Effect of temperature, salinity and light intensity on the growth of *Gracilaria* spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India

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Species of *Gracilaria* Greville were collected from Japan, India and Malaysia. Growth rate experiments were conducted at different temperatures, salinities and light intensities. *Gracilaria vermiculophylla* was found to be the fastest growing species with a growth rate of 22.32%. The growth rates of all species varied with salinities, but most of them attained their optimum growth rates at a normal seawater salinity 35‰. During the temperature tolerance experiments, it was observed that only *G. vermiculophylla* had a high temperature tolerance limit of up to 35°C. The temperate and the tropical species of *Gracilaria* were divided into three groups depending upon their optimum growth rates at the optimum temperatures. Group I consisting of *G. arcuata* and *G. textorii* from Japan with optimum growth rates at 20°C. Group II consisting of *G. vermiculophylla*, *G. incurvata* from Japan and *G. foliifera*, *G. corticata* from India with optimum growth rates at 25°C, while group III consisting of *G. edulis* from India and *G. lichenoides* from Malaysia with optimum growth rates at 30°C.

The genus *Gracilaria* Greville, belonging to the division Rhodophyta is one of the most extensively studied alga throughout the world, because of its economic importance as an agarophyte. This genus consists of more than 100 species with a worldwide distribution except in the Arctic Ocean. *Gracilaria* spp. account for more than half of the world agarophyte production and are cultivated on a commercial scale in Chile, South Africa, China, Taiwan and Israel. Some of the *Gracilaria* species are used as food, for the extraction of research grade agar-agar and are also being used in the preparation of pharmaceutical compounds.

The overexploitation of the wild biomass of economically important agarophytes has led to the development of techniques for cultivation of *Gracilaria* to meet industrial demands. The success achieved in *Gracilaria* cultivation is attributed to its domestication, which has been accomplished rapidly, and efficiently in recent years. However, there should have been more focus on developing improved strains to meet the increasing market demands. The selection of the strains should be based on certain agronomically important traits like adaptability to wide environmental variations, high biomass production and better quality and quantity of agar. In order to select the desirable strains, basic knowledge on the growth rates of wild biomass under varied environmental factors is a primary requisite.

Changing temperatures, salinities and light intensities are a part of the marine environment. A strain can be selected by assessing its response to these abiotic factors. In this study growth rates of different *Gracilaria* species collected from Japan, Malaysia and India have been investigated under ranges of various environmental factors viz. temperature, salinity and light intensity and compared with each other. The main aim of this study was to identify fast growing strains and also to prepare a detailed comparative account of *Gracilaria* species from tropical and temperate regions to the above mentioned conditions. In this study a comparison of growth rates of different species of *Gracilaria* from two different biogeographical regions of the world viz., from the western tip of Japan which can be considered as the warm temperate zone and from the Malaysian and the Indian coasts which can be considered as the tropical zone have been undertaken.

**Materials and Methods**

*Culture and maintenance of plant material*—Matured cystocarpic plants of *Gracilaria* species (Table 1) were collected from Nagasaki (Japan), Penang (Malaysia) and Diu, Gopnath and

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Temperature experiments—The effect of temperature on the growth of different species was investigated at 10, 15, 20, 25 and 30°C. The experiments were conducted in incubators (NK System Biotron, Osaka, Japan). Once the optimal temperature for each species was known, salinity experiments were initiated at their respective optimum temperatures.

Salinity experiments—Salinity experiments were carried out at 10, 15, 20, 25 and 30‰. Different salinities were made by adding distilled water to filtered seawater to get the desired salinity, which was checked using salinometer (Salinometer S10 Iuchi No.4, Osaka, Japan). It was then autoclaved and enriched with PES before being used for the culture experiments.

Light intensity experiments—Growth rates of the different species of Gracilaria were investigated at three different light intensities viz. 10, 40 and 80 µmol m⁻² s⁻¹ at their optimum temperatures and salinities. The light intensity was measured using a photometer (Conmic 100B, Osaka, Japan).

Growth measurements—The growth was recorded as changes in the fresh weight of the fragments. Before weighing the fragments were blotted with paper towel. The weights were taken at the start of the experiment, initial weight (Wi) and after 1 month at the end of the experiment, final weight (Wf), using analytical digital balance (Mettler, Toledo PB153, Switzerland). The daily growth rate, DGR (%) was calculated by using the formula

\[ DGR = \ln\{Wi/Wf\} \times 100/d \]

where \( \ln \) = natural logarithm, and \( d \) = number of days.

