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The life cycle of *Tinocladia crassa* (SURINGAR) KYLIN (Phaeophyta, Chordariales) without a haploid gametophyte from Kuchinotsu, Kyushu, Japan

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The life cycle and cytology of a strain of *Tinocladia crassa* growing at Kuchinotsu, Nagasaki Prefecture, Japan, was investigated in culture. Biflagellate zoospores liberated from unilocular sporangia of macroscopic plants from the natural habitat developed directly, without fusion, into sporophytic plants identical to the mother plants. The chromosome number of both macroscopic plants in nature and cultured germlings of zoospores from unilocular sporangia was found to be about 2n=24. It is thought that meiosis failed to occur in the unilocular sporangia and that the strain of *T. crassa* at Kuchinotsu has no haploid phase in its life cycle.

Key Index Words: *Chordariales*; *development*; *life cycle*; *cytology*; *Phaeophyta*; Tinocladia crassa.

Tinocladia crassa (SURINGAR) KYLIN is one of the edible brown algae of Japan. It is distributed widely in the warm temperature regions. ARASAKI (1941) investigated the life cycle of this alga using material collected at Ise Bay, Mie Prefecture and Mikawa Bay, Aichi Prefecture. According to him, *T. crassa* has an alternation of heteromorphic generations, with a microscopic gametophyte and a macroscopic sporophyte. YOTSUI(1978) confirmed the same life cycle using *T. crassa* collected at Nomozaki, Nagasaki Prefecture. However, a similar culture study using material growing at Kuchinotsu and some other localities of the north western coast of Kyushu showed another type of life cycle without a haploid gametophyte. In the present paper, this type of life cycle in *T. crassa* is reported.

Materials and Methods

Macroscopic *Tinocladia* plants were collected in May 1979, at Kuchinotsu on the southern end of the Shimabara Peninsula where the plants grew on stones from 0 to 1 meter below the low tide mark. Material was also collected at Nomozaki and Sasebo, Nagasaki Prefecture, and Nishinoura, Fukuoka Prefecture (Fig. 1), for comparison of the life cycle. In the laboratory about ten mature plants were selected and each was put into a small glass vessel containing sterilized sea water and placed in the light. Within a few hours, zoospores were liberated from unilocular sporangia and swam to the darker side of the vessel. The liberated zoospores were rinsed several times with sterilized sea water and allowed to settle on a small glass plate. The zoospores liberated from one individual were cultured in a vessel.

Culture were carried out under the following two conditions; a) in a culture cabinet

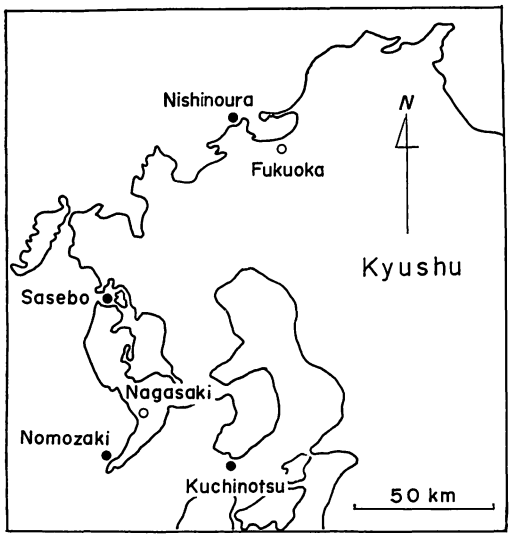

Fig. 1. A map of north-west Kyushu showing the collection sites (solid circle) of *Tinocladia crassa*.

kept at 12-14°C and illuminated with fluorescent lamps of 4000 lux light intensity for 10 hours a day; b) in a room of the laboratory exposed to ambient temperature ranging from 22°C to 30°C, and the illumination kept at 3000 lux in early summer and at 500 lux in mid summer. The culture medium used was Schreiber's solution enriched with modified Pl solution (FUJITA 1965).

For chromosome counts, the aceto-iron-haematoxylin-chloral hydrate method (WITTMAN 1965) was employed.

