MICRO-ORGANISMS ON CAVE WALLS IN THE DEEP TWILIGHT ZONE

GUY COX*
Sydney University Speleological Society

Abstract

A greenish tinge is often observable on cave walls well into the 'deep twilight zone', where human vision requires considerable dark adaptation. To study the organisms responsible for this colouration a site in Hennings Cave, Jenolan, where daytime illumination does not exceed 0.002 lux was chosen. In the surface layer of rotten limestone four photosynthetic micro-organisms have been recognised. Electron microscope studies reveal that two of these show extreme modifications for photosynthesis in this very low light intensity. Various non-photosynthetic organisms are also found in the colonies. A fungus is occasionally seen, and there is a range of gram-negative and gram-positive bacteria one of which also forms extensive colonies on limestone and mud in the dark zone. Another resembles no type previously described, and may have evolutionary significance. These organisms together represent a complex ecosystem which has never previously been studied.

Introduction

Cave entrances harbour a variety of shade and damp-loving plants. Flowering plants (angiosperms) are found only in the outermost area, being succeeded by ferns, which often form a luxuriant growth just inside the cave entrance. Where soil is sparser or light intensity lower mosses succeed the ferns, and here the classical ecologists' sequence ends. In the totally dark region making up the larger part of most caves, whitish or yellowish patches are often seen on the walls. These have been assumed to be colonies of fungi or bacteria, but do not seem to have received much study. (Cubbon (1976) suggests that they are actinomycetes – fungus-like bacteria). Between the entrance zone and the dark zone is a region of deep twilight where human vision needs a lot of dark adaptation, and where nobody seems to have looked for plant life. Yet it has been known for a long time that blue-green algae (cyanophytes) can live in very low light intensities, and also that they have a special affinity for limestone – some species deposit it, while others dissolve it.

The entrance chamber of Henning's Cave, Jenolan, NSW, is a large boulder collapse, behind which is a chamber lit very dimly by light filtering through the boulders. A greenish tinge is noticeable on the walls, suggesting the possible presence of photosynthetic micro-organisms, and this prompted the present study.

Collection Sites

All the 'deep twilight' material described here was collected from one site in the Hennings entrance chamber. The illumination at the site was measured around midday in bright sunshine, using a sensitive battery-powered photometer, and the maximum level recorded 0.002 lux. The surface few millimetres of rock at this site were relatively soft and friable; a similar green tinge was, however, also seen on more solid rock.

Samples of the white microbial colonies of the dark zone were also taken, from both mud and solid rock.

Techniques

Fresh material was examined using both light and scanning electron microscopes. Most work, was however, carried out on sectioned material. Samples were fixed in glutaraldehyde and osmium tetroxide, with the addition of Na EDTA to remove some of the limestone, dehydrated in ethanol series, centrifuged and embedded in epoxy resin. Sections were cut at 1 μm thickness for light microscopy, and 0.07 μm thickness for electron microscopy. Electron microscope sections were stained with lead citrate and uranyl acetate.

* Electron Microscope Unit, University of Sydney, NSW 2006

Photosynthetic Organisms

Green alga.

A unicellular green alga was the only photosynthetic eukaryote (organism with a true nucleus) present. Cells were small (around 6 μm diameter) with an irregular 'castellated' shape and dense contents (Fig. 1). The cell wall was up to 1 μm thick. The most interesting feature was the structure of the chloroplasts, with thylakoids (the membranes that carry out the light reaction) packed extremely closely together (over 100 per μm), as seen in Fig. 2. These cells were isolated in culture (by Dr H. Marchant, at ANU) and identified as a species of *Chlorella*. When grown in culture, under lighting approximating to cloudy daylight, the chloroplasts still showed very densely packed thylakoids. By comparison, *Chlorella fusca* grown in culture has a thylakoid density across the chloroplast of only 15-20 per μm. Clearly the cave *Chlorella* has adapted considerably for an existence in minimal light, and cannot revert to a more normal structure in higher light conditions (though, at least in pure culture, it grows well under high light levels).

Blue-green algae.

Blue-green algae (cyanophytes) are not algae in the true sense of the term. They are allies of the bacteria; prokaryotes with no true nucleus or cell organelles. A range of types was seen, but only three were sufficiently distinctive and common for detailed study.

