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Studies on the Life History of *Ulothrix* - III.*

On the Life History of *Ulothrix acrorhiza* KORNMANN

By
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The present series of this study has been made in order to define the life history of marine species of the genus *Ulothrix* in Japan. In regard to *Ulothrix acrorhiza* KORNMANN, KORNMANN (1964, 1965) had already reported the asexual reproduction, but the sexual reproduction has not been difined so far. The present culture study shows that *U. acrorhiza* has both the asexual and the sexual reproduction and then the life history of this species is almost clarified.

The present paper deals with some observations on the growth and formation of the reproductive cells and the structure of the frond of *U. acrorhiza* in incubators.

Materials and Methods

Ulothrix acrorhiza was collected at the mouth of the Isuzu River in Atsumi Gun (District), Aichi Prefecture, twice in January and March, 1975.

The materials collected for the culture study were rinsed to get rid of epiphytes with the culture medium. After rinsing they were cut and divided into 20 to 50 cell fragments. These fragments were rinsed with a capillary pipette several times and a few pieces of fragments were transferred to the culture vessels.

The culture medium used for this investigation was prepared by SWM-III (OGATA, 1970) without soil extract and liver extract and was sterilized by the autoclave (ca 120°C, 1kg/cm^2) for fifteen minutes. The medium in culture vessels was renewed every five days.

The culture was carried out in incubators regulating temperatures, luminous intensity (3500—4000 lux) and photoperiods. The incubators were kept under the following conditions : 10 hours' and 14 hours' light daily at 10°C ; 10 hours', 12 hours' and 14 hours' light daily at 15°C with white fluorescent lamps.

Most figures and plates of the present paper were based on living materials. The observation and measurement of swarmers were made on living materials as well as on materials fixed with formalin vapour for 10—20 seconds. The flagella of swarmers were stained with Gentian-violet.

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Observations

Habitat and Frond Structure : This species was found at the beginning of December and showed luxuriant growth during January and March, but it disappeared at the mouth of the Isuzu River in Aichi Prefecture in April. It was usually observed adhering to rocks and stakes in the intertidal zone. The frond is the filamentous thallus, one to three centimeters in length and is green in color. Each of vegetative cells makes a form of cylinder with 12—18 μ in diameter. Its length is from one-third to the same as the diameter. The chloroplast is an incomplete ring. One or rarely two pyrenoids are present in each cell (Fig. 1, a ; Plate I, A).

Formation and Germination of Zoospores : Each of cut frangments developed into filamentous thalli mainly in the following two ways: One was the way that the rhizoid expanded from the lower part of fragments and the upper cells of fragments divided repeatedly, and the other was the way that the cells of the cut fragments except the cells of both ends formed zoosporangia, from which zoospores were liberated and they settled at the substratum and grew. When ten to fifteen days had passed since culture started, green fronds which grew adhering to the substratum of culture vessels were visible to the naked eye. These filamentous thalli grew about one centimeter long and zoosporangia were formed in each cell (Plate I, B).

The formation of zoospores was observed under all of culture conditions. The zoosporangia were formed in most of the cells except in the part of basic cells. The zoospores formed in each zoosporangium was 4, 8 and 16 in number, among which zoosporangia with 8 zoospores dominated (Fig. 1, b). The liberation of zoospores was observed one to three hours after illumination. The zoospores were dischared from the zoosporangium as a mass enclosed in a hyaline sac through a pore formed on the side wall of the cell. A few seconds after liberation the zoospores acquired motility in the sac outside the mouth of the pore and then swam away. The zoospores were pear-shaped in general, although some of them were sphere-shaped. They had four flagella of equal length at the anterior end and one eye-spot, and possessed a basal chloroplast with a single pyrenoid (Fig. 1, c ; Plate I, D). As a result of measurement for one hundred zoospores immediately after liberation, the zoospores were 3.8—5.4 μ in diameter and 7.4—9.5 μ in whole length and the flagella were 10.0—13.5 μ long. The zoospores showed strong positive phototaxis, After swimming in the culture vessel from twenty minutes to one hour, they settled its anterior end on the substratum. The settled zoospores were 4.9—7.7 μ in diameter (Fig. 1, d ; Plate I, E). After settling, cell membrane was formed and immediately the settled zoospores began to germinate ; first they became long and narrow, and the rhizoid expanded from the lowest part of a cell (Fig. 1,e) and after two days it was divided above and below into two parts with septum (Fig. 1, f ; Plate I, F). At this time, there were observed some germlings in which an eye-spot remained. After three days, the number of cells was three to five (Fig. 1, g ; Plate I, G), and more seven to ten days passed, the frond became over one contimeter long and finally formed the reproductive cell again.

