Life Histories of Species of *Chondrus* in Unialgal Culture

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Abstract

The life histories of *Chondrus pinnulatus* f. *armatus* (Harvey) Yamada et Mikami, *C. yendoi* Yamada et Mikami, *C. giganteus* Yendo and *C. ocellatus* Holmes from the coastal waters of Japan were completed in unialgal culture. All exhibited a Polysiphonia-type life history, except for a single instance of apparent apomixis in *C. pinnulatus* f. *armatus*. Each species clearly showed gametophytic and tetrasporophytic phases to be distinguishable by the resorcinol reagent as previously reported with *C. crispus* Stackhouse.

Introduction

Along the Atlantic coast of North America, *Chondrus crispus* is being harvested commercially. This plant is an important source of carrageenan colloids which are widely used in dairy products, pharmaceuticals and cosmetics (Witt 1985). In the northern Pacific Ocean, in the coastal waters of Asia, there are six species of *Chondrus* in addition to *C. crispus* (Mikami 1965) and all have been used as foodstuffs and as stucco-paste in wall plaster for decades in Japan (Okazaki 1971). Little is known regarding the life history of these species and it would be valuable to learn the developmental sequence, since all the plants are commercially
important. Chondrus crispus has been shown in culture (Chen and McLachlan 1972) to have a Polysiphonia-type life history and this has confirmed the life history inferred from plants in nature (cf. Taylor and Chen 1973). In addition, C. crispus has been shown to produce different types of carrageenan in different reproductive phases (Chen et al. 1973; McCandless et al. 1973). There have been no reports on the type of carrageenan in the Asian Chondrus plants except for C. ocellatus from China (Shi et al. 1986).

Peristenko (1980) did not accept Mikami's inclusion of C. armatus as a form of C. pinnulatus on the basis of more or less unilateral proliferaton of branchlets and the appearance of C. pinnulatus in waters at higher levels in the intertidal zone.

The present communication describes the isolation of C. pinnulatus f. armatus, C. yendoi, C. giganteus, and C. ocellatus from the coastal waters of Japan and reports their developmental sequence in culture and type of carrageenan in different reproductive stages.

**Materials and Methods**

Specimens of healthy fronds with carposporangial sori or tetrasporangial sori from C. pinnulatus f. armatus and C. yendoi were collected from the Oshoro Bay, Japan and brought to Halifax, Nova Scotia by Dr. J. Kaneko in September 1978. Chondrus giganteus was collected by the author in the intertidal zone in Hakodate, Hokkaido, in October 1980, while specimens of C. pinnulatus f. armatus and C. ocellatus were collected at Shimofuro, Amori Prefecture, Japan and sent to Halifax by air express from Hakodate by Dr. H. Yabu in August 1982. All the specimens were fertile, with mature, healthy tetrasporangial and carposporangial sori. Unialgal cultures of the four species of Chondrus were established either from carpospores or tetraspores (Chen and McLachlan 1972).

Three different constant temperatures (10, 15 and 20°C) with 8:16, 12:12 and 16:8 photoperiods and various light intensities were selected.
approximately corresponding to conditions in the locations from which the four species were collected.

Culture medium D-11 (Table 1) was used for developmental studies of *Chondrus* spp. The medium was changed weekly in the early developing stages and then biweekly after the first dichotomous branch was found.

A simple test for the presence of 3,6-anhydrogalactose as an indicator of k-carrageenan using a resorcinol reagent in *C. crispus* (Craige and Leigh 1978, Chen et al. 1982) was applied to all species cultured. In this test, the production of a dark red colour indicates that k-carrageenan is present and that the plant is a gametophyte. On the other hand, if no red colour is produced, k-carrageenan is absent and the plant presumed to be a sporophyte.

**Results**

Released carpospores from all species currently isolated in unialgal culture gave rise to discoid holdfasts from which upright fronds developed. After a few (2-4) dichotomous branches formed tetrasporangial sori developed. The discharged tetraspores germinated to form male and female gametophytic plants, similar in appearance to their tetrasporic plants. Each species retained its distinguishing morphological characteristics in culture. Released tetraspores from *C. giganteus* and *C. pinnulatus f. armatus* gave rise to male and female gametophytes.

