The Microanatomy and Ecology of '*Chlorochromatium aggregatum*' in Two Meromictic Lakes in Tasmania

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The motile ectosymbiotic consortium '*Chlorochromatium aggregatum*' occurs in large numbers in two meromictic lakes in Tasmania. In its structure it resembles specimens previously described, except that vesicles or mesosomes are present in the central bacterium. The population occupies a narrow stratum near the chemocline, but may occur either above or below it, in microaerobic or anaerobic conditions, respectively. Its presence under aerobic conditions need not invalidate previous hypotheses concerning the mutualistic relationship between the two partners of the consortium.

INTRODUCTION

Several bacterial consortia have been described in which a central, chemoorganotrophic, motile bacterium is covered by synchronously dividing sulphur bacteria (Trüper & Pfennig, 1978). The *Chlorobiaceae* are the only bacteria known to form such ectosymbiotic associations. The consortia were originally described as species. However, this is 'without standing in nomenclature' (Trüper & Pfennig, 1971). The names are still useful, however, and are freely used in the literature; where they appear here they are placed in inverted commas.

The best example of such consortia is '*Chlorochromatium aggregatum*' Lauterborn, in which cells of green sulphur bacteria are regularly arranged around a large, colourless, polarly flagellated, rod-shaped bacterium of unknown physiological activity and taxonomic position (Stanier *et al.*, 1981). There is little doubt that the green bacteria belong to the genus *Chlorobium* (Trüper & Pfennig, 1971). Growth and division of all cells occur synchronously, indicating a high degree of metabolic interdependence.

Stanier *et al.* (1971) and Gorlenko *et al.* (1977) have published light micrographs of '*C. aggregatum*' and Caldwell & Tiedje (1975) have published electron micrographs of ultrathin sections. Pfennig (1980) has discussed it in a review article of syntrophic mixed cultures and symbiotic consortia. In the latter work, the hypothesis is presented that the central bacterium is either a sulphate- or sulphur-reducing bacterium. However, repeated attempts at culturing the central bacterium in isolation have failed. One possible reason for this is that the central bacterium of the consortium functions as an electron donor, and is obligately dependent on the green sulphur bacteria as electron acceptors: growth of the central bacterium in pure culture would therefore be impossible (Pfennig, 1980).

'*Chlorochromatium aggregatum*' and other, less known consortia (see Starr & Schmidt, 1981) exhibit chemo- and phototactic responses and accumulate in conditions of optimum sulphide concentrations and light intensity (Pfennig, 1978). They have been found only in freshwater habitats, and may occur both in shallow pools above the mud and in the layers of green or purple sulphur bacteria in stratified lakes.

'*Chlorochromatium aggregatum*' occurs in two dystrophic, meromictic lakes in the south-west wilderness area of Tasmania, Australia. The two lakes, Lake Fidler and Sulphide Pool, both have severe physico-chemical stratification at a depth of 2-3 m, with a finely structured microbial array across this chemocline. The general features of the lakes and their micro-

organisms have been described elsewhere (King & Tyler, 1981, 1982, 1983; Croome & Tyler, 1983a, b, 1984). '*Chlorochromatium aggregatum*' is a prominent member of the stratified microbial community in both lakes, occurring at dimly lit depths in the vicinity of the chemocline. Here, we describe its microanatomy and ecology in these meromictic lakes.

METHODS

Most of the field and analytical methods have been published in previous descriptions of the lakes (e.g. King & Tyler, 1981; Croome & Tyler, 1984). The physico-chemical zonation and accompanying microbial array was sampled with a close-interval pneumatic sampler (Croome & Tyler, 1984). Specimens for scanning electron microscopy were exposed to 1% osmium tetroxide vapour for 20–30 s, placed on polylysine-coated glass coverslips (Marchant & Thomas, 1983), dehydrated in an acetone series, critical point dried using CO₂ as the intermediary fluid, sputter coated with gold, and viewed using a Philips 505 scanning electron microscope. Material for ultrathin sectioning was concentrated by centrifugation, fixed in 1% glutaraldehyde and 1% osmium tetroxide, dehydrated in acetone, and embedded in Spurr's resin. After sectioning, the material was placed on Formvar-coated copper grids, stained with 1% uranyl acetate and Reynold's lead citrate, and viewed using an Hitachi H-300 transmission electron microscope.