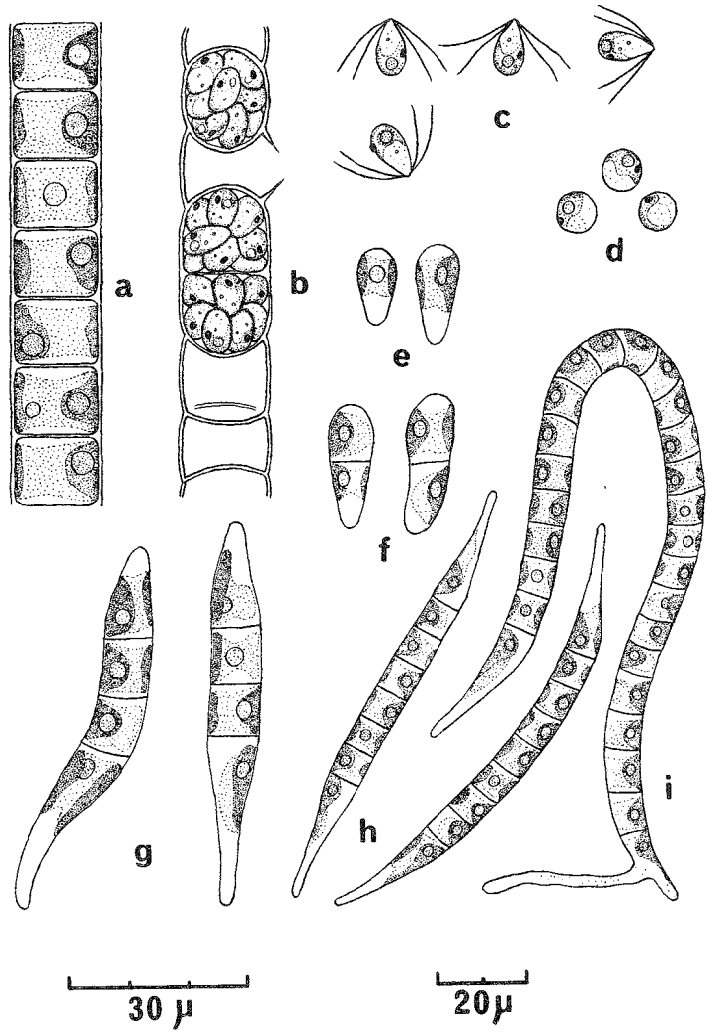

Fig. 1. Vegetative cells, zoosporangia, zoospores, and succesive stages in the germination of zoospores in *Ulothrix acrorhiza* KORNMANN. a : vegetative cells ; b : zoosporangia and empty cells ; c : zoospores ; d: settled zoospores ; e, f : germlings after 1 to 2 days cultivation ; g : after 3 days ; h : after 4 days ; i : after 5 to 6 days. (a-g : drawn to 30 µ scale ; h, i : drawn to 20 µ scale).

The first process for development of zoospores of this species showed the same tendency to other species of this genus. However, in case of gemlings with over five cells, the rhizoid expanded even from the uppermost cell (Fig. 1, g, h ; Plate II, G, H), an it touched the substratum, where it came to settle (Fig. 1, i).

Though some of zoospores were observed remaining in zoosporangia without liberation, they expanded a rhizoid in zoosporangia and showed the same development as liberated zoospores (Plate I, C).

