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Analysis of a cyanobacterium *Synechocystis* sp. PCC 6803 genome by application of chlorophyll fluorescence video-imaging system

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Introduction

Since whole genomic sequence of a cyanobacterium, *Synechocystis* sp. PCC 6803, was determined in 1996 (Kaneko et al.), the function or interaction of genes has been targeted for elucidation. Though the expression of whole genes can be simultaneously analyzed by means of DNA microarrays, the phenotype of each gene-disruptant must have been analyzed one by one. For the screening of photosynthesis-related genes, 2D monitoring of chlorophyll fluorescence has been successfully applied (e.g. Li et al. 2000). Fluorescence of photosynthetic pigments is a good index of the condition of photosynthetic machinery, and was widely used for the physiological study (for a review, see Maxwell et al. 2000). In cyanobacteria, unlike higher plants, photosynthetic and other metabolic pathways are not separated in organelles, and seem to have interaction with each other through redox changes. Therefore, monitoring the redox state of photosynthetic electron transport chain in cyanobacteria has potentiality to detect the phenotype of the disruptants of various genes even if they are not directly involved in photosynthesis. Combined with two-dimensional fluorescence video-imaging system, chlorophyll fluorescence emitted from multiple cyanobacterial colonies can be simultaneously determined. Here, we show that this system can be applied for analysis of various gene disruptants. It seems to be possible to classify the mutants according to the function of the disrupted genes.

Materials and methods

Wild type (WT) and gene disruptants of *Synechocystis* sp. PCC 6803 were grown at 30 ^{o}C. Cells were once pre-cultured under 60 μE m^{-2} s^{-1} and then replaced on fresh BG-11 agar plate and cultured under 200 μE m^{-2} s^{-1}. In case of the mutants by transposon-mediated random insertion, the pre-culture was carried out in liquid BG-11. The culture with adjusted OD_{730} was dropped on BG-11 agar plate. After 2-6 days, time course of chlorophyll fluorescence emission was monitored by fluorescence video-imaging system (FluorCam, Photon Systems Instruments, Czech Republic). Cells on plates were dark adapted for 5-15 minutes, and actinic light was

applied for 3-6 seconds to monitor the fluorescence. Intensity of the fluorescence was normalized with initial value at the start of actinic light (Fo). For preparation of targeted gene-disruptants, two types of strains (PCC and GT) were used as genetic background. GT strain was used for the mutants made by transposon-mediated random insertion.

Table1 Gene disruptants used in this study and their phenotype (chlorophyll fluorescence kinetics)

	Gene name	Gene function or gene production	Background	*Phenotype
sll1968	*pmgA*	modulation factor of PSII/PSI ratio	GT	△
slr0574	*P450*	cytochrome P450	GT	-
slr1139	*trxA2*	thioredoxin	GT	-
sll1057	*trxM2*	thioredoxin M	GT	-
slr0233	*trxM1*	thioredoxin M	GT	△
slr1562	*grxC1*	glutaredoxin	GT	△
sll1732	*ndhF3*	NADH dehydrogenase subunit 5	GT	△
sll1349	*cbbZp*	phosphoglycolate phosphatase	GT	△
sll0223	*ndhB*	NADH dehydrogenase subunit 2	GT	○
slr0051	*icfA*	carbonic anhydrase	GT	△
slr1655	*psaL*	photosystemI subunit 6	GT	△
ssr2831	*psaE*	photosystemI subunit 4	GT	△
sll0374	*braE*	a.a. transport ATP binding protein	GT	△
sll0040-1		nitrate transport 45kDaprotein	GT	△
slr0530-1		membrane bound sugar transport protein	GT	△
slr1890	*bfr*	bacterioferritin	GT	△
sll1341	*bfr*	bacterioferritin	GT	-
sll0223	*ndhB*	NADH dehydrogenase subunit 2	PCC	-
sll1987	*katG*	catalase	PCC	-
ssl0707	*glnB*	nitrogen regulatory protein P-II	PCC	-
slr2102	*ftsY*	cell division protein FtsY	PCC	-
ssl0452-3	*nbl*	phycobilisome degradation protein NblA	PCC	△
slr1843	*zwf*	Glucose 6-P dehydrogenae	PCC	○
sll1479	*dev*	Glucose 6-P dehydrogenase	PCC	○
sll1988	*kmpkatG*	catalase	PCC	△
sll1732	*ndhF3*	NADH dehydrogenase subunit 5	PCC	○
sll1387	*pppA*	serine/threonine phosphatase	PCC	-
sml0001	*psbI*	PhotosystemII PsbI protain	PCC	△

*Category of “Phenotype” shows whether the difference from background strain in chlorophyll fluorescence kinetics was observed. ○: Difference from WT was confirmed, △: Difference might exist, but has not been confirmed, -: Difference was not detected.

