

Ecology

A niche for cyanobacteria containing chlorophyll *d*

The cyanobacterium known as *Acaryochloris marina* is a unique phototroph that uses chlorophyll *d* as its principal light-harvesting pigment instead of chlorophyll *a*, the form commonly found in plants, algae and other cyanobacteria; this means that it depends on far-red light for photosynthesis. Here we demonstrate photosynthetic activity in *Acaryochloris*-like phototrophs that live underneath minute coral-reef invertebrates (didemnid ascidians) in a shaded niche enriched in near-infrared light. This discovery clarifies how these cyanobacteria are able to thrive as free-living organisms in their natural habitat.

Acaryochloris marina was first isolated from extracts of didemnid ascidians^{1,2} and was presumed to be a symbiont, like the cyanobacterium *Prochloron* sp., which contains chlorophyll *a* and *b*, and is found inside didemnids³. *Acaryochloris marina* has been found on red algae⁴ and a free-living *Acaryochloris*-like organism has been discovered in a turbid saline lake⁵. This indicates that cyanobacteria containing chlorophyll *d* may be fairly widespread, yet little is known about their habitat and ecology.

In a microphotometric survey of the didemnid ascidians *Lissoclimum patella*,

Trididemnum paracyclops, *Diplosoma similis* and *Diplosoma virens*, we investigated the occurrence and distribution of cells containing chlorophyll *d* (for methods, see supplementary information). *Prochloron*, and some unicellular cyanobacteria containing chlorophyll *a* and phycobiliproteins, colonized internal cavities of the didemnids, but we found no evidence of chlorophyll *d* in the ascidians. However, biofilms growing on the underside of the didemnids contained clusters of pale, greenish-yellow *Acaryochloris*-like morphotypes: these had spectral absorption and fluorescence features that were characteristic of chlorophyll *d* (Fig. 1a, b; and see supplementary information).

We cultured these *Acaryochloris*-like cells from the biofilm. Sequence analysis of the isolate (results not shown) indicates that the genes that encode cells' light-harvesting protein (*pcbC*) and 16S ribosomal RNA correspond to those of *A. marina*: there is 100% identity with the 302-base-pair polymerase-chain-reaction fragment and 99% identity with the 392-base-pair fragment, respectively (NCBI database⁶).

Fibre-optic microprobe measurements⁷ in *D. virens* showed intense attenuation of visible light. Far-red light penetrated more efficiently through the ascidian tissue, and was enhanced relative to the incident light owing to light-trapping effects^{3,7}. Under all ascidians, visible light was strongly depleted but there was 10–20 times more far-red light,

providing an ideal niche for cyanobacteria containing chlorophyll *d*, which absorbs maximally at 700–720 nm (Fig. 1b).

We used variable chlorophyll fluorescence imaging to assess photosynthesis of the cyanobacteria containing chlorophyll *d* in their natural habitat. Maximal quantum yields of photosystem II (PSII) were 0.77 and 0.59 in zones comprising *Prochloron* and *Acaryochloris*-like cells, respectively (Fig. 1c). Maximal PSII quantum yields of 0.67–0.80 have been reported for *A. marina* cultures⁸.

As expected, the quantum yield of PSII decreased with increasing irradiance (Fig. 1d). Surprisingly, the *Acaryochloris*-like cells were, like *Prochloron*, able to sustain high photosynthetic activity at strong light intensity (Fig. 1e). A similar light adaptation is also evident in *A. marina*⁹.

It is an apparent paradox that *Acaryochloris*-like cells thrive in extreme shade but show features of adaptation to strong light. This unusual photoacclimation reflects the fact that they live in an environment rich in near-infrared light and that chlorophyll *d* is the main light-harvesting pigment that drives both photosystems I and II under these conditions^{7,10}.

We conclude that *Acaryochloris*-like cyanobacteria grow in biofilms beneath didemnid ascidians, where far-red is enhanced over visible light and is used for oxygenic photosynthesis. This explains the occurrence of epiphytic *A. marina* on the underside of red algae⁴. Cyanobacteria that contain chlorophyll *d* may thrive in other habitats with little visible light, but further microenvironmental controls may be important in defining the niche of these microorganisms.