Vegetative Reproduction : Sometimes there remained one or some vegetative cells in filamentous thalli which liberated zoospores and gametes. A few days later, a rhizoid expanded from the lower part of one cell or from the lowest cell of some in a row (Plate II, A, B). After the division was repeated in the upper cells, they grew into new filamentous thalli.

When some cells of the filamentous thallus perished out by some injuries, the cell just above the injured cell outgrew the rhizoid, which settled at the substratum, and the remaining upper part grew into the new filamentous thallus, too.

Gametes and their Conjugation : Gametes were formed in the same filamentous thallus as zoospores were. Formation of gametes was observed under the condition that photoperiod was more than 12 hours a day. Reproductive cells were almost all zoosporangia in the incubator kept at 10°C when photoperiod was 10 hours a day, but when it was 14 hours a day, gametangia were formed in 30—80 percents of all reproductive organs. The same result was obtained in the incubator kept at 15°C ; when photoperiod was 10 hours a day, reproductive cells which were formed were almost all zoosporangia, when it was 12 hours, gametes were formed in 5—10 percents of all reproductive organs, and when it was 14 hours, gametangia were formed in 50—80 percents of all.

Each gametangium, which as formed in almost all vegetative cells except basic cells of filamentous thalli, contained 16 (in rare cases), 32 or 62 gametes in number (Fig. 2, a ; Plate II, C). Like zoospores, liberation of gametes was observed for one to three hours after illumination (Plate II, D). The gametes were elongated pear-shaped and had two flagella of equal length at the anterior end, one eye-spot and a single chloroplast (Fig. 2, b ; Plate II, E). The gametes showed stronger positive phototaxis than zoospores. The gametes were 2.2—3.3 μ in diameter, the whole length was 4.3—6.5 μ and the flagella were 10.2—12.8 μ long. Conjugation may occur between a pair of the gametes of the same size or of different filamentous thalli (Fig. 2, c; Plate II, F). The gametes generally conjugated laterally, but some of them anteriorly.

Zygotes and their Development : After sexual fusion, the zygotes swam actively for a while and gradually became less active. They finally settled down on the substratum and lost flagella. They became spherical, and the cell membrane was formed. The settled zygotes were 4.9—6.4 μ in diameter (Fig. 2, d). They had two eyespots obviously, and immediately began to germinate.

The culture of zygotes was performed under two conditions : 10 hours' and 14 hours' light daily at 15°C. In the case of the former condition, after a few days, almost all zygotes continued to increase in size without cell devision retaining spheres but few zygotes formed