Results

Mature *Tinocladia* plants in nature bore only unilocular sporangia on the basal cells of assimilating filaments from mid April (Fig. 3A). Cultures of zoospores from unilocular sporangia were started early in May, 1979. The development of the zoospores from Kuchinotsu plants was as follows. The unilocular sporangia liberated biflagellate zoospores (6.5~8.0×3.0~4.0 μm in size) having a single chloroplast and an eyespot (Figs. 2A, 3B). They settled down on the substratum in a few hours and became spher-

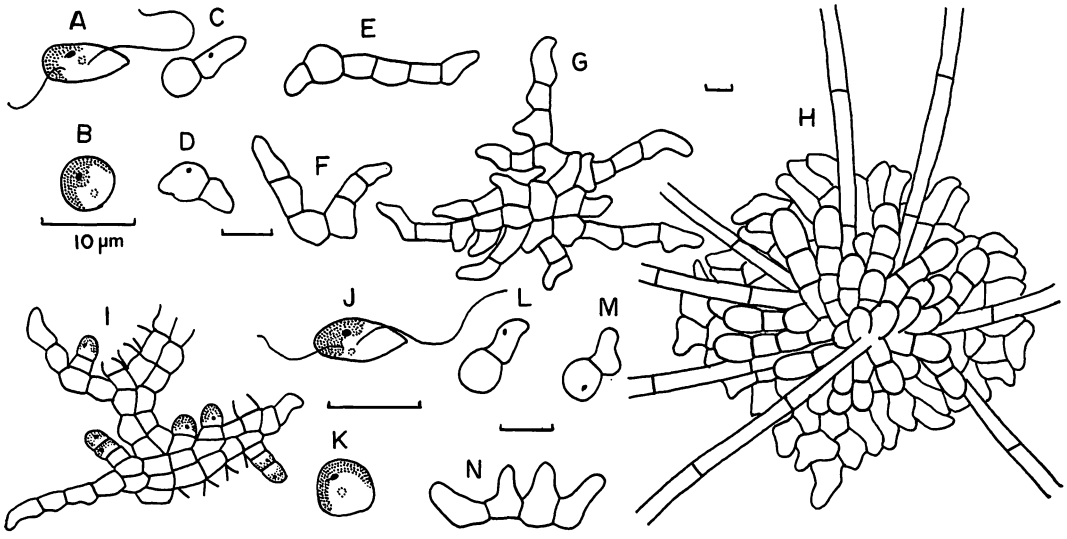

Fig. 2. Development of zoospores from *Tinocladia crassa* collected at Kuchinotsu. A-H, culture at low temperature; I-M, culture at high temperature. A. Zoospore from unilocular sporangia; B. Settled zoospore; C-G. Germination and early developmental stage of the zoospore; H. Formation of hairs and erect assimilators; I. Formation of plurilocular sporangia on the sporeling at high temperature; J. Zoospore from plurilocular sporangia; K. Settled zoospore; L-N. Early developmental stage of zoospore from plurilocular sporangia.

