STUDIES ON MECHANISM TO COPE WITH HARSH ENVIRONMENTS IN FILAMENTOUS CYANOBACTERIA

(Doctoral qualification by Coursework, Form 7)

Abstract of Dissertation

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Abstract :

Cyanobacteria play a key role in food webs as primary producers performing oxygenic photosynthesis. Cyanobacteria are photoautotrophic prokaryotes that form oxygen-evolving photosynthesis with water as the primary electron donor. Cyanobacteria have very simple nutrient requirements e.g., light, water, carbon dioxide, and inorganic salts that allow these organisms to occupy highly diverse ecological niches. In our ecosystem, cyanobacteria are inevitably exposed to multiple stresses, and it can be a good model organism to study the impact of environmental conditions on the physiology, metabolism, and morphology of microbial cells. To adapt the environmental stresses, microalgae and cyanobacteria can produce extracellular polysaccharides (EPSs) in their membrane lipids. Extracellular polysaccharides are molecules with a great ecological significance for the producing organisms, serving in a wide array of biological processes and increasing the organism tolerance to environmental stresses. In this study, microscopic analysis of cyanobacterium *Nostoc* sp. Strain SO-36 which found from Antarctic revealed that morphology of the cells grown at 30°C was filamentous and differentiated heterocysts, the specialized cells for fixation of gaseous nitrogen, under nitrogen deprived conditions, indicating that the strain can grow diazotrophically. The cells grown at 10°C have smaller cell size and shortened filament length with decreased chlorophyll content per cell. At 10°C, cells also got aggregated with extracellular polysaccharides (EPS), which is a common mechanism to protect cells from UV. These results imply that the segmentation to short filaments was induced by photodamage at low temperature. To understand the adaptation mechanisms for low temperature in *Nostoc* sp. strain SO-36 more in detail, the author conducted next generation sequence analyses. In most environmental stresses, signal transduction pathways are triggered by changes of a membrane physical state which can rigidify or fluidize under stress, for example changes in temperature or salinity. The membrane lipids could adapt to the stress due to the change its fatty acids composition inside the cell. Under conditions of low temperature, unsaturated fatty acids accumulated and the chain length of fatty acids in the glycerol moiety also decreased. The process that introduces a double bond

between two carbons in the fatty acid chain was namely desaturation and the enzyme that took a role is *desaturase*. In cyanobacterial membrane lipids, C18 fatty acids are the most abundant at the *sn*-1 position and C16 at the *sn*-2 position of the glycerol backbone. In model unicellular cyanobacteria, *Synechocystis* 6803, contains four acyl lipid desaturases, DesA, DesB, DesC, and DesD, which catalyze the desaturation at the Δ12, Δ15, Δ9, and Δ6 positions of C18 fatty acyl chains in membrane lipids, respectively. DesC is the initial desaturase that introduces a double bond at Δ9 positions of both C16 and C18 fatty acids. It has been reported that *Nostoc* sp strain SO-36 has two DesC enzymes (DesC1 and DesC2). The model filamentous cyanobacterium *Anabaena* sp. PCC 7120 also has two *desC* genes (*all1599* and *all4991*) whose functions are still unknown. In this study the author first expressed the *desC* genes from *Nostoc* sp. strain SO-36 and *Anabaena* sp. PCC 7120 in a model unicellular cyanobacterium *Synechocystis* sp. PCC 6803 that only have sole *desC* gene. No obvious phenotype was observed under the optimum growth condition and the fatty acid composition of transformants shows that DesC1 accumulated C18:1 (9) at *sn*-1 position in all membrane lipid and DesC2 accumulated C16:1 (9) at *sn*-1 position in DGDG and PG, and in both *sn*- position in MGDG. Next, knocked out the *Synechocystis desC* gene using the transformants described above. The transformants with *desC*1 gene was completely segregated but not with *desC*2 gene. The unsaturated fatty acids C16:1 (9) and C18:1 (9) of the mutant was lower compared to the wildtype. This result indicated that *desC2* is not enough to complement the original *desC* in *Synechocystis* sp. PCC 6803. On the other hand, *desC*1 that share more similarity in amino acid sequence with *desC* of *Synechocystis* sp. PCC 6803. Knock out mutant of *desC*1 and *desC*2 genes in *Anabaena* sp. PCC 7120 could not be isolated, suggesting that both *desC*1 and *desC*2 genes is essential in *Anabaena* sp. PCC 7120 and perhaps in filamentous cyanobacterium.