Structural Aspects of the Adaptation of *Nostoc muscorum* to Salt

Eduardo Blumwald and Elisha Tel-Or

Department of Agricultural Botany, Faculty of Agriculture, The Hebrew University, P.O. Box 12, Rehovot, Israel

**Abstract.** The physiological and biochemical changes during the adaptation of *Nostoc muscorum* to salt are accompanied by specific structural changes. Cells of *Nostoc muscorum* exposed to saline medium vary in size and envelope organization. There are also drastic changes in the intracellular organization of the thylakoidal assembly. The heterocysts exhibit a preferential tolerance to NaCl rather than mannitol. These findings suggest that *Nostoc muscorum* is equipped with a specific physiological capacity for NaCl tolerance.

**Key words:** Cyanobacteria - *Nostoc muscorum* - Cell envelope - Thylakoids - Heterocysts - Salt adaptation

Cyanobacterial cell structure and composition are well characterized when grown under optimal conditions (Lang 1968; Stanier and Cohen-Bazire 1977) but most diverse when grown under extreme conditions (Whitton and Sinclair 1975). A detailed description of the cyanobacterial cell envelope was presented by Drews (1973). The cell wall is composed of a typical Gram-negative type mucopolysaccharide. There is, however, no information on the possible modification of cell wall composition and changes in cell size and organization during environmental stress. The heterocyst is composed of additional external cell wall layers, rich in carbohydrates containing specific glycolipids (Haselkorn 1978). The ultrastructure of the thylakoids in cyanobacteria, is uniform (Oelze and Drews 1972). The thylakoidal sacs are longitudinal and concentric as described for *Nostoc muscorum* (Wildon and Mercer 1963), *Tolypothrix tenius* and *Fremyella diplosiphon* (Gantt and Conti 1969) and *Anacystis nidulans* (Allen 1968). Wildon and Mercer (1963), working with *Nostoc muscorum* grown in the dark, reported an obvious modification in the thylakoidal ultrastructure. Allen (1968) found changes in *Anacystis nidulans* thylakoid organization, affected by light intensity. *Chlorogloeopsis fritschii* thylakoids long and continuous at low light intensity, were fragmented and vesiculated at high light intensity (Findley et al. 1970).

We have recently studied the physiological and biochemical aspects of cyanobacteria adaptation to salt (Tel-Or 1980a, b), and analyzed the cellular composition of the fresh-water cyanobacterium, *Nostoc muscorum*, after exposure to a saline medium (Blumwald and Tel-Or 1982). In this report we examine the dynamic structural changes, such as cell size and envelope and thylakoidal organization, accompanying modifications in cyanobacterial cell composition and physiology occurring under exposure to ionic and osmotic stress.

**Materials and Methods**

For culture, growth conditions and analytical methods see Blumwald and Tel-Or (1982), (accompanying paper).

**N₂-Fixation**

Acetylene reduction activity was measured under 10% acetylene in air, with cells suspended in growth medium in vials sealed with rubber stoppers. Samples were illuminated on a shaker at 25°C, 1 = 5 Watt/m². Ethylene formation was followed with a Gow-Mac mod. 69-100 gas chromatograph equipped with a Porapack N column.

**Scanning Electron Microscopy**

Cell samples, fixed with glutaraldehyde (2%), dehydrated with water-alcohol solutions and coated with gold, were viewed with a JEOL JSM-35C scanning electron microscope.

**Transmission Electron Microscopy**

Cells were fixed with glutaraldehyde (2%), washed and stained with osmium tetraoxide (1%) and dehydrated with water-alcohol solutions. Samples, embedded in Epon, sectioned with a diamond knife and poststained with saturated uranyl acetate solution and lead citrate solution, were viewed with a JEOL 100CX transmission electron microscope.

**Results**

**Effect of Salt on Cell Structure and Function**

*Nostoc muscorum* cells have been shown to possess the physiological potential to adapt, to a certain degree, to a saline medium (Blumwald and Tel-Or 1982). Salt tolerance during N₂-fixation shown to be the most salt sensitive activity (Tel-Or 1980a) was much higher in salt-adapted *Nostoc muscorum* cells than in the control cells (Fig. 1). Heterocyst frequency dropped immediately after exposure to salt and returned to normal after the recovery phase (Fig. 2).

As the major goal of this work was to link physiological and structural evidence for salt adaptation, we have compared the form and structure of control and salt-adapted cells. Fig. 3a shows a culture of control cells, including heterocysts, which form long filaments. The average vegetative cell is 3 μm long, and 2 μm in diameter, with a smooth surface. Only the heterocyst, which is known to contain additional carbohydrate layers, external to the mucopolysaccharide layers, is

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Fig. 1. The response to salt of N₂-fixing activity, in control and salt-adapted cells of *Nostoc muscorum*. The activity of C₂H₂ reduction was measured as described in methods. Each sample contained cells containing 5-20 µg chlorophyll. Salt-adapted cells were grown in the presence of 0.2 M NaCl for 2 weeks.

Fig. 2. Heterocyst frequency of *Nostoc muscorum* in presence and absence of 0.2 M NaCl.

Fig. 3a–c. The effect of NaCl and KCl on the structure of *Nostoc muscorum* cells. a Control cells, V vegetative cell, H heterocyst. b Cells grown in the presence of 0.2 M NaCl. c Cells grown in the presence of 0.1 M KCl. Bar = 10 µm.

Fig. 4a–c. Effect of different NaCl concentrations on the structure of *Nostoc muscorum* cells. a Control cells, V vegetative cell, H heterocyst. b Cells grown in the presence of 0.1 M NaCl. c Cells grown in the presence of 0.2 M NaCl. Bar = 1 µm.