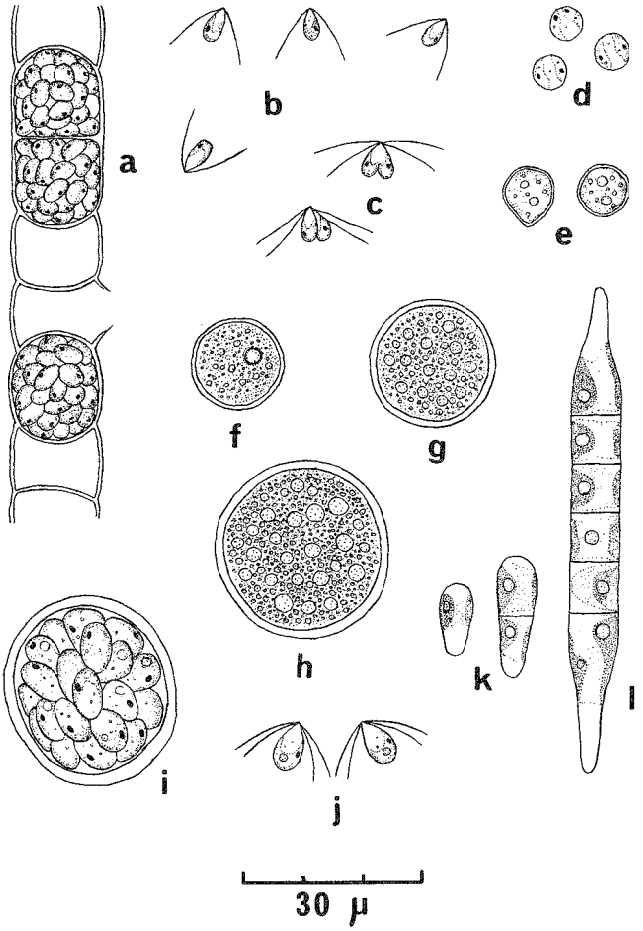

Fig. 2. Gametangia, gametes, conjugation of gametes, and successive stages in the development of zygotes in *Ulothrix acrorhiza* KORNMANN. a : gametangia ; b : gametes ; c : conjugation of gametes ; d : settled zygotes ; e : germination of zygotes, after 6 days cultivation ; f : after one month ; g : after 2 months ; h : after 3—4 months ; i : fertile cyst, showing formation of swarmers, after 4 months ; j : swarmers, liberated from cyst ; k,l :germination of swarmers.

germination tube. At this time, two eye-spots remained in many zygotes. After about one week, zygotes developed into 8—10 μ in diameter (Fig. 2,e) and many of them were spherical and some were pear-shaped or elliptical, and they had two pyernoids and in rare cases three. About one month later, zygotes grew about 15μ in diameter, and the cell wall got thick and the content became dark green (Fig. 2, f). After two months, they grew 20—25μ in diameter (Fig. 2, g ; Plate II, G). In three to four months, they developed into thick-walled cysts, measuring 25—30 μ diameter (Fig. 2, h). These cysts became yellowish-green and swarmers were formed (Fig. 2, i ; Plate II, H). The swarmers were 16 (in rare cases) or 32 in number. A pore was opened in a part of the cyst and swarmers were liberated one by one from there. On the other hand, under the latter condition, zygotes also showed the same germination-type. However, it took more than eight months to form swarmers, and some of then perished out.

The swarmers liberated from a cyst were somewhat smaller than the zoospores formed in filamentous thalli. Except for these size differences they had nearly same character ; they had quadriflagella at the anterior end, one eye-spot and a single chloroplast with one pyrenoid (Fig. 2, j). Swarmers liberated from a cyst showed positive phototaxis and settled at the substratum after swimming in the culture vessel for a while. On settling, they began to germinate and grew into filamentous thalli (Fig. 2, k, l).

Parthenogenesis : The gametes of both sexes, even if they missed an opportunity in finding a partner for sexual conjugation, developed parthenogenetically. Gametes swam in the culture vessel for one to three hours and settled at the substratum. The settled gametes were 3.6—4.3 μ in diameter. After settling, the cell membrane was formed and gametes immediately began to germinate. Showing the same germination-type as zygotes, they developed into cysts. The content of cysts had division and the swarmers with quadriflagella were formed after three to four months under the condition of 10 hours' light daily at 15°C. The characters of the swarmers were almost the same as those of zoospores formed in filamentous thalli. But some cysts bleached without forming swarmers. The liberated swarmers settled at the substratum and grew into filamentous thalli.

The culture of the swarmers liberated from one cyst was carried out in the incubator kept for 14 hours' light daily at 15°C. After two weeks to one month the swarmers developed into filamentous thalli, where formation of gametes was observed. Among these gametes, conjugation phenomenon has not so far observed.

Discussion

The life history of *Ulothrix acrorhiza* is summarized as shown in Fig. 3 based on this culture study. In this species, non-alternation of generation was reported by KORNMANN (1964). According to him, the filamentous thallus of this species produces only quadriflagellate zoospores which develop directly into filamentous thalli. However, when filamentous thalli were cultured under the long day condition, the formation of gametes was carried out and their conjugation could be observed. It is confirmed that in the present case, sexual reproduction is dioecious and isogamous. Zygotes developed into cysts and quadriflagellate swarmers were formed in the cysts. This shows clearly that dimorphic generations are found in this

species. Accordingly, the life hitory of this species is the same as those of *U. zonata* (DODEL, 1876), *U. rorida* (LIND, 1932), *U. subflaccida* (KORNMANN, 1964) and *U. implexa* (OHGAI *et al.*, 1975 ;OHGAI and FUJIYAMA, 1976). It is regarded that an alternation of generations is made among the filamentous thallus-plant producing quadriflagellate zoospores and biflagellate gametes and the cyst-plant producing quadriflagellate swarmers. It is thought that the one cycle in life of this species is about 12 months under the natural condition. On the other hand, it is 5—6 months under the culture condition. This result shows that the period of the one cycle of this species does not always require a fixed time and that it is generally influenced by various environmental factors.