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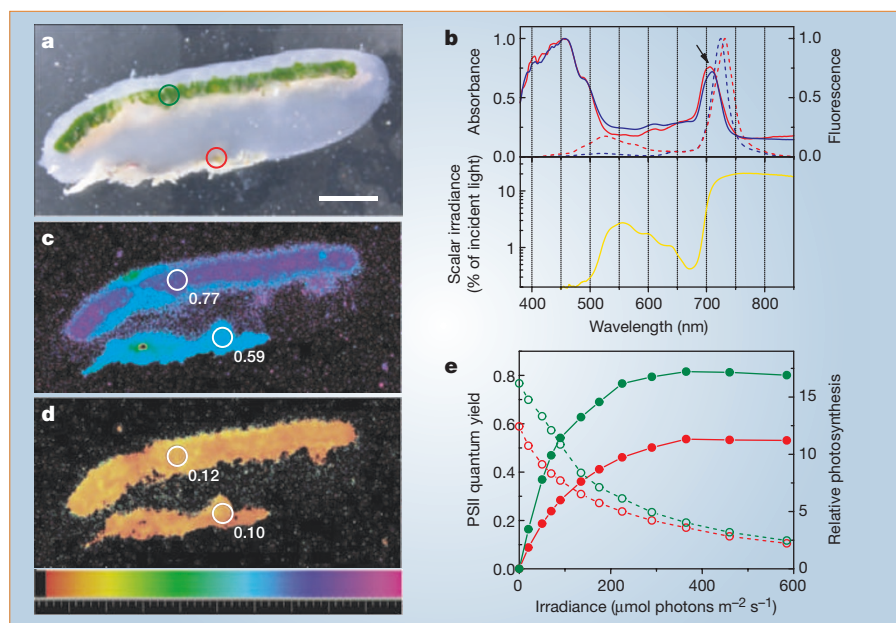


Figure 1 Distribution, spectral characteristics and photosynthesis of cells containing chlorophyll *d* that are associated with the didemnid ascidian *Diplosoma virens*. **a**, Vertical section through *D. virens*, showing the green cells of symbiotic cyanobacterium *Prochloron* sp. inside cavities, and a biofilm (white) patch of *Acaryochloris*-like cells growing on the underside of the ascidian (scale bar, 2 mm). **b**, Top, spectral absorbance (solid lines) and ultraviolet-excited fluorescence (dashed lines) of cells from the biofilm shown in **a** (red curves) and cells from an *A. marina* culture (blue curves). Arrow, absorption maximum of chlorophyll *d*. Data were normalized to the maximal absorbance and fluorescence, respectively. Bottom, spectral irradiance measured below *D. virens* after the biofilm had been removed, expressed as a percentage of downwelling irradiance at the tissue surface. **c**, **d**, Images as in **a**, but showing the maximal photosystem-II (PSII) quantum yield of the dark-adapted section (**c**) and the effective PSII quantum yield at an irradiance of 585 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (**d**). Both variables were scaled to the same colour gradient (0–1). **e**, PSII quantum yield (dashed lines) and relative rates of photosynthesis (solid lines) as a function of irradiance in *Prochloron* symbionts (green) and *Acaryochloris*-like cells (red), taken from areas circled in **a**.

- Miyashita, H. et al. *Nature* **383**, 402 (1996).
- Miyashita, H., Ikemoto, H., Kurano, N., Miyachi, S. & Chihara, M. *J. Phycol.* **39**, 1247–1253 (2003).
- Kühl, M. & Larkum, A. W. D. in *Symbiosis: Mechanisms and Model Systems* (ed. Seckbach, J.) 273–290 (Kluwer, Dordrecht, 2002).
- Murakami, A., Miyashita, H., Iseki, M., Adachi, K. & Mimuro, M. *Science* **303**, 1633 (2004).
- Miller, S. R. et al. *Proc. Natl Acad. Sci. USA* **102**, 850–855 (2005).
- Chen, M., Hiller, R. G., Howe, C. J. & Larkum, A. W. D. *Mol. Biol. Evol.* **22**, 21–28 (2005).
- Kühl, M. & Fenchel, T. *Microb. Ecol.* **40**, 94–103 (2000).
- Schiller, H., Senger, H., Miyashita, H., Miyachi, S. & Dau, H. *Fed. Eur. Biochem. Soc. Lett.* **410**, 433–436 (1997).
- Miyashita, H. et al. *Plant Cell Physiol.* **38**, 274–281 (1997).
- Boichenko, V. A., Klimov, V. V., Miyashita, H. & Miyachi, S. *Photosynth. Res.* **65**, 269–277 (2000).

Supplementary information accompanies this communication on Nature's website.

Competing financial interests: declared none.