High temperature tolerance of Gracilaria spp.—Temperature tolerance limits of each species was determined by culturing segments of plants in a test-tube containing 20 ml PES medium which in turn were placed in water baths maintained at different temperatures of 30, 32.5, 35 and 37.5°C. The experiment was continued for 10 days and the plants which were bleached of pigments were considered to be dead. During the experiments medium was changed after every 3 days.

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Table 1—Isolates of Gracilaria species: Place and date of collection

<table>
<thead>
<tr>
<th>Species Code</th>
<th>Place</th>
<th>Latitude, longitude</th>
<th>Date collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-jpn</td>
<td>Nagasaki, Japan</td>
<td>32°50'N, 129°55'E</td>
<td>17 May 1996</td>
</tr>
<tr>
<td>IN-jpn</td>
<td>Nagasaki, Japan</td>
<td>32°50'N, 129°55'E</td>
<td>10 Jun. 1996</td>
</tr>
<tr>
<td>TX-jpn</td>
<td>Nagasaki, Japan</td>
<td>32°50'N, 129°55'E</td>
<td>17 May 1996</td>
</tr>
<tr>
<td>LI-mal</td>
<td>Penang, Malaysia</td>
<td>5°20'N, 100°20'E</td>
<td>25 Aug. 1995</td>
</tr>
<tr>
<td>ED-ind</td>
<td>Mandapam, India</td>
<td>13°05'N, 80°17'E</td>
<td>12 Dec. 1996</td>
</tr>
<tr>
<td>FO-ind</td>
<td>Gopnath, India</td>
<td>20°54'N, 70°22'E</td>
<td>14 Nov. 1996</td>
</tr>
<tr>
<td>CO-ind</td>
<td>Diu, India</td>
<td>20°42'N, 70°59'E</td>
<td>08 Jan. 1996</td>
</tr>
</tbody>
</table>
Results

**Growth rates at different temperatures**—It was observed that the temperatures at which the species showed the maximum daily growth rates, DGR (%), corresponded to their regional optimum temperatures. Species from the Japanese waters showed their maximum DGR at temperatures between 20-25°C with the VR-jpn showing the highest DGR of 12.94% at 25°C while species from Malaysia and India showed their maximum DGR between 25-30°C (Fig. 1). Among the tropical species, the highest DGR of 16.39% at 25°C was observed in the isolate CO-ind followed by FO-ind, LI-mal and ED-ind. Statistical analysis using Students’ *t*-test showed that the results between two species were significant at \( p \leq 0.05 \).

**Growth rates at different salinities**—There was a varied response of all the species from both tropical as well as temperate regions to the varying salinities (Fig. 2). Especially the temperate species of *Gracilaria* from Japan differed greatly in its response. Isolates VR-jpn and TX-jpn could tolerate very low salinities (10‰) but for the isolates IN-jpn and AR-jpn, low salinities were lethal. The IN-jpn plants died at 15‰ and 15‰ salinities but from 20‰ to 30‰ salinity there was an abrupt increase in their growth rates. A similar response was observed with the isolate AR-jpn.

Among the tropical species, the isolate CO-ind from Diu, from the western coast of India, had better growth rates at lower salinities, but very low salinities below 15‰ were found to be lethal. It showed an optimal DGR of 10.92% at 25‰. At lower salinities of 10‰ the plants segments died after 2 days in culture. The other Indian tropical isolates, ED-ind and FO-ind, could survive very low salinities of even up to 10‰ but the DGRs were as low as 5.37% and 5.28% respectively. Growth increased with increasing salinities in both the cases. In the case of the isolate LI-mal it showed an optimal DGR (11.69%) at 25‰ and decreased at higher salinities. It was seen that LI-mal could survive low salinities of even 10‰ though the DGR was observed to be very low (4.64%). Statistical analysis using Students’ *t*-test showed that the results were significant at \( p \leq 0.05 \).

**Growth at different light intensities**—The difference between the growth rates at different light intensities was not much, except for LI-mal and AR-jpn (Fig. 3). Their growth rates increased with increasing light intensities. In fact all the species studied showed an increase in growth rate with increasing light intensity implying that *Gracilaria* species grew well at higher light intensities.