Type 1. Cell diameter 1.2 μm approx., cells solitary or in small colonies. Thick (0.1 μm) cell wall or sheath, which appeared very dense in the electron microscope, suggesting impregnation with lipid or other non-carbohydrate material. The thylakoids (photosynthetic membranes) are 4-6 in number, arranged concentrically around the cell. This is a common arrangement, found in many cyanophytes and characteristic of the often-studied *Chlorogloea fritschii* when grown in *high* light intensity (Peat & Whitton 1967). When the cells form colonies, the colony is not bounded by a single capsule or slime layer. No name has yet been assigned to this type.

Type II. Cells ovoid in shape, 2 x 4 μm in diameter in colonies of 4 or more cells together, bounded by a common capsule, 0.3 – 0.5 μm thick. Thylakoids are much more numerous than in Type 1, and are arranged in whorls filling much of the cell. This is again a typical cyanophyte pattern, and is seen in *Chlorogloea fritschii* grown in low light intensity. (Though the low intensity used was around 200 lux – 100,000 times higher than the illumination in the cave!) Type II has provisionally been assigned to the genus *Gloeocapsa* following Stanier and others (1971).

Type III. Very small cells, 0.5 - 1 μm in diameter, which occur in spherical colonies (c. 2 μm diameter) of 4 – 8 cells enclosed in a common sheath, of microfibrillar structure. The shape of each cell tends to show the plane of last division. Fig. 1 shows three such colonies living in association with a *Chlorella* cell, and apparently linked by a slime layer. The most striking feature is the unusual thylakoid arrangement, with the membranes densely (50 per μm) packed in parallel arrays (Fig. 3). Such an arrangement has not been reported before, and must be an adaptation to the extremely low light intensity of the deep twilight zone. This species seems to belong to the genus *Chroococcus,* following Stanier and others (1971).

Heterotrophic Organisms

A varied heterotrophic (non-photosynthetic) community is present. The only eukaryote seen is a fungus, but there is range of bacteria of which two merit individual mention.

1. One bacterium found in the zone (Fig. 4) resembles no organism previously described. (A note describing it is in preparation at present). It appears to be a gram-negative bacterium, occurring in colonies with a fibrillar sheath surrounding each cell and a layer of tubular scales surrounding the whole colony. The spiral arrangement of DNA in the nucleoid is particularly distinctive; this resembles closely the pattern seen in the most primitive eukaryotes (cells with nuclei) so that this organism may represent an evolutionary 'missing link'.

2. The bacterial colonies of the dark zone consist solely of one gram-positive bacterium (Fig. 5); this is also found as a 'stray' in the deep-twilight zone. The colonies consist of short chains of irregularly-shaped cells with spiky cell walls and dense granules (probably polyphosphate) in the cytoplasm. The nutrition of this bacterium is an interesting problem – the other bacteria of the deep-twilight zone presumably live saprophytically on the remains of the algae, but this forms pure colonies in the dark and must presumably survive on some component of limestone or cave mud.