In *C. pinnulatus f. armatus* which has a thick, cylindrical, linear frond (Fig. 1), tetraspores germinated to form discoid holdfasts. The first upright frond developed rapidly (approx. 4-5 wks) from the discoid holdfast (Fig. 2) and the initiation of first dichotomous branches (Fig. 3) occurred sooner (9 wks) than in the other species. The completion of its life history in culture, from tetraspore back to tetraspore again, normally required 12 months at 20°C, 50 μE.m⁻².s⁻¹ (Table 2) (approximately 16 months at 15°C), (20 months at 10°C). *Chondrus pinnulatus f. armatus* retained its characteristic morphology throughout growth and
reproductive maturity in culture (Fig. 4). However, in one case it was observed that the carpospore germinated to give rise to form a tiny (\(< 3 \text{ cm}\)) detached erect frond from which a tetrasporangial sorus developed within 13 weeks in culture. The released tetraspores developed the morphological characteristics of gametophytic plants.

A single plant derived from a tetraspore of *C. pinnulatus* f. *armatus* apparently formed carposporangial sori without the presence of male gametophytes. The released "carpospores" developed into a morphologically similar plant to those produced from normal carpospores. However, the plants derived from abnormal "carpospores" did not form sporangial sori.

The early development of sporocarps of *C. yenoid* (Fig. 5) is typically characteristic in that the germinated spore formed a discoid holdfast which was covered with numerous colourless hairs (Figs 6, 7). These hairs also formed along the surface of primary upright fronds and disappeared only after the subsequent erect frond developed from the holdfast.

The development of the first upright frond from both tetraspores and carpospores was slower than in *C. pinnulatus* f. *armatus* and the first dichotomous branch did not form until several subsequent upright fronds developed (Fig. 8). However, the discoid holdfast became more firmly attached than in *C. pinnulatus* f. *armatus*. The morphology of *C. yenoid* (Figs 9, 10) during the developmental sequence in culture was identical to that of the plants in nature (Fig. 5).

*Chondrus giganteus* (Fig. 11) has fronds somewhat flattened as in *C. yenoid*, but much longer and a dark greyish brown colour. In culture, these fronds reached a length of 20 cm (Fig. 12). The developmental pattern resembled that of *C. yenoid* and *C. pinnulatus* f. *armatus*, although the time required to complete the life history in culture under the same conditions (20°C) was longer than with the other species (Table 2). Perhaps more time is needed to develop the larger fronds. This plant was subsequently cultured and required only 22 months to complete its life history. The cultured plants
Life Histories of Species of *Chondrus* in Basic Culture.

resembled the plant from nature that provided the spores, although some irregularities did occur in the development of released carpospores: A detached ball-like mass of cells (Fig. 13) formed in some cases instead of normal discoid sporelings. This variation may parallel the previous report on *C. crispus* in which the germinating spores did not form a blanket-like sheath at early ontogeny (Chen and Taylor 1976) in culture, and thus failed to form normal discoid holdfasts. After 10 months in culture *C. giganteus* tetraspores germinated into detached irregular sporelings from which upright fronds developed. These fronds were irregularly shaped and did not fruit even after two years in culture.

*Chondrus ocellatus* (Fig. 14) was found to have a developmental sequence like the other species. It grew well in culture and developed normally in the temperature range 5-20°C to resemble wild plants. The time required to complete its life history in culture (Table 2), was shorter than for *C. giganteus* but longer than for either *C. pinnulatus* or *C. yendoi*.

When the resorcinol test for 3,6-anhydrogalactose was applied to *C. pinnulatus* f. armatus, *C. yendoi*, *C. ocellatus*, and *C. giganteus*, the gametophytic and tetraspophytic phases were clearly distinguishable just as reported in *C. crispus* (Chen et al. 1973, 1978; McCandless et al. 1973).

**Discussion**

The present culture investigation of four species of *Chondrus* showed that their complete life histories are similar to that of *C. crispus* (Chen and McLachlan 1972; Taylor and Chen 1973). Each of the species retained and exhibited its morphologically distinguishing characteristics in culture. It appears that the time required (Table 2) to complete the life histories was dependent on culture conditions rather than the genetic make-up of the particular species. This has been noted with *C. giganteus* when in repeated culture studies the time to complete its life history was shortened.

The single case of an
Figure Legends

Figs 1-4 *Chondrus pinnulatus* f. *armatus*.

Figure 1 *Chondrus pinnulatus* f. *armatus*, a natural field collected plant with tetrasporangial sori.

Figure 2 Four-week-old sporlings in culture.

Figure 3 A group of young sporlings in culture showing the first dichotomous branch of the upright frond (9 wks).

Figure 4 Mature tetrasporophyte with mature tetrasporangial sori from culture.

Figs. 5-10 *Chondrus yendoi*.

Figure 5 Field collected *C. yendoi* with numerous tetrasporangial sori.

