

SHORT COMMUNICATIONS

Leucothrix: Absence of Demonstrable Fixation of N₂

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Leucothrix mucor Oersted is a common marine epiphyte, probably ubiquitous on shores around the world. The long, unbranched filaments, which are usually attached to algae, taper somewhat from 2 μm wide at the base to less than 1 μm wide at the distal end. Asexual reproduction is effected by the abstriction and detachment of terminal segments or single cells, which are capable of slow gliding on solid substrates until they re-attach. In liquid cultures, rosettes of shorter filaments are commonly formed. Although apochlorotic, and presumably an obligate heterotroph, *Leucothrix* shows certain resemblances to blue-green algae such as *Calothrix*. Unlike *Calothrix*, however, it apparently lacks heterocysts. In spite of this, its possible affinity with blue-green algae suggested that *Leucothrix* might be able to fix N₂. This was tested by the acetylene reduction assay for nitrogenase activity (Stewart, Fitzgerald & Burris, 1967).

METHODS

A pure culture of *Leucothrix mucor* was established from an isolated epiphytic filament growing on a brown alga, *Ectocarpus*, at the sea-shore near Muroran, Hokkaido, Japan. It reproduces well in a simple sea-water medium (Lewin, 1959), requiring no vitamins or other specific growth factors. It can use glutamate as a source of combined nitrogen and carbon, or N and C compounds supplied separately as an ammonium salt and lactate respectively. In this respect *Leucothrix* differs physiologically from some 40 isolates of *Synechococcus* and *Aphanocapsa* spp., unicellular blue-green algae which were reported to be unable to utilize glutamate as a sole source of nitrogen (Wan & Floyd, 1974). We tested filaments grown with sodium glutamate (10.0 g/l) as a source of combined N and filaments cultured with sodium lactate (10.0 g/l) and all essentials except for a source of combined N. Flask cultures (1 l) were grown at 23 °C in air with constant gentle oscillation for 7 days. Those in the medium containing glutamate grew well, forming a stringy, milky suspension of filaments, rosettes and free cells. Those grown with lactate produced only a few macroscopic tufts of filaments, which had probably grown at the expense of the residual combined N carried over with the inoculum or N introduced as a contaminant. Nevertheless, we had sufficient N-starved material for the acetylene reduction assay.

Before the acetylene reduction tests, one flask each of the glutamate-grown culture and the lactate-grown culture were sparged with N₂ for 20 h to provide time for possible synthesis of the N₂-fixing enzyme complex in the absence of O₂.

Approximately 50 mg (fresh wt) samples of the glutamate-grown cells were transferred with 1.0 ml medium to 7 ml serum bottles. The lactate-grown organisms were concentrated by gentle filtration on 25 mm glass-fibre filters, which were then similarly transferred with 1.0 ml of the culture filtrate to serum bottles. Bottles were flushed with gas mixtures con-

taining 0, 0.05, 1.0 or 20% (v/v) O₂ in argon, introduced through a hypodermic needle inserted through the septum stopper; a second needle served as a vent. One ml of purified acetylene (scrubbed of contaminating acetone vapour) was injected into all samples and the excess pressure was immediately released by piercing the stopper. The samples were incubated for 2 h at 23 °C and the reaction terminated by the addition of 1.0 ml of N-H₂SO₄. Controls, included for every treatment, consisted of identical samples of medium containing cells which were inactivated with acid immediately after addition of acetylene. Samples (0.25 ml) of the gas phase were analysed for ethylene production on a Varian (series 1400) gas chromatograph equipped with a Porapak N column and flame ionization detector.

As 'positive controls' we also tested scrapings of blue-green algae – predominantly *Calothrix* (?) *scopulorum*, a species known to fix nitrogen – scraped from rocks in the marine supralittoral zone at La Jolla, California. The samples contained approximately 50 mg (fresh wt) and were tested in the same way as the *Leucothrix*.

RESULTS

Acetylene reduction was not detected under aerobic, microaerophilic or anaerobic conditions in any of the 72 samples containing 25 to 700 µg protein N, from cultures grown with or without a combined N source, pre-conditioned for 20 h under N₂, or pre-incubated for 3 h in various argon + O₂ mixtures before the addition of acetylene. The samples of *Calothrix* reduced acetylene at the rate of 36 nmol/mg N/h under aerobic conditions in the laboratory.

This lack of demonstrable nitrogenase activity in *Leucothrix mucor*, corroborating the severely limited growth of the filaments in nitrogen-free medium, provides strong evidence that this organism is unable to fix N₂.

DISCUSSION

Reports of acetylene reduction by a unicellular blue-green alga, *Gloeocapsa* sp. (Wyatt & Silvey, 1969; Rippka, Neilson, Kunisawa & Cohen-Bazire, 1971), and by the filamentous, non-heterocystous blue-green alga *Plectonema boryanum* (Stewart & Lex, 1970) suggest that among cyanophytes N₂-fixation is not necessarily restricted to those which possess heterocysts. Although *P. boryanum* does not normally fix N₂ in aerobic conditions, under anaerobiosis it develops acetylene-reducing activity within 30 min (N. Weare, personal communication). Other aerobic, N₂-fixing micro-organisms exhibit maximum nitrogenase activity at a pO₂ considerably lower than 0.2 atm (Millbank, 1970; Drozd & Postgate, 1970). Since our *Leucothrix* showed no activity either under argon or at low pO₂ it is unlikely that this organism is capable of synthesizing the N₂-fixing enzyme complex. Presumably, under natural conditions, exudates from the algae on which this epiphyte grows supply it with sufficient fixed N compounds, as well as organic substrates, to permit its normal development.

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