Involvement of *slr0081*, a Two-Component Signal-Transduction System Response Regulator, in Acid Stress Tolerance in *Synechocystis* sp. PCC 6803

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Abstract: Two-component signal transduction is the primary signaling mechanism used for global regulation of cell response to changes in the environment. DNA microarray analysis identified genes up-regulated by acid stress in cyanobacteria *Synechocystis* sp. PCC 6803. Several of these altered genes are thought to be response regulators that are directly involved in this type of stress. Deletion mutants of response regulator genes were constructed and survivability was compared between the cells transfected with mutant and wild-type genes in a low-pH medium. Among these, deletion of *slr0081* affected the growth rate under conditions of acid stress (pH 6.0). We examined the genome-wide expression of genes in Δ*slr0081* mutant cells by using DNA microarray in an attempt to determine whether *slr0081* is involved in the regulation of other acid stress responsive genes. Our findings by quantitative real-time RT PCR revealed that down regulation of acid responsive genes *slr0967* and *sll0939* occurs by deletion of *slr0081.*

Keywords: Low-pH; Cyanobacteria; Stress response; Two-component system

Introduction

Plants were exposed to environmental stress such as temperature, light, salts concentration, heavy metal density and more because those have superior acclimation ability to environmental change. Recently, the acid rain is most serious environmental stress. It causes acidification of lakes and streams and contributes to damage of plants, algae, and cyanobacteria in many parts of the world. Rhizotoxicity in acid soil, which involves the action of Al^{3+} has been well investigated (Jones *et al.*, 1995). Nevertheless, little has been done to elucidate the basic set of adaptations necessary for acid tolerance in plants, algae, or cyanobacteria.

Several species of cyanobacteria serve as model organisms for elucidating both functional and regulatory aspects of photosynthesis. Above all, *Synechocystis* sp. PCC 6803 was the first photosynthetic organism for which a complete genome sequence became available (Kaneko *et al*., 1996), and DNA microarrays have been used to examine gene expression in response to various kinds of stress such as redox, oxidative, osmotic, salinity, and high light stress(Kanesaki *et al*., 2002; Hihara *et al*., 2001).

DNA microarray analysis of *Synechocystis* sp. PCC 6803 cells revealed that acid stress induced the expression of putative stress-related proteins, such as chaperones, regulatory factor, and function unknown protein (Ohta *et al.*, 2005). Gene of slr0967 and *sll0939* continuously increase 7 and 16-fold after 4 h of acid stress (Ohta *et al.*, 2005).

In this study, based on DNA microarray analysis in acid stress condition, we constructed deletion mutant cells. In acid stress condition, deletion mutants of *slr0081*, which encoded response regulator involved in phosphate limitation (Suzuki *et al.*, 2004), were weaker than wild-type cells. From analysis of quantitive real-time RT PCR, we revealed that

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Slr0081 up-regulated acid responsive genes (*slr0967* and *sll0939*). Our observations suggest that Slr0081 plays an important role for *Synechocystis* to survive in acid condition.

Material and Methods

Strain and culture conditions of Cyanobacteria

Wild-type strain of *Synechocystis* sp. PCC6803 and *slr0081*-disrupted mutants, created by inserting the chlo-ramphenicol-resistance cassette, were grown at 30 °C in BG-11 medium (Stanier *et al.*, 1971) with 5 mmol TES-NaOH (pH 8.0) under continuous illumination by fluorescent lamps. Cells growing in the exponential phase were subjected to acid stress by centrifuging the cell cultures and resuspending the cell pellets in a pH-adjusted BG-11 medium. BG-11 medium was acidified using MES (pH 6.0) buffer instead of TES (pH 8.0) buffer. Cultures were streaked onto pH-adjusted BG-11 plates and cultured for 5 days. Experiments were performed in duplicates at least 3 times.

Generation of insertion mutants

Mutants with impaired expression of selected genes were generated by reverse genetic techniques. The coding and neighboring sequences as well as the chloramphenicol cassette gene were amplified by PCR. These PCR products were then cloned into pUC19 (Toyobo, Osaka, Japan). The primers for amplification were designed using the complete genome sequence of *Synechocystis* (Kaneko *et al.*, 1996), and sequences that contained appropriate restriction sites were selected to improve cloning efficiency of the fragments. Transformants were initially selected on a medium containing 10 μg CM mL^{-1} (Wako Pure Chemical, Osaka, Japan), whereas the clones were segregated by restreaking (at least 3 transfers) of primary clones on plates supplemented with 50 μg Cm mL^{-1}. During the culture of mutants, Cm was added to the liquid media.

RNA isolation and quantitative real-time RT PCR

Total RNA was isolated from *Synechocystis* cells using the RNeasy Midi kit (Qiagen) as described by Hihara *et al.* (2001). The extracted RNA was reverse-transcribed using PrimeScript^{tm} RT regent kit (Takara Bio). Real-time PCR with SYBR Green I was performed using SYBR Premix EX Taq (Perfect Real Time) (TAKARA). Each real time-PCR was performed in triplicate with *rnpB* as the internal standard.

