FL.)	Light brownish. Slender short and slightly flattened branches. Dichotomous and two dimensional branching.
GFL-TKG	G. tikvahiae		U.S.A. (FL.)	Green, slender, long branches. Flexible cylindrical thallus.

Table 2: Some growth and carbohydrate aspects of different *Gracilaria* strains.

Strain	Branching Index (branches/gr FW)	Epiphytic Coverage (% of T.area)	Dry Weight (% of FW)	Agar Content (% of DW)	Gel Strength (gr/cm^2)
SGY - 2	202	5	11.5	17.3	992±33
SGYC- 3	115	10	10.7	16.6	678±20
SGSC- 6	48	60	12		
SGSC-11	43	60	12.4	16.6	432±22
GY -12	48	60	12.4		
GSC -14	35	20	12.5	18.3	778±77
P	52	60	10.7		
FL -VR	92	0	12.3	17.7	117±06
FL -TK	242	5	10.7		
FL -TKG	183	10	11.7		

Table 3: Chlorophyll-a (μg/mg DW) in 5 strains subjected to different combinations of light intensity (PFD) and temperature (gradient table).

PFD $\mu E/m^2/s$	Temp.(°C) → Strain	10	15	20	25	30
40						
	SGSC-6	-	-	1.41	1.08	1.01
	SGYC-3	-	0.95	1.18	0.85	1.01
	GSC -14	-	0.8	1.11	0.62	0.75
	SGY -2	-	1.45	1.74	1.2	1.05
	GFL -VR	0.861	1.13	2.146	2.043	1.716
80						
	SGSC-6	-	0.98	0.86	0.94	0.72
	SGYC-3	-	1.01	1.06	0.66	-
	GSC -14	-	0.93	1.25	1.1	-
	SGY -2	-	1.56	1.4	0.81	-
	GFL -VR	0.885	1.149	1.302	1.526	1.485
180						
	SGSC-6	-	0.58	0.91	0.73	-
	SGYC-3	-	0.78	0.92	0.93	-
	GSC -14	-	0.73	0.91	0.95	-
	SGY -2	-	1.13	-	1.15	-
	GFL -VR	-	0.99	1.095	1.313	-
280						
	SGSC-6	-	0.315	0.75	0.6	-
	SGYC-3	-	-	0.75	0.76	-
	GSC -14	-	0.47	0.75	0.76	-
	SGY -2	-	-	1.2	0.85	-
	GFL -VR	-	0.717	0.757	0.94	0.22

Table 4: Carbohydrate characteristics of different *Gracilaria* strains and of agarose. The results of the different strains are an averege of measurements done at different temperatures (10-30°C) and PFDs (40-280 $\mu E/m^2/s$).

Sample (strains or compounds)	CRBH (% of DW)	% of total carbohydrate content				
		T.GLU	T.GAL	GAL/GLU	αGAL	βGAL
SGY -2	30.0±7	11.6±4	88.4±4	7.6	29.3±3	32.0±6
GSC -14	37.5±12	24.3±12	69.0±22	2.8	21.0±3	35.0±9
SGSC-6	32.6±7	25.0±11	75.2±11	3.0	22.0±6	33.0±12
Agarose	100.0±0	0.5±0	99.5±0	199.0	16.7±1	25.6±1

CRBH - carbohydrate.
T.GLU - total glucose.
T.GAL - total galactose.
GAL/GLU - galactose/glucose.
αGAL - α-galactose.
βGAL - β-galactose.

Table 5: Agar quality markers: Relative quantities of 3 carbohydrate fractions (% of total carbohydrate (CRBH)) as they appeared on the chromatograms in different strains, and in agarose. Significant differences (t- test) between the strains are indicated (A; B; C), ($P < 0.05$).

CRBH fractions → Strains or compound		0.92		1.013		6-O-MG
SGY-2	B	2.982±1.151	B	3.592±1.064	B	8.158±3.761
GSC-14	A	2.133±0.460	A	1.889±0.690	A	3.746±2.537
SGSC-6	A	1.427±0.737	C	1.213±0.667	A	4.254±4.315
Agarose		23.241±1.633		14.272±0.244		9.611±0.313

6-O-MG - 6-O-methylgalactose.