Among the temperate species from Japanese waters, isolate VR-jpn showed the highest optimal DGR of 22.32% at 80 μmol m⁻²s⁻¹ and the least was showed by the isolate TX-jpn from Japanese waters at 10 μmol m⁻²s⁻¹ having a DGR of 1.55%. *Gracilaria corticata* from the tropical waters of India, at 10 μmol m⁻²s⁻¹ had a much better DGR 12.78% and had the best DGR of 16.39% at 80 μmol m⁻²s⁻¹ among the tropical species. Among the tropical species the growth rates remained almost steady from
10-80 µmol m$^{-2}$s$^{-1}$ except for the isolate LI-mal where there was a sudden increase from 10-40 µmol m$^{-2}$s$^{-1}$ (6.63% -12.19%) and thereafter remaining almost steady. Statistical analysis using Students’ $t$-test showed that the results were significant at $p\leq0.05$.

High temperature tolerance limits—During this experiment it was observed that the temperate isolate VR-jpn could tolerate high temperatures of up to 35°C whereas the isolate AR-jpn could tolerate the highest of up to 30°C (Table 2). Remaining other species from the temperate and the tropical regions showed a high temperature tolerance limit of 32.5°C. The species CO-ind from Indian waters showed slight bleaching at 32.5°C. This was also the case with isolate LI-mal from Malaysian waters.

Thus, VR-jpn showed the maximum daily growth rate, followed by CO-ind, ED-ind, AR-jpn, LI-mal, IN-jpn, FO-ind, and TX-jpn at their optimum culture

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**Fig. 2**—Comparison of percentage daily growth rates of *Gracilaria* species from Japan, Malaysia and India at different salinities at 20°C temperature and 60 µmol m$^{-2}$s$^{-1}$ irradiance

**Fig. 3**—Comparison of percentage daily growth rates of *Gracilaria* species from Japan, Malaysia and India at different light intensities with 20°C temperature and 30‰ salinity
conditions of temperature, salinity and light intensity (Table 3).

Discussion

Growth rate studies of *Gracilaria verrucosa* and *Gracilaria* species were reported under laboratory conditions\(^2\,\text{12}\). Growth rate studies of *G. verrucosa* and *G. salicornia*, and *G. gracilis*, were also carried out in outdoor as well as indoor culture\(^{13,\text{14}}\), but studies on comparative growth rates between different species of *Gracilaria* from different geographical regions are scanty. Several studies have reported that temperature\(^{15-17}\), salinity\(^{1,\text{18}}\) and light\(^{19}\) are the most important factors determining the growth and distribution of benthic marine algae. Majority of the marine algae are confined mostly to the intertidal region where there are wide fluctuations in these factors. In the present study, different species of *Gracilaria* showed various responses with regards to their growth rates with the changing environmental factors like temperature, salinity and light intensity.

All the temperate species showed a decrease in their growth rate above 25°C. This behaviour of the species may be attributed to their adaptation to their natural habitats. The *Gracilaria* species were found to be abundant when the seawater temperatures fluctuated from 20-27°C. The experimental results also coincide with environmental conditions. Similarly, the optimal temperature range shown by the *Gracilaria* species from tropical areas (India and Malaysia) is in accordance with the seawater temperatures of their respective areas. Seawater temperatures in the Arabian Sea, range from 26 to 30°C, that of Bay of Bengal 24 to 30°C and Malaysia 26 to 28.5°C (ref. 20-22). Temperatures below this range would hinder the growth and lower temperatures became lethal. It thus suggests that, lower winter temperatures as transit towards the temperate region act as barriers to the distribution of species from tropical regions.

Among the tropical species of *Gracilaria*, two of the isolates which were collected from the northwestern part of India, viz., FO-ind from Gopnath and CO-ind from Diu, had optimal growth rates of 10.62% and 13.36% respectively at 25°C. The isolates collected from Mandapam, the southeastern part of India, and from Penang, Malaysia, ED-ind and LI-mal respectively had best growth rates at 30°C, thus indicating that the optimum temperature for optimum DGR goes on increasing from temperate to tropical region. The maximum growth rates of the isolates, VR-jpn, 22.32%; LI-mal, 11.69%; ED-ind, 12.26%; FO-ind, 10.62%; and CO-ind, 13.36% are greater than those observed in previous studies\(^{14,\text{23}}\), and thus these can be considered as potential strains for cultivation.