RESULTS

Light micrographs and scanning electron micrographs of the consortium are shown in Fig. 1. By light microscopy there is no perceptible difference between consortia from the two lakes. The consortium appears as a slightly knobbly, square to rectangular shape (Fig. 1a, b), grey under phase contrast microscopy, which slowly revolves about its long axis as it swims along an undulating path. Recently divided consortia are approximately cubical, about 2 µm long by 1.5 µm wide. Dividing consortia are more elongate. Scanning electron microscopy shows that in non-dividing consortia the outer, phototrophic bacteria are arranged more or less at right angles to the long axis (Fig. 1c), though in elongate, dividing consortia some cells may be randomly orientated (Fig. 1e) or almost longitudinally arranged (Fig. 1d). Although the consortia are motile, we have never seen flagella by either light or electron microscopy.

By transmission electron microscopy (Fig. 2) the internal structure of the green phototrophic bacteria of the outer layer is similar to that of the free-living *Chlorobium* populations in the lakes (see below). The cells are of the *Chlorobium limicola* type and the characteristic chlorobium vesicles are quite apparent.

The central bacterium is comparatively long, occasionally exceeding 3 µm in length, and of the order of 0.5–1.0 µm in diameter. The cell wall is often in close contact with those of the phototrophic bacterial cells around it, and displays structures similar to those described by Caldwell & Tiedje (1975) as hexagonal cups, postulated to play a role in maintaining the integrity of the consortium (Fig. 2a, b). The matrix of the central bacterium is lacking in detail, but large vesicles or mesosomes were observed near the cell wall (Fig. 2c, d). Such structures have not been reported previously for the central bacterium, and their function is unknown.

Lake Fidler and Sulphide Pool display abrupt changes in physical and chemical conditions across the chemocline, typical of meromictic lakes. Seasonal variation in these conditions has been described by King & Tyler (1982, 1983). Fig. 3 shows a typical example, from Lake Fidler, of this physico-chemical zonation and of the accompanying microbial array. The sharpness of the chemocline is best demonstrated by the almost horizontal redoxcline, above which there is usually a microaerobic zone. The microbial array consists of the flagellates *Trachelomonas volvocina* Ehrenb. and *Scourfieldia caeca* (Korsh.) Belcher et Swale, the colourless sulphur bacteria cf. *Achromatium* and *Beggiatoa*, the free-living phototrophic bacterium *Chlorobium*, and '*Chlorochromatium aggregatum*'. All of these except '*C. aggregatum*' maintain a more or less constant position, relative to the chemocline, diurnally and throughout the year; '*C. aggregatum*', on the other hand, varies its position from high in the microaerobic zone (0.25 m above the redoxcline), at an oxygen concentration of 0.4 mg l⁻¹ and a redox potential of + 350 mV, to well within the anaerobic zone, with a sulphide concentration of 30 mg l⁻¹ and redox of

Fig. 1. Whole mounts of *'Chlorochromatium aggregatum'*. (a, b) Light micrographs of live, dividing consortia. (c, d, e) Scanning electron micrographs of single and dividing consortia. Bars represent 1 μ m.

Fig. 2. Ultrathin sections of '*Chlorochromatium aggregatum*'. (a) Transverse section of a consortium; bar, 1 μ m. (b) Central bacterium enlarged to show surface structures (arrow); bar, 0.5 μ m. (c) Oblique section of a consortium; bar, 1 μ m. (d) Central bacterium enlarged to show vesicles or mesosomes (arrows); bar, 0.5 μ m. (e) Longitudinal section of a dividing consortium; bar, 1 μ m.