Table 6: Carbohydrate characteristics of different Gracilaria strains subjected to different PFD (40, 80, 180, and 280 $\mu E/m^2/s$), and temperature (20, 25, and 30°C) conditions. (T. CRBH- total carbohydrate content as % of DW; T. GAL- total content of galactose as % of T. CRBH; αGAL- content of α-galactose as % of T. CRBH; βGAL- content of β-galactose as % of T. CRBH).

PFD $\mu E/m^2/s$	Strain	T. CRBH			T.GLU			GAL/GLU			αGAL			βGAL			6-O-MG			0.92 RRT*			1.013 RRT		
Temp. °C		20	25	30	20	25	30	20	25	30	20	25	30	20	25	30	20	25	30	20	25	30	20	25	30
40																									
	SGSC-6	27	24	21	25	11	8	3	8	12	22	24	25	40	53	52	1	1	1	2	1	2	1	1	3
	GSC-14	21	14	34	10	6	12	9	16	7	23	23	23	52	42	40	0	6	9	3	2	3	3	3	2
	SGY-2	49	28	22	6	6	9	16	16	10	27	35	26	20	27	39	18	6	4	5	3	4	2	3	5
80																									
	SGSC-6	39	26	33	37	23	20	2	3	4	17	17	28	35	31	27	3	8	4	1	1	2	1	1	1
	GSC-14	32	38	30	25	21	11	3	4	8	24	19	23	33	35	49	3	2	3	2	2	2	1	2	3
	SGY-2	32	29	33	8	10	9	12	9	10	29	29	28	42	38	32	10	10	6	2	3	5	5	5	3
180																									
	SGSC-6	43	33	39	47	21	25	1	4	3	15	14	33	26	20	21	1	14	3	1	0	2	1	1	1
	GSC-14	42	52	42	45	32	25	1	2	3	17	22	24	22	29	30	3	1	3	2	2	3	2	1	2
	SGY-2	28	28	20	20	10	17	4	9	5	31	25	29	32	29	35	5	8	7	2	2	3	3	3	5
280																									
	SGSC-6	-	-	40	-	-	31	-	-	2	-	-	24	-	-	20	-	-	9	-	-	2	-	-	1
	GSC-14	60	43	42	43	35	26	1	2	3	12	18	21	25	31	31	1	3	3	1	2	2	2	1	2
	SGY-2	30	26	34	17	15	14	5	6	6	30	37	25	33	26	30	11	10	7	2	3	2	2	2	3

* Two unidentified compounds marked by their retention time relative to α-glucose (as it appeared on the gas chromatogram).

