

Chlorophyll *d* as a major pigment

SIR — Chlorophyll *d* was reported first as a minor, green and magnesium-containing pigment in various species of red macroalgae in 1943 (ref. 1). It was thought by some, however, that chlorophyll *d* could be an artefact produced by the pigment extraction process, as it is not found in all algae, and one of the oxidation derivatives of chlorophyll *a* has an absorption spectrum identical with that of chlorophyll *d*^{2,3}. It remains unclear as to whether chlorophyll *d* does occur *in vivo*. We have now isolated a previously undescribed oxygenic photosynthetic prokaryote containing chlorophyll *d* as a major green pigment: it has only a small amount of chlorophyll *a*.

We isolated the new organism from a suspension of algae squeezed out of *Lissoclinium patella*, a colonial ascidian, collected in 1993 from the marine coast of the Palau islands in the western Pacific Ocean. Cells are unicellular and spheroidal or ellipsoidal, 1.5–2.0 µm in diameter and 2.0–3.0 µm in length. They are photoautotrophs, and have evolved in the presence of oxygen. We used electron

microscopy to demonstrate that the cell is a prokaryote (Fig. 1a, b). About 10 to 15 layers of thylakoid-like membranes are stacked peripherally (Fig. 1b), and we observed no protein entities similar to phycobilisomes on the membranes (Fig. 1a). These ultrastructural features are very similar to those of the Prochlorophyta (Oxychlorobacteria), oxygenic photosynthetic prokaryotes, containing chlorophylls *a* and *b* without phycobiliproteins, rather than those of the Cyanophyta (Cyanobacteria), containing chlorophyll *a* with phycobiliproteins.

The cell suspensions are green and have an absorption maximum in the red region at 714–718 nm (Fig. 1c), whereas all known oxygenic photosynthetic organisms have a characteristic absorption maximum around 675 nm due to a bathochromic shift of chlorophyll *a*⁴. Pigment analysis by HPLC reveals that a green pigment with three absorption maxima at 400, 455 and 697 nm in methanol is a predominant pigment of the cell in addition to only a small amount of chlorophyll *a*. The absorption spectra of the predominant pigment and of its acidification product (peaks at 392, 421 and 692 nm in diethyl ether) are the same as those reported for chlorophyll *d* and phaeophytin *d*¹, respectively. We confirmed that the predominant pigment is chlorophyll *d* by NMR and fast atom bombardment mass spectrometry (Fig. 2).

The chlorophyll *d* makes up about 80% of the total lipid-soluble pigment of the cell and more than 2% of the cell dry weight. The amount of chlorophyll *a*, on the other hand, is less than 0.08% of the cell dry weight, whereas it ranges from 0.3% to 3% in other oxygenic photosynthetic organisms⁵. The ratio of chlorophyll *a* to chlorophyll *d* in the

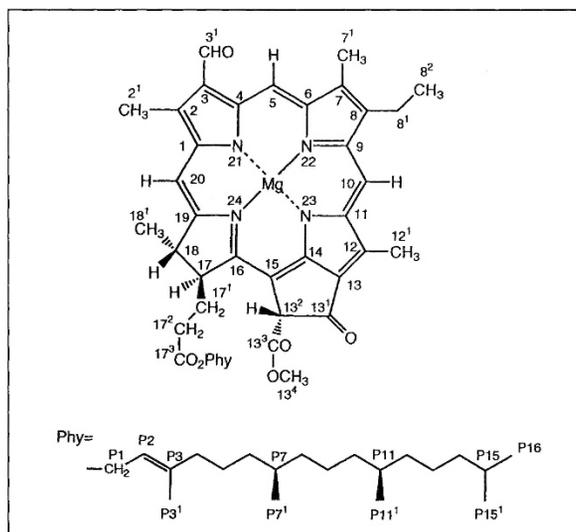


FIG. 2 Structure of chlorophyll *d*.

cells varies between 0.027 and 0.092, depending on the culture conditions.

Our results confirm the natural occurrence of chlorophyll *d* in a newly isolated, oxygenic, photosynthetic prokaryote. Moreover, the higher content of chlorophyll *d* and the low ratio of chlorophyll *a* to chlorophyll *d* in this organism suggest the existence of a unique and as yet unknown light-harvesting system which uses chlorophyll *d*. We propose for the new organism the name *Acaryochloris marina* Miyashita et Chihara gen. et sp. nov., to be formally described elsewhere (manuscript in preparation).

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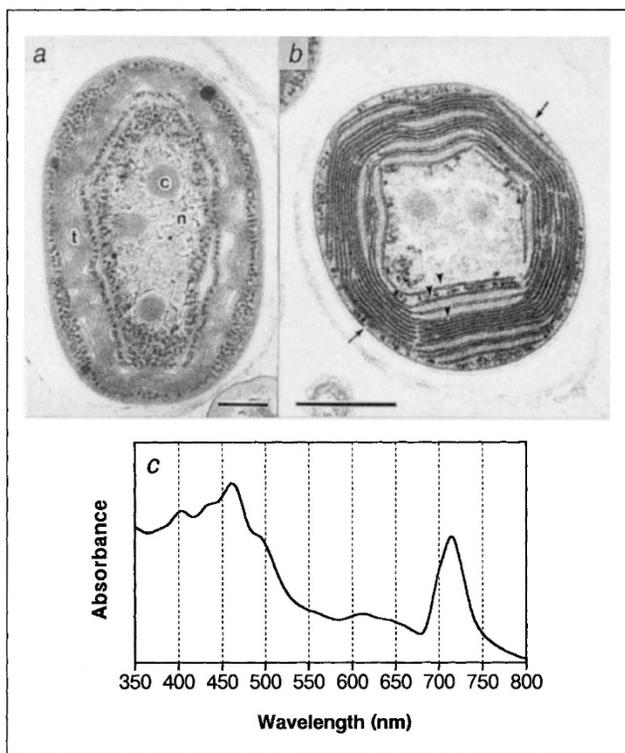


FIG. 1 a, Electron micrograph of a longitudinal section of a cell, showing the cytoplasmic space. The preparation was prefixed with 1% glutaraldehyde and postfixed with 2% osmic acid. c, carboxysome-like structure; n, nucleoid; t, thylakoid-like membranes. Scale bar, 0.3 µm. b, Electron micrograph of transverse section showing thylakoid-like membranes and plasmalemma. Cell prefixed with 1% glutaraldehyde and postfixed with 1% potassium permanganate. Arrowheads, thylakoid-like membranes; arrows, plasmalemma. Scale bar, 0.5 µm. c, Absorption spectrum of cells suspended in fresh culture medium.

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