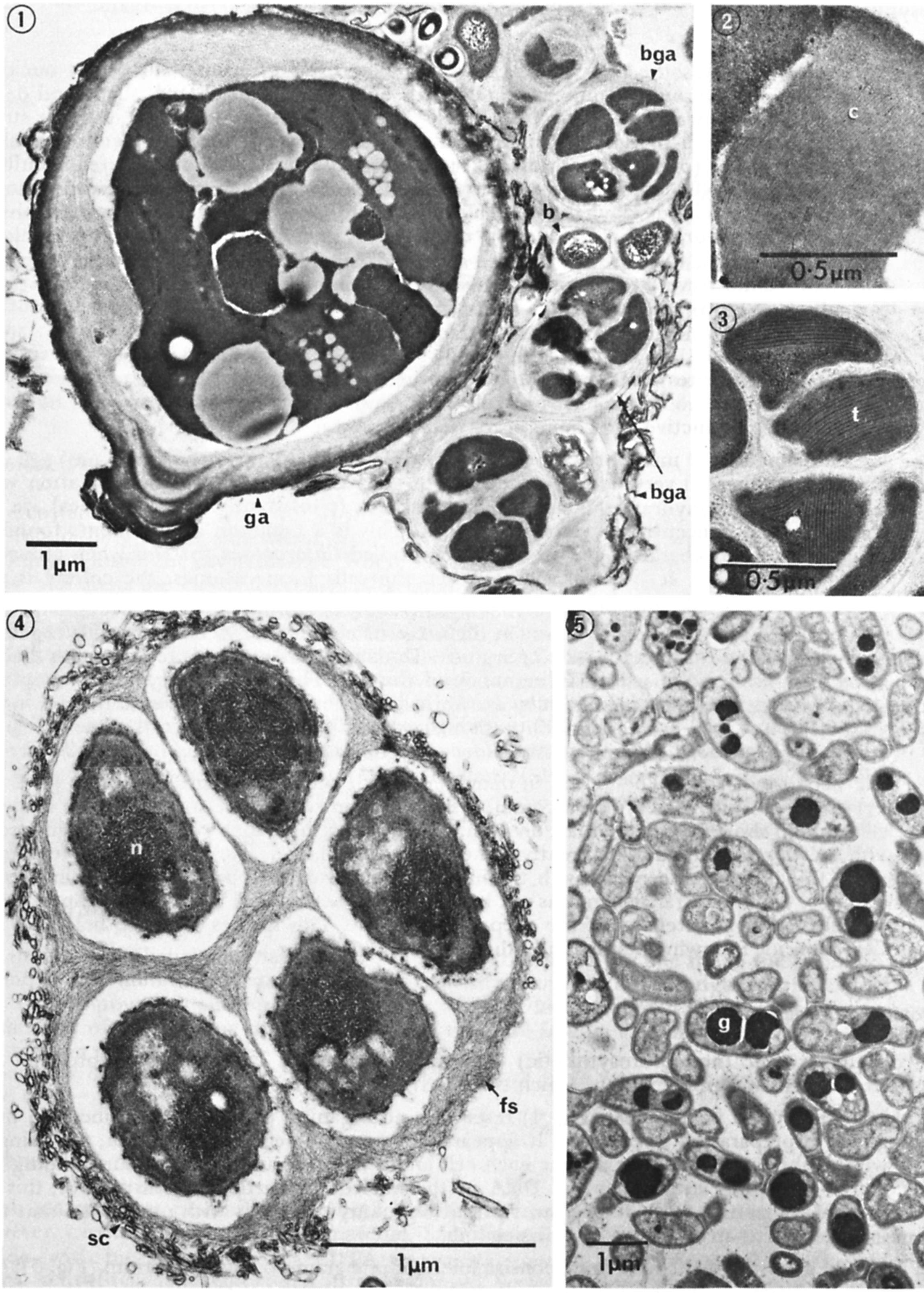

Fig. 1. A single green algal cell (ga) with attached colonies of blue-green algae (bga) and bacteria (b). x 12000

Fig. 2. Part of the green alga shown in Fig. 1. Part of the chloroplast (c) is shown; the photosynthetic membranes are very densely packed. x 45000

Fig. 3. Part of one blue-green algal colony from Fig. 1. Each cell contains close-packed thylakoids (photosynthetic membranes) (t). x 35250

Fig. 4. Bacterial colony from the deep-twilight zone. Cells have spiral-structured nucleoids (n) and are embedded in a fibrillar sheath (fs) which is surrounded by scales (sc). x 32250

Fig. 5. Bacteria from the dark zone. Many show large dense granules (g) which are probably polyphospate. x 10500

All figures are transmission electron micrographs of thin sections.

Conclusions

The little-studied deep twilight zone supports a varied, but probably slow-growing, flora. A green alga (*Chlorella* sp.) and a blue-green alga (*Chroococcus* sp.) seem to be highly adapted to the low-light environment; other blue-green algae may be casual strays from better-lit regions of the cave entrance. Bacteria form a second step in the food-chain; those marked 'b' in Fig. 1, for example, seem to be living saprophytically on the blue-green algal slime. The dark-zone bacterium, however, must either live saprophytically on the organic content of cave limestone or be a chem-autotroph. The whole represents a fascinating ecosystem in an exceedingly minimal environment; adaptation to this limited environment may also have provided an ecological haven from some evolutionary relics.

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