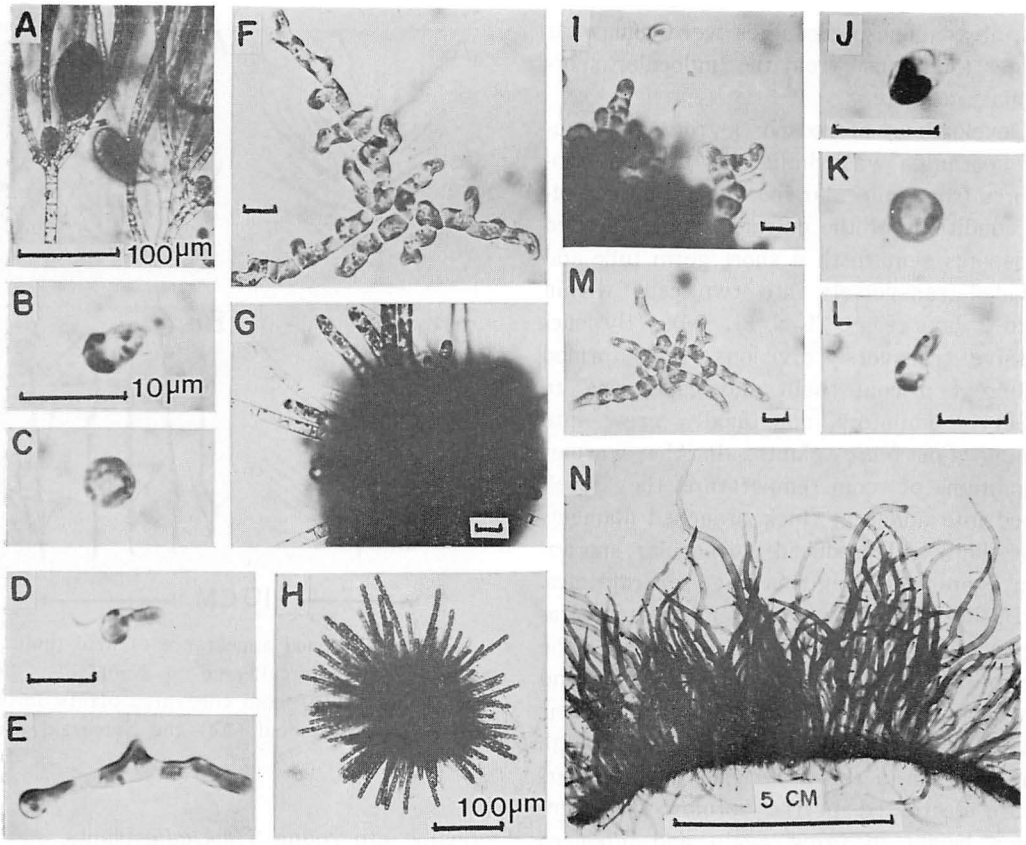

Fig. 3. Development of zoospores from *Tinocladia crassa* collected at Kuchinotsu. A-H, Culture at low temperature; I-M, Culture at high temperature; N. Cultivation in the sea. A. unilocular sporangia on the macroscopic thalli in nature; B. Zoospore from unilocular sporangia; C. Settled zoospore; D-F. Germination and early developmental stage of the zoospore; G.H. Formation of erect assimilators; I. Formation of plurilocular sporangia on the sporeling of zoospore at high temperature; J. Zoospore from plurilocular sporangia; K. Settled zoospore; L.M. Germination and early developmental stage of the zoospore; N. Macroscopic *Tinocladia* thalli growing on synthetic string.

ical (Figs. 2B, 3C). At this stage they measured 4.0 to 6.0 μm (average 4.9 μm) in diameter. In the cooler conditions of the culture cabinet, they sent forth a short germ tube and divided transversely into two cells within 1 or 2 days (Figs. 2C, D, 3D). By successive transverse cell divisions, the germlings continued to grow in length and branched thickly in the horizontal direction and formed colorless hairs (Figs. 2E-G, 3E, F). In 2 or 3 weeks, the germlings became creeping discs which formed a series of erect assimilators in the central areas and within 2 months they developed into young *Tinocladia* plants reaching up to 2 mm in height (Figs. 2H, 3G, H). Development of the zoospores liberated from other material examined showed the same results.

On the other hand, in the high room temperatures, the germlings of zoospores did not form erect assimilators and remained as adherent thick branched filamentous thalli. These creeping filamentous thalli frequently bore plurilocular sporangia (Figs. 2I, 3I). The plurilocular sporangia liberated biflagellate zoospores having a single chloroplast and an eyespot (Figs. 2J, 3J). They settled down in a few hours, with no sign of fusion, and at this stage they measured 4.5 to 6.5 μm (average 5.5 μm) in diameter (Figs.

2K, 3K). These zoospores were somewhat larger than those from the unilocular sporangia.

Development of zoospores from plurilocular sporangia was similar to that of zoospores from unilocular sporangia. In the cooler conditions of the culture cabinet, settled zoospores sent forth a short germ tube and divided transversely into two cells within 1 to 2 days (Figs. 2L-N, 3L, M). By successive transverse divisions, they formed adherent discoid thalli and gave rise to erect assimilators and finally grew into young *Tinocladia* plants. In the warmer conditions of room temperature, they developed into adherent thick branched filamentous thalli and produced plurilocular sporangia again, repeating this stage several times.

During mid summer, under high temperatures of above 28°C in the laboratory, the germlings of zoospores from unilocular and plurilocular sporangia ceased to grow. Their cells became spherical and passed through the summer in this state. As room temperature decreased in the autumn, the germlings began to grow again and produced plurilocular sporangia and released zoospores which grew directly into young *Tinocladia* plants. After removal of the germlings to the sea on a synthetic string, they grew to adult macroscopic thalli (Fig. 3N).

The same mode of development of zoospores from unilocular sporangia was observed in culture experiments using material collected at Sasebo and Nishinoura.