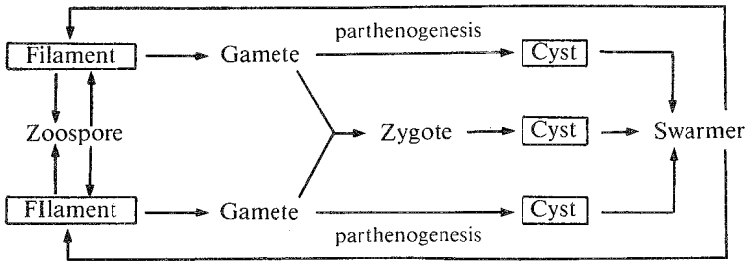

Fig. 3. Diagram of life history of *Ulothrix acrorhiza* KORNMANN.

As KORNMANN (1964) pointed out, in the process that zoospores of this species developed into filamentous thalli, the rhizoid is formed in the upper part of the cell after a germling had several cells. It is maintained first that this was caused under the artifitical condition. However, as compared with the other species of *Ulothrix* under the same condition, this phenomenon was observed only in this species. Therefore, this may be one of the characters of this species.

Gametes which have not the opportunity for conjugation show quite the same germination as that of zygotes by parthenogenesis. Such type of parthenogenesis of gametes was already reported in the papers on *Monostroma angicava* (TATEWAKI, 1969), *Monostroma grevillei* (KORNMANN, 1962), *Urospora mirabilis* (NAGATA, 1971), *Ulothrix* sp. (KORNMANN, 1963), *Ulothrix implexa* (OHGAI and FUJIYAMA,1976) and so on. Consequently, the parthenogenesis of gametes is a part of the life history of this species. Swarmers are formed in cysts, into which gametes develop because of the parthenogenesis, likewise in cysts, into which zygote developed and then into filamentous thalli. In the filamentous thalli are formed zoospores and gametes, and in this case it is doubtful whether the gametes are the same sex with above-mentioned gametes or not. The previous reports showed that gametes formed those of the same sex again in *Urospora mirabilis* (NAGATA, 1971), *Monostroma grevillei*(KORNMANN, 1962) and so on and such tendency was shown in this culture experiment. Further investigation will be required on this problem.

Summary

Ulothrix acrorhiza has a dimorphic alternation of generations ; one generation is the filamentous thallus which produces zoospores and gametes, and the other is the unicellular cyst producing swarmers. The zoospores formed in filamentous thalli are observed under both of short and long day conditions. The zoospores are pear-shaped, and have four flagella, one pyrenoid and one eye-spot. The settled zoospores immediatly germinate and develop into filamentous thalli. After 7—10 days culture, they form reproductive cells again. The gametes are formed mainly under the long day condition. The gametes are elongated pear-shaped, and have two flagella and one eye-spot. The conjugation of gametes takes place isogamously between gametes from the different filamentous thallus. After 3—4 months culture, zygotes develop into cysts, which form quadriflagellate swarmers. The characters of these swarmers are almost the same as those of zoospores formed in filamentous thalli. These swarmers develop into filamentous thalli. The gametes of both sexes develop parthenogenetically into cysts as zygotes did. These cysts form quadriflagellate swarmers, which grow into filamentous thalli.

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PLATE

PLATE I

Plate I. *Ulothrix acrorhiza* KORNMANN, zoospores and their development.

A. Filamentous thallus, ×480.
B. Zoosporangia formation of a filamentous thallus, ×480.
C. Germination of zoospores remaining in zoosporangia, ×480.
D. Liberated zoospores stained by Gentian-violet, ×660.
E. Settled zoospores, ×500.
F. Germination zoospores after 2 days old, ×500.
G. 3—4 days germling, ×500.
H. 5 days old, ×100.