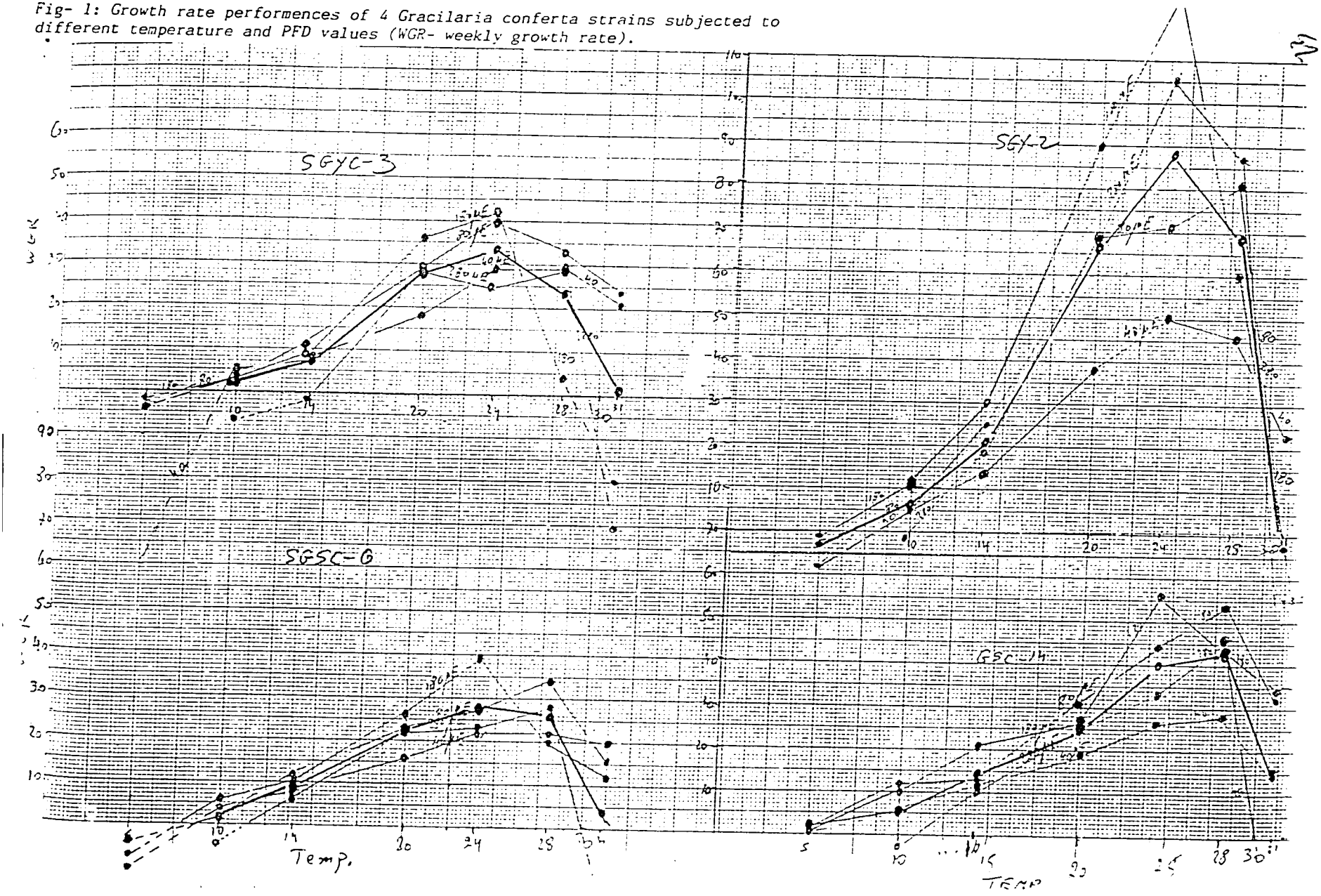
Table 7: Galactose yield constituents in different Gracilaria strains grown at different light and temperature conditions (WGR- weekly growth rate in %; DW- dry weight in % of fresh weight; T.CRBH - total carbohydrate; T.GAL - total galactose).