On the other hand, as previously reported (YOTSUI 1978), regarding the material collected at Nomozaki, zoospores from unilocular sporangia developed into adherent discoid thalli in the early stages in a similar manner to those of the Kuchinotsu plants. Later, the germlings did not produce erect assimilators, but continued to grow as adherent branched thalli and formed plurilocular gametangia. The plurilocular gametangia released gametes and they fused to form zygotes. The zygotes germinated into adherent discoid thalli and produced erect assimilators in the early stage, developing finally into young *Tinocladia* plants.

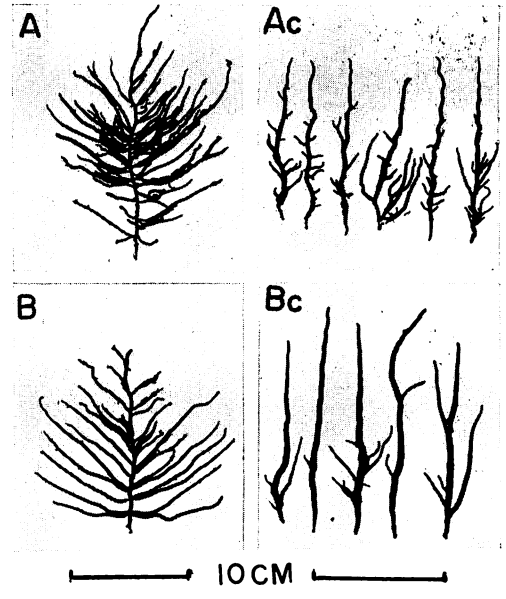

Fig. 4. External appearance of field thalli of *Tinocladia crassa* collected at Kuchinotsu (A) and Nomozaki (B), and cultivated plants derived from the Kuchinotsu (Ac) and Nomozaki (Bc) field thalli.

The external appearence of field material from Kuchinotsu seemed more slender and thickly branched than the Nomozaki material, and the same difference in the morphology also appeared in cultured plants derived from Kuchinotsu and Nomozaki field thalli (Fig. 4).

Cytological observations on the chromosome number were carried out on the cells of macroscopic thalli from nature and on those of the cultured germlings of zoospores from unilocular sporangia. As far as could be ascertained, the chromosome number of macroscopic thalli in nature was about 24 (2n) in the plants from Kuchinotsu, Nomozaki (Fig. 5A, C) and the other two localities. In the germlings of zoospores from unilocular sporangia, the chromosome number was about 24 (2n) in Kuchinotsu (Fig. 5B), Sasebo and Nishinoura plants, but it was about 12 (n) in Nomozaki plants (Fig. 5D). The chromosome number of the germlings of zoospores from unilocular sporangia of No-

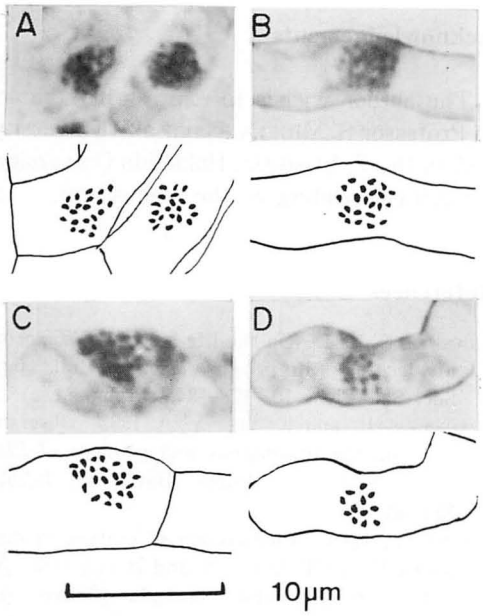

Fig. 5. Chromosomes of *Tinocladia crassa*. A. Macroscopic field thalli from Kuchinotsu (a chromosome number 20 or 21 was counted); B. Germling of zoospore from unilocular sporangia of Kuchinotsu (22); C. Macroscopic field thalli from Nomozaki (24); D. Germling of zoospore from unilocular sporangia of Nomozaki (12).

mozaki plants was reduced to about half of that of macroscopic thalli in nature. On the other hand, in the plants from Kuchinotsu and the other two localities the chromosome number was the same for both macroscopic thalli in nature and the cultured germlings of zoospores from unilocular sporangia.

Discussion

ARASAKI (1941) studied the life cycle of *Tinocladia crassa* collected at Ise Bay, Mie Prefecture, and Mikawa Bay, Aichi Prefecture, and found that zoospores from unilocular sporangia germinated into adherent, thickly branched gametophytes. These gametophytes produced plurilocular gametangia which released gametes. The gametes conjugated isogamously to form zygotes and the zygotes gave rise to *Tinocladia* plants. YOTSUI (1978) observed the same developmental pattern using material collected at Nomozaki, Nagasaki Prefecture.