PLATE I

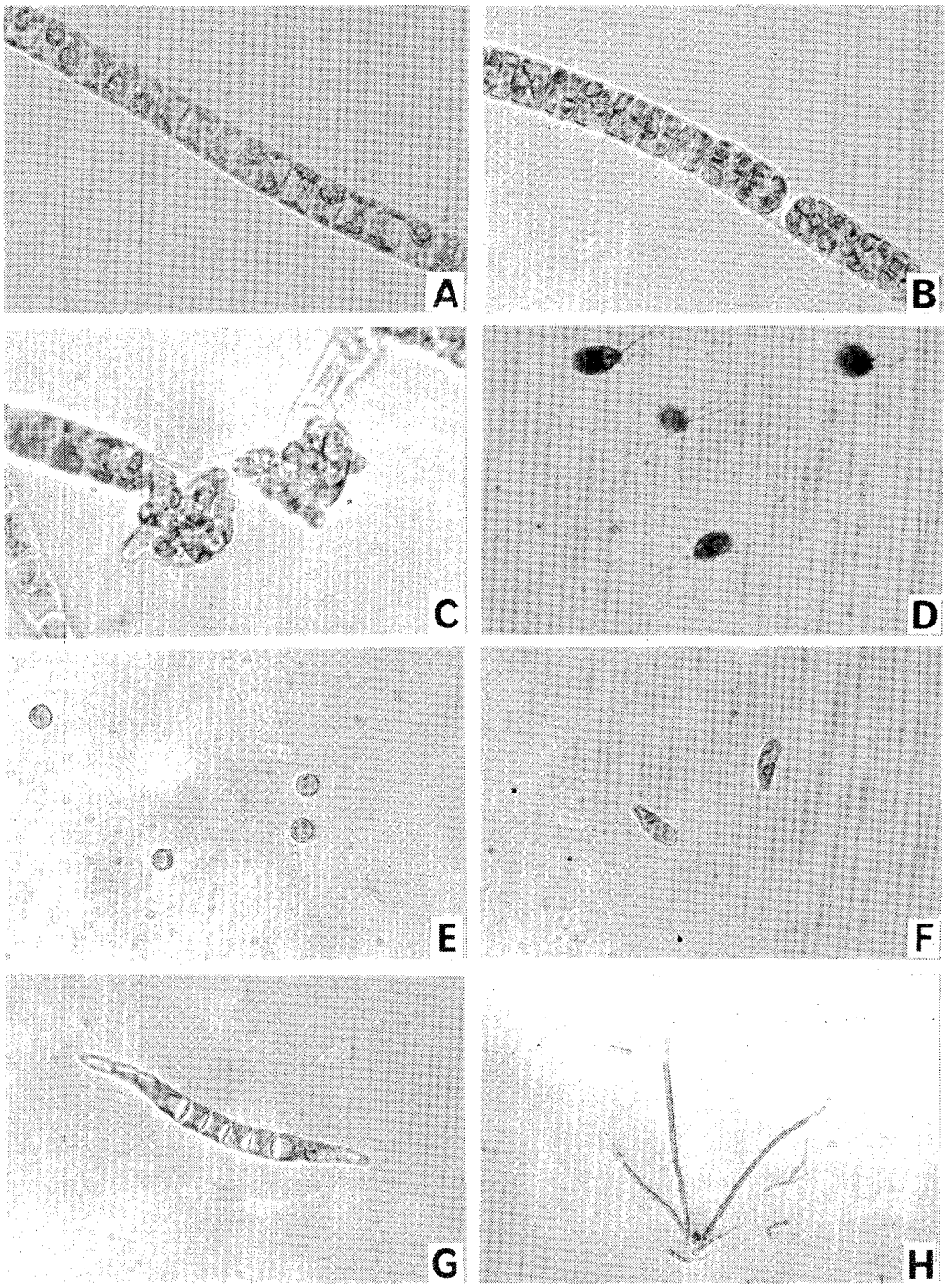

PLATE II

Plate II. *Ulothrix acrorhiza* KORNMANN, gametes and development of zygotes.