Figure 6 A group of 4-week-old sporlings showing colourless hairs (dark field).

Figure 7 A sporling showing hairs developing on the surface of the upright frond (dark field).

Figure 8 Four-month-old plant with many upright unbranched fronds.

Figure 9 Mature cultured tetrasporophyte with tetrasporangial sori (arrow) in culture.

Figure 10 A cultured gametophyte with mature carpogonial (arrow)sori developing on fronds.

Figs. 11-13 *Chondrus giganteus*.

Figure 11 A five-month-old plant of *C. giganteus*.

Figure 12 A cultured plant with tetrasporangial sori showing the size of the plant attained in culture.

Figure 13 A ball-like tetrasporophyte from culture, with germinated tetrasporlings on the surface.

Figure 14 A cultured plant of *C. ocellatus* with mature carpogonial sori.
unusual developmental pattern observed in *C. pinnulatus* f. *armatus* has its parallel in the broad-frond form of *C. crispus* in culture (Chen 1977), where it was suggested that, in fact, two tetraspores probably germinated together at a very early stage without this having been detected.

A single apical portion of a *C. pinnulatus* f. *armatus* frond, which had been forming carposporangial sori, was cut away well beyond the region of any soral development and cultured in complete isolation. This was repeated using both field-collected and cultured specimens. After four and six months, respectively, in isolated culture at a 20°C, 60 µE.m⁻².s⁻¹ and 16:8 photoperiod, carposporangial sori developed again. One must speculate that either the carposporangial sori formed on gonimoblast filaments that already penetrated into the segment before it was excised and these later produced carposporangial sori, or it is possible that they were formed apomictically from the female gametophytic tissue. The apomictic pattern of development has been reported as not uncommon in the Gigartinales and there is a number of reports of cases of apparently apomictic life histories. These include: *Gigartina stellata* (Chen et al. 1974; Rueness 1978; Dion and Delépine 1979), and *Gigartina cordata* var. *splendens*, *G. cornucopiae*, *C. rosea* (Kim 1976). Thus, *C. pinnulatus* f. *armatus* may also exemplify this phenomenon.

Of the species reported by Mikami (1965) in his systematic study of *chondrus* in Japan, those studied in culture all appear to follow the same patterns in life history and carrageenan as shown in *C. crispus*. Van der Meer et al. (1983) reported that several male plants from widely separated geographic locations produced carposporangial sori-like structures whose origin does not appear to be sexual. The released carspore-like cells germinated to give rise to plants closely resembling normal tetrasporic plants which released no spores. I have not observed examples resembling the case reported, however, I have observed apomictically developed *C. crispus* which in appearance closely resembled tetraspores.
Table 1. Composition of D-11 culture medium.

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>NaNO₃</td>
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</tr>
<tr>
<td>NH₄NO₃</td>
<td>0.25 mM</td>
</tr>
<tr>
<td>Na₂SiO₃·9H₂O</td>
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<tr>
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</tr>
<tr>
<td>FeEDTA</td>
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<tr>
<td>FeCl₃·6H₂O</td>
<td>1 µM</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
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</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>5 µM</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>5 µM</td>
</tr>
<tr>
<td>NaH₂PO₄·H₂O</td>
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<tr>
<td>CaCl₂·2H₂O</td>
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</tr>
<tr>
<td>KCl</td>
<td>2 µM</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>0.1 µM</td>
</tr>
<tr>
<td>Pyridoxine-HCl</td>
<td>0.1 µM</td>
</tr>
<tr>
<td>p-Hydroxyphenylacetic acid</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>V-3a</td>
<td>2 mL</td>
</tr>
<tr>
<td>PI-5Xb</td>
<td>2 mL</td>
</tr>
<tr>
<td>Seawater b</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

₁The medium was refer to D-5 medium (Chen 1982).

₂The pH was adjusted to 7.5 and the medium was sterilized by filtration (0.2-µm pore size).
Table II. The time required to complete the life history of four species of *Chondrus* cultured at 20°C with 50 μE·m⁻²·s⁻¹, 12:12 in D-11 medium.

<table>
<thead>
<tr>
<th>Months</th>
<th>From tetraspore to gametophyte</th>
<th>From carpospore to tetrasporophyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. pinnulatus f. armatus</em></td>
<td>6.5</td>
<td>5.5</td>
</tr>
<tr>
<td><em>C. yendoi</em></td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td><em>C. giganteus</em></td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><em>C. ocellatus</em></td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

Further cytological study is needed to clarify their true nature.

Acknowledgements

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References


