Environmental Conditions and Morphological Variation in the Blue-Green Alga *Chlorogloea fritschii*

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SUMMARY

The effect of environmental conditions on the morphology of the blue-green alga *Chlorogloea fritschii* is described. Availability of reduced carbon substrate, light and nitrogen all caused alteration in cell type, as did increase in temperature. The two major cell types were irregular clumps of cells (aserial), and filaments; in photoautotrophic conditions the former predominated during exponential growth at 34 °C. The presence of sucrose imposed aserial morphology in both phototrophic and heterotrophic cultures. The development of differentiated cells (heterocysts) following deprivation of nitrate and the interrelationships between different cell types are described.

INTRODUCTION

Chlorogloea fritschii, like certain other blue-green algae, exists in more than one morphologically recognizable form. The first descriptions of this organism described filamentous and irregular clumps of cells (Mitra, 1950; Mitra & Pandey, 1966) and the transitions between cell types led to the suggestion of a 'life-cycle' in which the filamentous character gave way to large groups of polygonal cells (Fay, Kumar & Fogg, 1964; Fogg *et al.* 1973). The change from mobile, filamentous cells to larger immobile aserial types forms the basis of a complex life-cycle in *Nostoc muscorum* G (see Lazaroff, 1973), changes between those morphological types being initiated by red light and reversed by green light. The availability of reduced carbon substrates, light and temperature all impose morphological changes on *C. fritschii*, and we describe here the cell types associated with photoautotrophic, photo-heterotrophic and heterotrophic growth under conditions of nitrogen fixation and where nitrate is supplied. It is not suggested that these constitute a life-cycle in the normal sense of the term. The different cell types observed are better described as the products of particular environmental conditions than as stages in an ordered developmental sequence.

The taxonomy of *C. fritschii* is in dispute, and because of its increasing use in experimental studies, a brief synopsis of the historical position is worthwhile. The first description of this blue-green alga was by Mitra (1950) who isolated it from Indian soil and designated it as a new species of the genus *Chlorogloea*, family Entophysalidaceae, order Chroococcales. The observation that when grown in the absence of nitrate the specialized type of cell known as heterocyst was present (Fogg, 1960; Pandey & Mitra, 1962), led Fay & Fogg (1962) to suggest that it might be classified as a *Nostoc* species. On the grounds that well-defined mucilaginous sheaths were absent and the vertical cell division amounted to true branching, Mitra & Pandey (1966) proposed that it represented a new genus, naming the alga *Chlorogloeopsis fritschii*. This designation was accepted by Stanier *et al.* (1971), but it was opposed by Gupta (1971). Bourrelly (1970), in his work on blue-green algal taxonomy, did not accept the need for a new family and transferred *C. fritschii* to the

genus *Nostoc*. All workers agree, however, that heterocysts are present and that the organism cannot therefore be a member of the Chroococcales. The DNA base ratio of *C. fritschii* was 41 to 42 % G + C (Craig, Leach & Carr, 1969), very close to the restricted range, 42 to 43 % G + C, found for six species of *Nostoc* (Edelman *et al.* 1967).

METHODS

Organism. *Chlorogloea fritschii* Mitra was obtained from Dr P. Fay, Department of Botany, Westfield College, London, in 1967. It is held in the Culture Collection of Algae and Protozoa, Cambridge (No. 1411/1).

Growth conditions. The growth medium employed was medium C (Kratz & Myers, 1955) supplemented with NaHCO_3 (0.1 %). Sucrose (10 mM) was included where indicated and a nitrate-free medium was constructed by substitution of chloride salts for nitrate salts. Growth medium for solid-phase culture was solidified with 1.5 % (w/v) agar.

For photoautotrophic growth the mineral salt medium, gassed with 5 % CO_2 -air, was used. Cultures were incubated at 34 °C either in an illuminated orbital shaker (Gallenkamp Ltd) containing three 30 W Grolux fluorescent lamps emitting about 1030 lm/mm² or in a vessel placed in a glass water-bath at 34 °C and illuminated by a 100 W tungsten bulb placed 125 mm from the culture and emitting about 1800 lm/mm². In the latter case cultures were agitated with a magnetic stirrer. Photoheterotrophic growth conditions were as above, except that sucrose was included in the growth media. *Chlorogloea fritschii* grew in light in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (10^{-5} M) and sucrose, indicating that the latter was a source of both reducing potential and carbon. This organism may therefore be classed as a facultative photoheterotroph (Stanier, 1973).

For heterotrophic growth, in which the doubling time increased from about 12 h to about 80 h, the cultures were incubated without agitation in the dark at 34 °C.

