Physiological analysis of heterocyst specific glycolipid and production of its aglycone, fatty alcohol in Anabaena sp. PCC 7120

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文要旨：

Abstract：

Cyanobacteria are a large group of gram-negative prokaryotes that perform oxygenic photosynthesis. These microorganisms are widely distributed and occupy a wide range of environmental conditions because many of them also have ability to convert nitrogen molecule to available form of ammonia using nitrogenases. However, photosynthesis and nitrogen fixation are incompatible each other because nitrogenase can be inactivated by oxygen. Cyanobacteria mainly use two mechanisms to separate these activities. Some cyanobacteria strictly separate those two processes temporally by expressing their nitrogenase only in dark when the photosynthesis is inactive and the intracellular oxygen pressure is low. Instead, the others, such as filamentous cyanobacteria, use a spatial separation by making a compartment to conduct nitrogen fixation in highly special cells, called heterocysts.

Anabaena sp. PCC 7120 (hereafter referred as *Anabaena*) is a multicellular cyanobacteria and has long filament consists of hundred or more vegetative cells in the presence of combined nitrogen source in the medium. When the concentration of combined nitrogen is low in the environment, *Anabaena* the representative filamentous heterocystous cyanobacterium, develops heterocyst cells to separate oxygen-labile nitrogen fixation from oxygen-evolving photosynthesis in vegetative cells. These cells are surrounded by glycolipid layer (heterocyst specific glycolipid, Hgl) to protect nitrogenase from oxygen diffusion

from outside of the cells. Heterocyst differentiation requires a number of genes involved in the different step of the developmental process. There are three group of genes cluster that involved in heterocyst differentiation: (1) Sensory genes which perceive the signal of nitrogen starvation in the environment; (2) genes that are responsible for initiating the heterocyst differentiation; and (3) group of genes which play role in heterocyst morphogenesis and function. Among of these genes, *hglT*, encodes the heterocyst glycolipid synthase and involved in the maturation of heterocyst. Heterocyst glycolipid synthase, HglT, catalyzes the final step of the Hgls synthesis, a glucose transfer reaction to the aglycone (fatty alcohol).

To clarify the physiological function of HglT protein under nitrogen-replete and –depleted conditions, the author isolated the complete knock out mutants of *hglT* gene. The *hglT* mutants grew comparable to that of the wild type under nitrogen-replete conditions, indicating that *hglT* gene is not an essential gene at least in the nitrogen-replete conditions. *hglT* mutants formed morphologically indistinguishable heterocyst under nitrogen starved conditions. Alcian blue staining of heterocyst cells from both the mutants and wild type indicated that the cells were surrounded by heterocyst polysaccharide layer (Hep). The mutants, however lacked detectable amount of Hgls, and accumulated fatty alcohol (aglycone). Nitrogenase activity of the mutants reached to the maximum level but one fourth of those of the wild type 48 hours after nitrogen step-down. Protein accumulation and gene expression of a subunit of nitrogenase in the mutant was similar level to the wild type, indicating that the retarded nitrogenase activity is due to the failure of heterocyst to maintain the micro-oxic environment. These results suggest that the mutants can fix nitrogen without Hgls in the presence of oxygen and thus, fatty alcohol can complement the function of Hgls, not perfectly but sufficiently in the heterocyt cells.

To increase the amount of fatty alcohol accumulated in the *hglT* mutant, the author then combined mutation of *hglT* with *patS* and *hetN*. It is known that PatS and HetN are involved in the heterocyst development and maintain the pattern of heterocyst formation. Both of genes are necessary to prevent heterocyst differentiation, and knock out mutants of these two genes are known to form multiple heterocyst under nitrogen starvation. Similar to the *hglT* single mutant, the triple mutants differentiated heterocyst cells under nitrogen starved conditions. As expected the multiple heterocysts and high frequency of heterocysts formation were observed in the triple mutant compared to the *hglT* mutant and wild type.

Interestingly, the triple mutant showed slight increase of growth and total chlorophyll content under nitrogen starved conditions compared with the *hglT* mutant. A plausible interpretation would be that the increased heterocyst frequency in the triple mutant might promote the recovery of chlorophyll biosynthesis and improve the energy flowing efficiency from phycocyanin to chlorophyll a. In addition, higher amount, almost two fold, of fatty alcohol was accumulated in the triple mutant. These fatty alcohols are probably substituting the function of Hgls as has been characterized in the *hglT* mutant. The increase of heterocyst frequency of the triple mutant, reached to three times higher compare with *hglT* mutants, resulted in accumulation of fatty alcohols. In addition, aeration to the culture increased the accumulation of Hgl in the wild type and fatty alcohol production in the single and triple mutant of *Anabaena*. These results suggest that filamentous cyanobacteria can be a good tool to provide fatty alcohol.