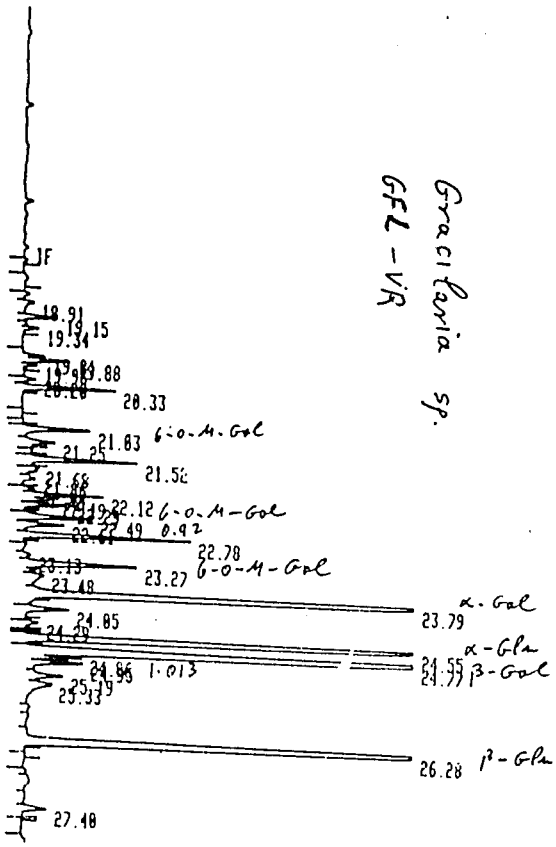
Temp. °C →		20				25				30			
PFD µE/m²/s	Strain	WGR	DW	T.CRBH	T.GAL	WGR	DW	T.CRBH	T.GAL	WGR	DW	T.CRBH	T.GAL
40													
	SGSC-6	15	12	27	75	22	12	24	89	20	12	21	92
	GSC-14	19	12.5	21	90	26	12.5	14	94	17	12.5	34	88
	SGY-2	37	11.5	49	94	50	11.5	28	94	30	11.5	22	91
80													
	SGSC-6	22	12	39	63	27	12	26	77	22	12	33	80
	GSC-14	30	12.5	32	75	46	12.5	38	79	39	12.5	30	89
	SGY-2	69	11.5	32	92	74	11.5	29	90	25	11.5	33	9
180													
	SGSC-6	26	12	43	53	39	12	33	79	15	12	39	75
	GSC-14	26	12.5	42	55	54	12.5	52	68	37	12.5	42	75
	SGY-2	90	11.5	28	80	110	11.5	28	90	25	11.5	20	83
280													
	SGSC-6	22	12	-	-	23	12	-	-	0	12	40	69
	GSC-14	25	12.5	60	57	35	12.5	43	65	3	12.5	42	74
	SGY-2	69	11.5	30	83	100	11.5	26	85	10	11.5	34	86

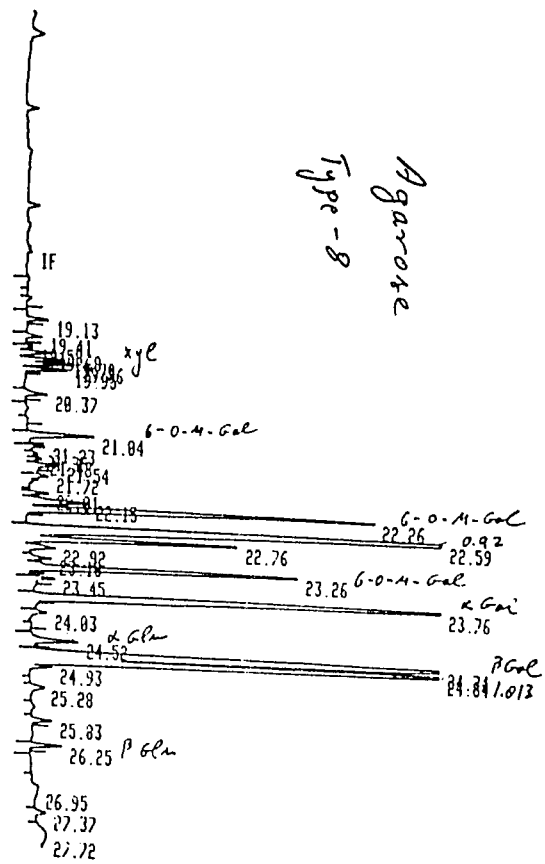
Table 8: Galactose yield (expressed as gr galactose/week or month/1 gr fresh initial weight) after 1 & 4 weeks of growth of different *Gracilaria* strains in different light and temperature conditions.