RESULTS

Morphology

When *C. fritschii* was grown photoautotrophically with nitrate as a source of fixed nitrogen, several cell types appeared in exponential culture (Fig. 1*a*). Type A cells were large, rather granulated cells existing either singly or as small clumps containing two or more cells which had arisen from divisions in up to three planes; type B cells, termed aseriate after Lazaroff (1973), were found in clumps which combined larger groups of cells apparently surrounded by a mucilaginous sheath; type C cells were small, and found in short filaments; and type D cells were larger and found in filaments in the process of dividing. The establishment of the relationship between these morphological forms has been considerably helped by studying the various types of nutrition of which the organism is capable.

Enrichment of particular cell forms

A culture of *C. fritschii* which had been subcultured for several years in total darkness with nitrate, using sucrose for heterotrophic growth, consisted of uniformly aseriate cells of type B (Fig. 1*c*). The accessory photopigment, phycocyanin, was reduced to approximately 5 % of that found in phototrophic cultures, and consequently the cells were a pale green colour. When sucrose was added to a photoautotrophic culture the aseriate-type cell became predominant, although type A cells were sometimes present. When a culture was maintained photoheterotrophically on sucrose agar, a uniform population of type A cells

Fig. 1. Cultures of *C. fritschii*. (a) Photoautotrophic culture, indicated cell types are shown in the text; (b) photoautotrophic culture after three weeks' dark incubation; (c) heterotrophic culture; (d) photoautotrophic culture 24 h after transfer to 45 °C.

Fig. 2. Photoheterotrophic culture of *C. fritschii*, after growth in solid medium. Arrow indicates discarded heavy sheath material.

was eventually obtained. This type of culture provided the initial stages for the examination of the changes imposed on *C. fritschii* by environmental conditions.

When these single cells of type A commenced further growth, a heavy outer layer was sometimes discarded (Fig. 2), this sheath-like material being translucent in transmitted light. This stage of culture may have been that referred to as an 'endospore' by other workers.

When a photoautotrophic culture (plus nitrate) was held in the dark (Fig. 1*b*) for three weeks, growth ceased and the cells became small and filamentous (type C); these cells were viable for at least three weeks, and grew when re-introduced to light. A similar filamentous cell type was obtained when either a photoautotrophic or photoheterotrophic culture was grown at 45 °C (Fig. 1*d*). Under these growth conditions type C cells or filaments similar in appearance to those shown in Fig. 3*b* were formed.

Photoautotrophic growth

When type A cells (Fig. 2) were transferred to illuminated-growth conditions lacking sucrose, three morphological changes were observed as the culture adapted to these photoautotrophic growth conditions. After 48 h most of the culture had changed to cell-type C (Fig. 3*a*). A further 48 hour period led to the formation of filaments whose cells were dividing transversely and longitudinally (Fig. 3*b*). Aseriate cells (type B) were formed after a third 48 h period of illumination (Fig. 3*c*) and these began to disrupt, releasing small filaments of type C. Some large filaments disrupted to yield single granulated cells (type A) and the culture began to attain the typical mixed appearance of photoautotrophic growth (Fig. 1*a*).

Photoheterotrophic growth

Growth in the light in the presence of sucrose did not lead to the development of filamentous forms. Thus type A cells (Fig. 2) gave rise, after 36 h, to small clumps (Fig. 4*a*), which after a further 36 h had developed into typical aseriate (type B) cells with external

Figs. 3(a, b, c). Sequence of changes following transfer of a photoheterotrophic culture of *C. fritschii* (Fig. 2) to photoautotrophic growth.

Figs. 4 (a, b). Transfer of a photoheterotrophic culture of *C. fritschii* from solid (Fig. 2) to liquid medium.

mucilage (Fig. 4b); this latter morphology was predominant as the culture continued to grow, with varying numbers of single cells present.

The quantitative decrease in filamentous cells in photoheterotrophic culture, relative to that of photoautotrophic culture, is illustrated in Fig. 5; this culture was inoculated with cells of type A at a stationary phase, which may account for the slower growth rate observed. Even photoautotrophic cultures did not acquire a predominantly filamentous character until in their late stages of growth, when growth rate had begun to decrease.

The development of heterocysts

The removal of nitrate from cultures of *C. fritschii* led to the development of heterocysts which were characterized by their paler colour, lack of granulation, polar bodies, and smaller

Fig. 5. Photoheterotrophic (●) and photoautotrophic (○) growth of *C. fritschii* measured by dry weight determination of removed samples. The percentage of filaments, as distinct from clumps of cells, at different stages of the cultures are expressed as solid (photoheterotrophic) and open (photoautotrophic) histograms.

size. When a photoheterotrophic culture (with sucrose) was deprived of nitrate, the aseriate morphology of the cells did not permit direct observation of heterocysts. However, gentle mechanical disruption of the clumps led to some disintegration of vegetative cells so that heterocysts were visible.