In the present study, *T. crassa* collected at Kuchinotsu and two other localities showed a different type of life cycle, in that zoospores from unilocular sporangia grew directly into sporophytic plants without passing through a gametophyte generation. In unfavorable warmer conditions, the thalli remained adherent branched filamentous forms producing plurilocular sporangia and giving rise to plethysmothalli which produced several successive generations. Similar development of sporophytes in unfavorable culture conditions has been reported for *Nemacystus decipiens* (MIGITA and YOTSUI 1972) and *Cladosiphon okamuranus* (SHINMURA 1977).

The development of zoospores from unilocular sporangia directly into sporophytic plants was also reported for a few related species. KORNMANN (1962) reported that zoospores from unilocular sporangia of *Chordaria flagelliformis* developed directly into *Chordaria* plants. However cytological evidence of such an alternation was not provided. BLACKLER and KATPITIA (1963) reported

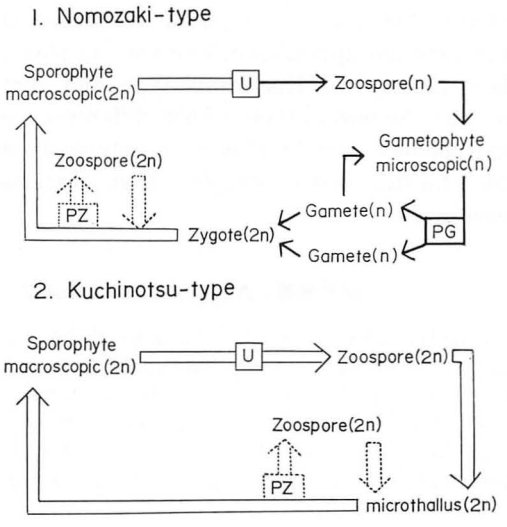

Fig. 6. Diagram showing the two types of life cycle of *Tinocladia crassa*.
U: unilocular sporangium, PG: plurilocular gametangium, PZ: plurilocular sporangium.

that zoospores from unilocular sporangia of *Elachista fucicola* developed into sporophytes and mentioned the probability of the plants being haploid.

In the case of *T. crassa* collected at Kuchinotsu and two other localities, both the germlings of the zoospores from unilocular sporangia and macroscopic plants from the field had the same number of chromosomes and are thought to be the diploid phase. Here the gametophyte generation is eliminated from the life cycle. On the other hand, in the case of *T. crassa* collected at Nomozaki, the germlings of the zoospores from unilocular sporangia became haploid gametophytes, and it is thought that the meiosis occurs in the unilocular sporangia on the macroscopic sporophytes.

From the present and the previous studies (YOTSUI 1978), it is clear that *T. crassa* has two types of life cycle; one is a heteromorphic alternation between microscopic gametophytes, and macroscopic sporophytes, as seen in the Nomozaki population (Nomozaki-type) and the other is a type of cycle with only a diploid sporophyte, as seen in the population from Kuchinotsu and two other localities (Kuchinotsu-type) (Fig. 6).

Comparison of specimens from the field shows that there is a slight difference in the external appearance between the plants belonging to the Kuchinotsu-type and that of the Nomozaki-type. This difference in morphology will be closely investigated in the future, using samples from various localities.

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四井敏雄：九州ロノ津産フトモズク（褐藻類ナガマツモ目）の配偶体のない生活環

長崎県ロノ津産フトモクズの生活環を室内培養によって調べた。天然の大型藻体は生殖器官として単子嚢を形成する。これから放出される遊走子は着生後直ちに発芽して仮盤状の発芽体となる。この発芽体は，低温下では直立同化糸を形成して直接フトモズク体に生育するが，高温下では仮盤状のまま生長して複子嚢を形成し，その遊走子は再び仮盤状の体になる発生を繰り返す。染色体数は，天然の大型藻体，単子嚢の遊走子発芽体とも 2n＝約24が数えられた。ロノ津産フトモズクは配偶体世代をもたず，複相の胞子体世代のみをもち，同様の生活環は長崎県佐世保湾，福岡県西ノ浦産のものでも認められた。 (851-05 長崎県西彼杵郡野母崎町　長崎県水産試験場増養殖研究所)