Temp. °C →		20		25		30	
PFD µE/m²/s	Strain	week	month	week	month	week	month
40							
	SGSC-6	0.028	0.042	0.031	0.057	0.028	0.048
	GSC-14	0.028	0.047	0.021	0.041	0.044	0.070
	SGY-2	0.072	0.186	0.045	0.153	0.033	0.071
80							
	SGSC-6	0.036	0.064	0.031	0.062	0.039	0.070
	GSC-14	0.032	0.086	0.055	0.171	0.046	0.125
	SGY-2	0.057	0.276	0.052	0.275	0.043	0.084
180							
	SGSC-6	0.034	0.069	0.043	0.116	0.040	0.061
	GSC-14	0.036	0.073	0.068	0.248	0.054	0.139
	SGY-2	0.049	0.336	0.061	0.563	0.024	0.047
280							
	SGSC-6	-	-	-	-	-	-
	GSC-14	0.053	0.104	0.047	0.116	0.040	0.044
	SGY-2	0.048	0.233	0.051	0.407	0.037	0.050

Fig- 1: Growth rate performences of 4 Gracilaria conferta strains subjected to different temperature and PFD values (WGR- weekly growth rate).







RUN # 16 MAY/09/88 14:42:32
WORKFILE ID: C
WORKFILE NAME:

AREA%

	RT	AREA	TYPE	AR/HT	AREA%
	19.15	16165	VV	0.050	0.452
	19.88	22071	VV	0.051	0.616
	20.33	43210	PB	0.049	1.207
6-O-M-G	21.03	34367	BP	0.056	0.960
	21.25	11869	PB	0.051	0.332
	21.52	50098	BB	0.050	1.399
	22.12	39461	VV	0.053	1.102
	22.19	14134	VV	0.049	0.395
6-O-M-G	22.29	21509	VB	0.051	0.601
	22.49	34144	BV	0.053	0.954
0.92	22.61	20864	VV	0.056	0.583
	22.78	74072	VB	0.047	2.069
6-O-M-G	23.27	68805	VV	0.065	1.924
	23.48	16819	VV	0.116	0.470
α Gal	23.79	644440	VB	0.058	17.998
	24.05	22051	BB	0.058	0.616
α Glu	24.55	482380	BB	0.051	13.472
β Gal	24.77	1174500	BV	0.052	32.800
1.013	24.86	25444	VV	0.048	0.711
	24.95	36101	VV	0.068	1.008
	25.19	30803	VV	0.089	0.860
	25.33	35158	VV	0.138	0.982
β Glu	26.28	647760	VB	0.053	18.090
	27.40	14375	VB	0.065	0.402

TOTAL AREA= 3580700
MUL FACTOR= 1.0000E+00

RUN # 11 MAY/09/88 09:58:49
WORKFILE ID: C
WORKFILE NAME:

AREA%

	Ri	AREA	TYPE	AR/HT	AREA%
	19.68	12000	VV	0.050	0.346
	19.79	17689	VV	0.047	0.510
	19.86	22381	VV	0.049	0.646
	19.95	18609	VB	0.047	0.537
	20.37	11559	BB	0.055	0.333
6-O-M-G	21.04	36433	PB	0.058	1.051
	21.54	12252	PB	0.054	0.353
	22.15	40792	VV	0.072	1.177
6-O-M-G	22.26	164720	VB	0.050	4.751
0.92	22.59	987320	BV	0.054	28.479
	22.76	104630	VV	0.052	3.018
	22.92	20463	VV	0.078	0.590
	23.10	13076	VV	0.060	0.377
6-O-M-G	23.26	142260	VB	0.056	4.103
α Gal	23.45	11009	BB	0.052	0.318
	23.76	670320	PB	0.050	19.335
α Glu	24.52	25984	PB	0.061	0.750
β Gal	24.74	604400	BV	0.052	17.434
1.013	24.84	397760	VV	0.051	11.473
	24.93	10131	VP	0.052	0.292
	25.28	13114	PV	0.090	0.378
β Glu	26.25	15972	BB	0.054	0.461
	26.95	28734	VV	0.306	0.829
	27.37	23078	VV	0.139	0.666
	27.72	62135	I VH	0.368	1.792

TOTAL AREA= 3466800
MUL FACTOR= 1.0000E+00

Fig- 2: Gas Chromatograms of agarose and some Gracilaria strain material with the retention times of the different compounds and their relative content (Area %).