### Table 2 — High temperature tolerance of thalli of different *Gracilaria* species

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>VR-jpn</td>
<td>++</td>
</tr>
<tr>
<td>TX-jpn</td>
<td>++</td>
</tr>
<tr>
<td>AR-jpn</td>
<td>++</td>
</tr>
<tr>
<td>IN-jpn</td>
<td>++</td>
</tr>
<tr>
<td>LI-jpn</td>
<td>++</td>
</tr>
<tr>
<td>ED-ind</td>
<td>++</td>
</tr>
<tr>
<td>FO-ind</td>
<td>+</td>
</tr>
<tr>
<td>CO-ind</td>
<td>+</td>
</tr>
<tr>
<td>++: No damage; +: Slight damage; -: Dead</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 — Maximum growth rates of *Gracilaria* species at their optimum culture conditions of temperature, salinity and light intensity

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Maximum daily growth rate (%)</th>
<th>Temperature (°C)</th>
<th>Salinity (‰)</th>
<th>Light intensity (µmol m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-jpn</td>
<td>22.32</td>
<td>25</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>AR-jpn</td>
<td>11.73</td>
<td>20</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>IN-jpn</td>
<td>11.18</td>
<td>25</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>TX-jpn</td>
<td>8.13</td>
<td>20</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>LI-mal</td>
<td>11.69</td>
<td>30</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>ED-ind</td>
<td>12.26</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>FO-ind</td>
<td>10.62</td>
<td>25</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>CO-ind</td>
<td>13.36</td>
<td>25</td>
<td>25</td>
<td>80</td>
</tr>
</tbody>
</table>

The range of temperature, salinity and light intensity investigated in this study were 10-30°C, 10-30‰, and 10-80 µmol m\(^{-2}\) s\(^{-1}\) respectively.
Gracilaria spp. which are growing in the subtidal region are always subjected to great variations in light intensities, sometimes due to the turbidity of water, cloudy sky, or overcrowding by other algae, and positioning of the substratum. Exposure to full light in tidal pools has deleterious effects on young plants. Gracilaria plants mature well in the infralittoral zone and have been seen growing luxuriantly in the intertidal zone and also in the pools, but young plants were not commonly seen in the tide pools. During growth rate experiments with G. sordida, it responded to light at all temperatures and that the growth increased at higher photon flux densities.

In the present study, only isolates AR-jpn and LI-mal showed much variation in their growth rates in response to various light intensities. Isolate LI-mal showed an initial spurt in the growth rate and thereafter remained almost constant, suggesting that light does not limit the growth of Gracilaria species. Gracilaria spp. are not usually limited for growth by low light in nature. This kind of response may be attributed to the turbid waters in their natural environments.

In conclusion, we found that, temperature and salinity play a major role in the growth and distribution of Gracilaria plants. Each species responded in accordance with the environmental conditions of their inhabitant regions of growth. For some species like VR-jpn, salinity did not act as a limiting factor while for some others like AR-jpn, it was a limiting factor. Growth was not restricted by light, probably because the species are adapted to low light intensities in their natural environments.

Based on the optimum DGR at their optimum temperatures, the species studied were divided into 3 groups viz.

Group I: Consisting of isolates AR-jpn and TX-jpn from Japan having optimum growth rates (9.33% and 8.13% respectively) at 20°C.

Group II: Consisting of isolates VR-jpn, IN-jpn from Japan and FO-ind and CO-ind from India having their optimum growth rates (12.94%, 9.07%, 10.62% and 13.36% respectively) at 25°C.

Group III: Consisting of isolates ED-ind from India and LI-mal from Malaysia having their optimum growth rates (10.62% and 12.26% respectively) at 30°C.

The group I species are temperate and can grow to their maximum potential only in the temperate zone. The group II species suggests that they are subtropical and might grow in the temperate and the tropical waters. Group III species suggest that they are purely tropical species and would not be able to tolerate temperatures above 32.5°C and below 20°C and could be grown to their maximum potential only at 30°C. Thus the possibility of temperate species growing in tropical waters cannot be neglected but the vice-versa may not be possible.

Based on the results of the experiments on temperature, salinity and light intensity we could conclude that the Gracilaria species under investigations can be separated into 3 groups. The group II species, isolate VR-jpn, (Table 3) which has shown consistent growth rates even in wide temperature, salinity and light intensity tolerances seems to be a potential cultivar for the temperate as well as the tropical regions. Cultivation of this isolate seems to be possible at sites like river mouths where considerable salinity fluctuations take place and also in the open sea since it has a wide salinity tolerance range.

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References


22. Toh H S, Physico-chemical and biological factors that affect the level of pollution in Penang Island, Master thesis, School of Biological Sciences, Universiti Sains Malaysia, 1996.