A.B. Rhizoid from vegetative cells, ×460.
C. Gametangia, ×460.
D. Liberation of gametes from gametangia, ×460.
E. Liberated gametes stained by Gentian-violet, ×680.
F. Conjugation of gemetes, ×1700.
G. Cysts, development of zygotes after 2 months culivation, ×500.
H. Fertile cysts, after 4 months cultivation, ×500.

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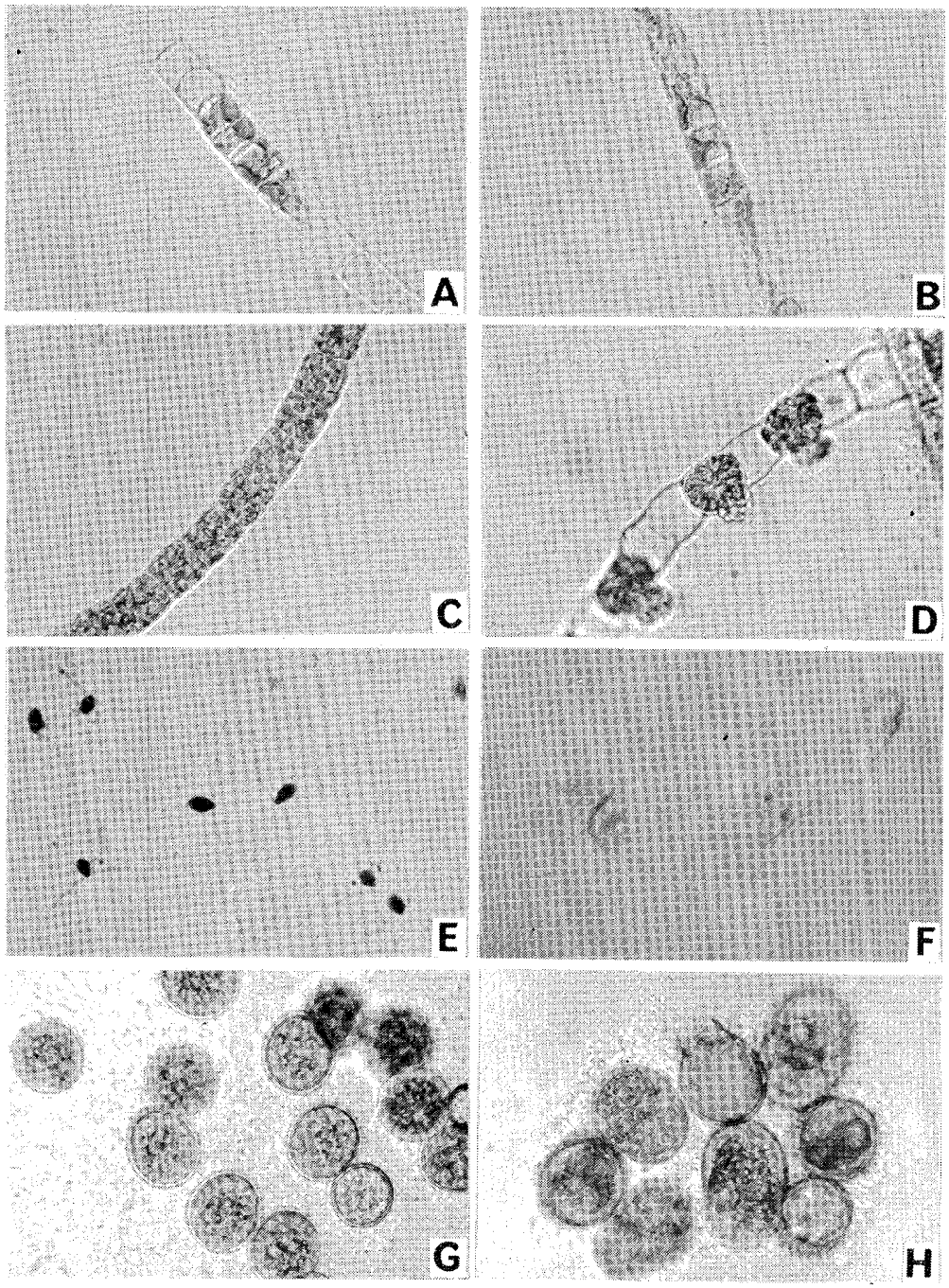

