Ecophysiological studies on *Spirulina platensis*
Effect of temperature, light intensity and nitrate concentration on growth and ultrastructure

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The ultrastructure of the cyanobacterium *Spirulina platensis* was studied in relation to temperature, light intensity and nitrate concentration. The organism was able to grow in media supplied with nitrate in concentrations up to 250 mM. High nitrate concentrations increased the yield and growth rate at temperatures above 35°C. Occurrence, distribution and abundance of cyanophycin granules, polyglucan granules, cylindrical bodies, carboxysomes and mesosomes varied widely in relation to the factors studied. At low temperatures (up to 17°C) cyanophycin was the abundant organelle, especially at high nitrate concentrations, whereas in the temperature range 17–20°C polyglucan was found in large quantities particularly at low nitrate concentrations. Special attention was paid to the cylindrical bodies, the ultrastructure of which was dependent on temperature. Three types of ultrastructure were distinguished each with several possible shapes.

**INTRODUCTION**

An abrupt metabolic change at 17–20°C was observed during studies on the morphology and ultrastructure of the cyanobacterium *Spirulina platensis* in relation to temperature and light intensity. At temperatures above 17°C, cyanophycin was replaced by polyglucan as the abundant storage material (Van Eykelenburg, 1979b).

The utilization of nitrate is based upon an active nitrate-reducing system which catalyzes the reduction of nitrate via nitrite and hydroxylamine to ammonium ions. The activation of this system is mediated by light (Huffaker et al., 1976; Tischner and Hüttermann, 1978). The enzymes involved, nitrate and nitrite reductases, are molybdoproteins. Reduced ferredoxin is probably the
electron donor in the cyanophytes (Hewitt, Notton and Garner, 1979 and the review by Hewitt and Notton, 1980). The amount of nitrogen storage products such as cyanophycin (Shively, 1974) and biliproteins (Stewart and Lex, 1970; Boussiba and Richmond, 1979) depends largely on the activity of the nitrate-reducing system. This activity, in its turn, is dependent on temperature, light intensity and nitrate concentration, the effects of which are reported in the present paper.

MATERIALS AND METHODS

Culture methods

*Spirulina platensis* (see Van Eykelenburg, 1977) was cultivated in a mineral medium (see Zarrouk, 1966; Van Eykelenburg, 1979b) using an “ecobox” as described by Van Eykelenburg (1979a). The ecobox used in the present study consisted of 10 × 10 cylindrical compartments. The incident light intensity from cool white fluorescent lamps (Philips TL-33, 40 Watt) ranged from 0.3 to 21 klux, and the culture temperature ranged from 15 to 39°C. All cultures were harvested after seven days unless otherwise indicated. Sodium nitrate (0–300 mM) was used as a source of nitrogen.

Preparation for ultra-thin sectioning

The cells were prefixed in a mixture of 2.0% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 for two hours at 20°C. After several washings in buffer, the cells were fixed for two hours at 20°C in 1% OsO₄ dissolved in the same buffer, washed again, dried in increasing concentrations of ethanol and 1,2-propylene oxide embedded in Epon resin and sectioned on an LKB-Ulrotome. Sections were stained with uranyl acetate in 50% ethanol (20 minutes) and lead citrate (5 minutes). The sections were examined using a Philips EM 201 electron microscope.

Nitrate measurements

Nitrate concentrations were measured by a nitrate ion electrode (model 92–07: ORION Res. Inc.) using a double junction reference electrode with a filling solution of 0.01 M KCl in the outer chamber.

Growth measurements

Growth was measured by determining dry weight. Each day contents of ten compartments, differing only in temperature, were harvested, washed and air-dried at 70°C for 24 hours.