Results and Discussion

Characterization of the slr0081 deletion mutant in acid condition

On the basis of the results of DNA microarray analysis in acid stress conditions, we constructed deletion mutant cells. Wild-type *Synechocystis* sp. PCC6803 was transformed with *slr0081* that had been interrupted with a cassette conferring resistance to chloramphenicol. Slr0081 is a reported response regulator of a two-component system that regulates the expression *phoA* gene for alkaline phosphatase under phosphate-limiting condition in *Synechocystis* (Suzuki *et al*., 2004). In normal BG-11 medium at pH 8.0, the mutant cells exhibited a similar photoautotrophic doubling time (Fig. 1), suggesting that the deletion did not affect growth in normal conditions. However, under acid stress conditions, deletion mutants of *slr0081* were weaker than the wild-type cells (Fig. 2). In addition, a growth curve showed that the growth of Δ*slr0081* mutant cells in acid stress conditions (pH 6.0) was significantly inhibited compared with that of wild-type cells (Fig. 1). These results suggest that Slr0081 plays an important role in survival of *Synechocystis* under conditions of acid stress. Slr0081 is reported to be a response regulator involved in phosphate limitation, thus *Synechocystis* in acid stress may cause phosphate limitation.

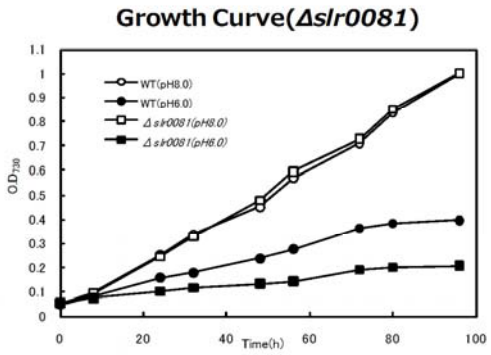

Fig. 1 Growth curves of wild type and *slr0081* deletion mutants at pH 8.0 and pH 6.0.
Cell density was measured at OD_{730}. The pH of the BG-11 medium was adjusted using 5 mmol TES-NaOH (pH 8.0) and the acid-culture used MES-NaOH (pH 6.0). This experiment was repeated 3 times.

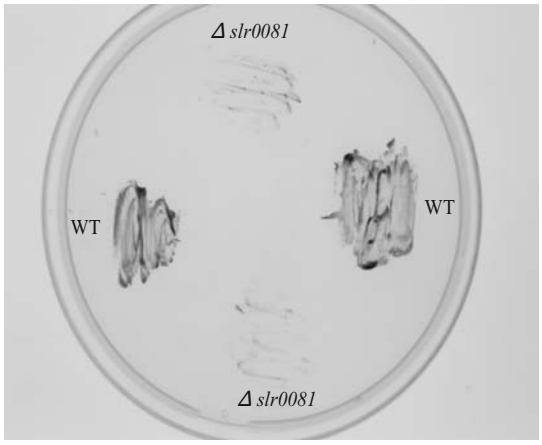

Fig. 2 Effect of WT and *slr0081* deletion on growth at pH 6.0. Wild-type and *Δslr0081* were streaked onto BG-11 plates containing MES-NaOH at pH 6.0 and cultured for 5 days. This experiment was repeated 3 times.

Quantitative real-time RT PCR analysis of slr0967 and sll0939 in slr0081 deletion mutants

To examine the effect of a deletion mutation of *slr0081* on acid tolerance of *Synechocystis* cells, we performed DNA microarray analysis of *slr0081* deletion mutant cells under acid stress conditions by culturing the mutant cells at a pH 3.0 for 30 min (data not shown). The expressions of *slr0967* and *sll0939* were induced by acid stress in wild-type, but not in *slr0081*-deleted cells. These results suggest that Slr0081 regulates transcription of *slr0967* and *sll0939*. To elucidate the relationship between *slr0081* and these genes, we performed quantitative real-time RT PCR analysis of the expression of *slr0967* and *sll0939* in *slr0081*-deleted mutants (Table 1). In the wild-type cells, the transcription level of *slr0967* and *sll0939* increased 7.78- and 33.15-fold, respectively, after acid stress treatment. In contrast, in *slr0081*-deleted cells, the expressions of these genes did not increase (1.21 and 0.64-fold, respectively) after acid stress treatment. These results indicate that Slr0081 up-regulated *slr0967* and *sll0939* genes.

Table 1 Expression levels of *slr0967* and *sll0939* after acid treatment (pH 3.0) in wild-type and *Δslr0081*, determined by *Quantitative real-time RT PCR*.

sample	*sll0939*	*slr0967*
WT(cont)	1	1
WT(acid)	7.78	33.15
Δslr0081(acid)	1.21	0.64

Wild-type and *slr0081* deletion mutants were incubated in 50 mmol Gly-HCl (pH 3.0) for 0.5 h.

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