Under photoautotrophic conditions the development of heterocysts in response to nitrate-deprivation was observed on populations of type A and type B cells. When type A cells (Fig. 2) were transferred from a sucrose slope to a liquid medium lacking sucrose or nitrate, an unequal division of the cells was observed (Fig. 6*a*). A second 24 h period led to discernible heterocysts attached to cells of type A (Fig. 6*b*). Aseriate cell types developed, which in turn gave rise to heterocyst-containing filaments as type C (Fig. 6*c*). Heterocysts were recognized by polar bodies, two for intercalary heterocysts (Fig. 6*d*) and one for terminal heterocysts. Multiplanar division eventually led to a chain of aseriate-like organisms (Fig. 6*c*).

DISCUSSION

The markedly different environmentally-influenced cell types in populations of *C. fritschii* give some indication of the difficulties of blue-green algal taxonomy in general.

Light, supply of reduced carbon, availability of fixed nitrogen, and temperature all affected the morphology of *C. fritschii*. It was fixed carbon (supplied as sucrose), rather than light, which determined the adoption of aseriate, rather than filamentous, morphology. Thus in the presence of adequate illumination, the inclusion of sucrose in the growth medium caused a change from the characteristic photoautotrophic appearance (Fig. 1*a*) towards that associated with heterotrophic growth (Fig. 1*c*). Interrelationships of population type, for cultures which did not contain heterocysts, are summarized diagrammatically in Fig. 7. The right-hand side of the diagram shows changes in cell morphology after phototrophic growth in the presence of sucrose; the aseriate form was dominant and filaments rarely seen. The left-hand side of the diagram summarizes the sequence of events that arise from deprivation of sucrose, elevated temperature or transition to dark; in the latter case only the first stage of filament formation was accomplished, possibly because under these conditions the organisms had only their reserve endogenous material as a source of energy. The entire diagram represents

Figs. 6(a, b, c, d, e). Sequence of changes following the transfer of a photoheterotrophic solid-phase culture of *C. fritschii* (Fig. 2) to a liquid photoheterotrophic medium which lacked nitrate. H indicates heterocysts.

the routes of changes between cell types during photoautotrophic growth; the proportion of each type alters at different stages of culture, with the filamentous form finally becoming predominant.

Peat & Whitton (1967) investigated the effects of light intensity and the supply of fixed nitrogen on the morphology and structure of *C. fritschii* after different times. In photosynthetic cultures the lamellae were arranged peripherally, whilst in dark heterotrophic cultures the reduced number of lamellae present were scattered throughout the cytoplasm. They noted that the morphological form of the organisms altered at different stages of growth and observed that filaments were absent from dark, heterotrophic cultures. Differences in lamellar arrangement were also observed by Findley, Walne & Holton (1970) when *C. fritschii* was grown with light intensities that varied from 226 to 7560 lux. These

Fig. 7. Diagrammatic representation of interrelationships between environmentally-induced cell types of *C. fritschii*. For explanation see text.

workers described the presence of 'large unicells', which appear to be analogous to the organisms in Fig. 2. In our experiments these were predominant in the terminal stages of solid-phase culture, and this is consistent with the long (20 day) culture period employed by Findley *et al.* (1970).

The increased proportion of filamentous cells after growth at elevated temperature was noted by Findley, Holton & Herndon (1968), who also found that growth at 45 °C was slightly faster than at 35 °C. The observation of unequal division, and the fact that heterocysts always originated from the smaller of the two cells were similar to the development of heterocysts in the filamentous blue-green alga *Anabaena catenula* (Mitchison & Wilcox, 1972).

Chlorogloea fritschii is similar to *Nostoc muscorum* (Lazaroff, 1973) in that the interchange between aseriate and filamentous forms is guided by environmental triggers. However, the characteristic swarming of hormogonia (short filaments) into large, moving, spiral aggregates found in *N. muscorum* was absent in *C. fritschii*, and heterocysts were not always terminal. *Chlorogloea fritschii*, in contrast to *N. muscorum*, always had some of its population as single cells and this may be nearly 100 % under certain environmental conditions (Fig. 2). That sucrose affects *N. muscorum* morphology at the macrocolony level, in contrast to its dominant affect on the cellular form of *C. fritschii* is a major difference in the effect of the environment on the morphology of these two blue-green algae.

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