GENETICS OF GRACILARIA - A JOINT UWI-IOLR PROJECT.

REPORT ON THE WORK DONE BETWEEN SEPTEMBER 1987 - DECEMBER 1987

As I have communicated before by telex, due to some mix up, the project could not be started earlier. However, once the project got started, we moved quickly to get the work going: In accordance with the first phase of the plan, several species/strains of Gracilaria were collected from different localities in Jamaica (Map): These are briefly described below.

Gracilaria cornea J. Agardh. Two strains of this species were collected: One from the Seaward side of the Palisadoes, Port Royal at low tide. This plant grows up to 20cm with pinkish to pale straw colour. The thallus is cylindrical and carlitagenous, abundantly branched. The second strain was collected from White Horses, about 30 km east of the first strain. This strain was more rough in texture and robust in appearance than the Palisadoes one: Both female and tetrasporic plants were collected: But so far, only carpospores could be successfully isolated.

Gracilaria daemaecornis. J. Agardh. Two strains of this species were collected from two distinctly different localities - White Horses bay on the south coast, in St. Ann's Bay, on the north coast. These plants were bushy and bright cherry red in colour. Only female plants could be collected.

Gracilaria crassissima Crowan. Collected from lover's beach in Ocho Rios on the north coast in shallow water. Growth habit of this alga makes it look like a coral-line encrusted rock. Reproductive Status could not be ascertained at present.

Gracilaria verucosa (Hydson) Papenfus. Female plants of this species were collected from Bowden on the South Coast. While the identification of this alga in tropical waters is a bit doubtful, identification was made according to the present toxonomic criteria. This alga grown in shallow water where a small rivulet opens.

Gracilaria cervicornis. Both female and tetrasporophytes were collected from Mamee Bay in the north coast and Palisadoes on the south coast. This is similar to what is also described as G. ferox. But now both are treated under G. cervicornis.

Gracilaria foliifera (Forskal) Borgesen. Female plant of this species were collected from St. Ann's Bay near a river outlet. These plants were growing at 2 - 3m depth.

Gracilaria domingensis Sorder. Two strains of this species were collected one from St. Ann's Bay on the north coast and the second from White Horses Bay on the south coast.

Gracilaria sjcestedii. Female plants of this species were collected from Bowden on the south coast from very shallow waters near a rivulet mouth.

Gracilaria mammilaris. Two strains of this species were collected - one from lover's beach in Ocho Rios and the second from Mammee Bay, both growing in very shallow waters. Reproductive status uncertain.

Gracilaria cylindrica Bergseen. This species was obtained from St. Ann's Bay. Reproductive state uncertain. This species has a distinct Rose-red colour.

Gracilaria sp. Female plants of an unknown species were collected from Roxborough beach on the north coast. This could possibly be G. armata but further characterisation is necessary for correct identification.

Cultures: All the above mentioned strains are currently under cultivation in 40 litres aquarium tanks with continuous aeration. Filtered seawater is used to maintain the cultures. The seawater is diluted to give a strength of 29% salinity for better growth of Gracilaria sp. Salinity is monitored daily. The epiphytes are hand cleaned and also various other methods are used to suppress the growth of epiphytes. These include (1) Antibiotic treatment for inhibiting blue green algae, (2) Germanium dioxide for diatoms, (3) partial air drying to inhibit some red and green epiphytes. Seawater is changed once in a week. The cultures are maintained at 26± 1°C.

Nutrition: The plants are pulsed once in a week with a solution of 0.02m $NaNo_3$,0.004m NaH_2Po_4 and micro-nutrients, coinciding with water change. The pulsing duration is usually 8 hours after which the algae are returned to the tanks.

Growth: While growth in the form of new branches is observed, it is too early to quantify the growth for all the species.

Spore isolation: Spore isolation has been attempted with tetrasporic plants where available and cystocarps. Various conditions were tried. These include Photoperiods. (8hL or 14hL), temperature (22°C or 28°C) and salinity (25%-33%). Initial experiments were successful in only two species with carpospores. Further work is in progress.