Fig. 3. Physico-chemical and microbial profiles across the chemocline in Lake Fidler, 31 January 1981. The broken line indicates the position of the redoxcline. dO₂, dissolved oxygen; E_h, redox potential; S²⁻, dissolved sulphide; BChl d, bacteriochlorophyll d. Organisms are in numbers of individuals per ml.

– 100 mV. Between January 1981 and April 1983 numbers ranged from 10^2 to 64×10^4 consortia per ml at the depth of the population peak, usually occupying a layer about 0.1 m thick. Because the population of '*C. aggregatum*' was usually outnumbered by that of *Chlorobium* in the same stratum, a discrete bacteriochlorophyll peak attributable to the consortium could not be discerned.

Though the position of the chemocline relative to lake surface varies with water level it is always at such a depth that light is severely attenuated and restricted to wavelengths > 550 nm by the dystrophic water (King & Tyler, 1982; Croome & Tyler, 1984). The population of '*C. aggregatum*' always receives much less than 1% of surface photosynthetically-active radiation.

'*Chlorochromatium aggregatum*' also occurs in Sulphide Pool but it was not observed there before April 1982. It occurs there under similar environmental conditions and occupies the same niche as in Lake Fidler. It is possible that it was introduced to Sulphide Pool by sampling equipment previously used in Lake Fidler.

DISCUSSION

The structure of '*C. aggregatum*' from Lake Fidler is similar to that found by previous workers, with cells of *Chlorobium* enveloping and being in close contact with a large central bacterium. It is also similar in its ultrastructure, the phototrophic symbionts possessing distinct chlorobium vesicles, and the central bacterium being surrounded by structures which may help to keep the consortium together (Caldwell & Tiedje, 1975). The vesicles or mesosomes observed in the central bacterium have not been reported previously, and their function is unknown.

The consortium '*C. aggregatum*' has been found in large numbers in several other stratified lakes. In Lake Sakovo (Gorlenko & Chebotarev, 1981) and Lesnaya Lamba, Karelia, USSR (Dubinina & Kuznetsov, 1976) it occurred at the same depth as free-living green phototrophic bacteria, in numbers up to 420 000 and 100 000 consortia ml^{-1} , respectively. In Lake Nesoytjern, Norway, it was recorded at 30 000 consortia ml^{-1} (Blakar, 1979), and in Burke Lake, Mich., USA, it comprised 10% of the phototrophic bacterial community (Caldwell & Tiedje, 1975). It has also been reported in Lakes Mary and Rose, Wis., USA (Parkin & Brock, 1980) and in Estonian lakes (Gorlenko & Lokk, 1979). In all cases, it has been reported as inhabiting strictly anaerobic regions.

In Lake Fidler, where concentrations of '*C. aggregatum*' up to 640 000 consortia ml^{-1} have been recorded, the largest population maximum yet reported for this 'organism', it has been found under both aerobic and anaerobic conditions. This is interesting in the light of the recent hypothesis (Pfennig, 1980) as to the relationship between the two members of the consortium, that the central organism is a sulphate- or sulphur-reducing bacterium utilizing extracellular sulphur produced by the phototrophic bacteria, which in turn use dissolved sulphide, produced by the central bacterium, as an electron donor for their anoxygenic photosynthesis. At the same time the *Chlorobium* cells gain motility, enabling positive phototactic responses. The presence of '*C. aggregatum*' in the microaerobic zone of Lake Fidler in no way negates this hypothesis, but enhances it. When present in the anoxic zone the metabolic activity of the consortium could be just as supposed above. Its presence in the microaerobic zone most probably results from a positive phototactic response, and although the reducing conditions required for anaerobic photosynthesis would not be present outside the consortium, they could well prevail between the cells within it. Why '*C. aggregatum*' should be found high in the microaerophilic zone in one month, and well into the sulphide zone in another, is not known. Its location is presumably a response to light, redox potential, nutrients and sulphur distribution. We presume (Croome & Tyler, 1984) that the presence of viable cells of the other phototrophic constituent of the microbial array, free-living *Chlorobium*, above the redoxcline under microaerobic conditions indicates inefficient depth regulation by some members of the population.

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