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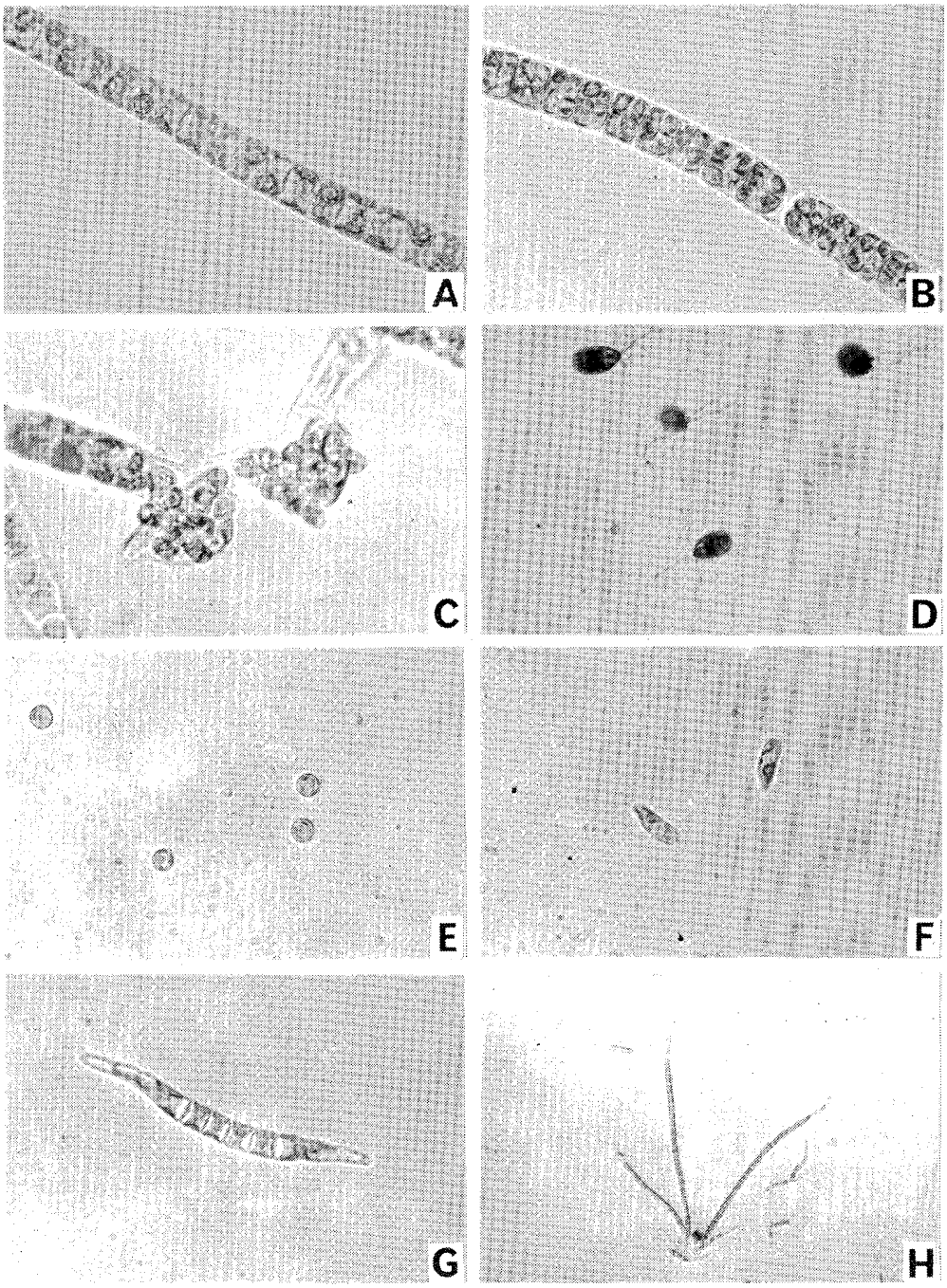

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