Results and discussion

Monitoring chlorophyll fluorescence kinetics emitted from targeted gene-disruptants

First, we monitored chlorophyll fluorescence of 28 gene disruptants as described in materials and methods. Different chlorophyll fluorescence kinetics from that of WT were observed among many gene disruptants. Among them, 4 disruptants showed reproducible difference (Table 1). As shown in Figure 1A, Δ*zwf*, Δ*devB* and Δ*ndhF3* strains in PCC background showed delayed appearance of primary peak compared with WT. Both *devB* and *zwf* encode glucose 6-phosphate dehydrogenase in pentose phosphate pathway. The result suggests that same type of chlorophyll fluorescence alteration is detected when genes having similar function are disrupted. Δ*ndhB* in GT background showed earlier appearance of primary peak (Figure 1B). *ndhB* and *ndhF3* are both involved in respiration, but their function seems to be different from one another. *ndhF3* were reported specifically in the CO_2 concentrating mechanism in

cyanobacteria (Ohkawa et al. 2000). We can therefore conclude that the 2D-imaging system of chlorophyll fluorescence employed here enable us to analyze the phenotype of non-photosynthetic genes. Δ*ndhF3* did not show detectable difference from WT when background was GT. GT strain itself showed delayed peak appearance compared with PCC strain, and slight delay of peak appearance was almost indistinguishable when background was GT. The change in peak position was not observed in Δ*ndhB* in PCC background. This may be due to the incomplete segregation in this mutant. In the mutants tested here, only the alteration of primary peak position was observed. To test whether various mutants truly can be classified into multiple categories, we carried out the following experiments.

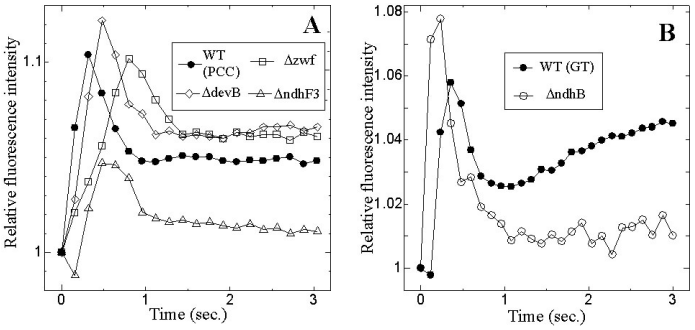

Fig.1 Chlorophyll fluorescence kinetics of gene disruptants. A: Δzwf, ΔdevB and ΔndhF3 showed the delayed peak relative to WT, B: ΔndhB (background strain is GT) showed earlier peak appearance than WT. Cells were cultured under 200 $\mu Em^{-2}s^{-1}$ for 2 days after pre-culture.

Chlorophyll fluorescence kinetics of mutants prepared by transposon-mediated random insertion

To analyze more gene disruptants, we monitored the chlorophyll fluorescence kinetics of 134 mutants prepared by transposon-mediated random insertion. Genomic DNA fragments used for transposon-mediated random insertion and subsequent genetic recombination are shown in Table 2. The 134 mutants include a maximum of 72 disrupted ORFs. WT and these mutant strains were cultured and their chlorophyll fluorescence kinetics was monitored as described in materials and methods. As a result, 9 out of 134 mutants showed different fluorescence kinetics from that of WT, and they were classified into 5 types (Fig. 2, category I~V). The features observed in each category are as follows. I: primary peak is apparently lower than that of WT. Category I includes 2 mutants (1418-5, 1418-25). II: primary peak is extremely lower and decrease of fluorescence intensity from peak is smaller than that of WT. Category II includes one mutant (1418-32). III: peak appearance is slightly earlier and fluorescence decay is slower than that of WT. Category III includes 3 mutants (1111-28, 1111-40, 1111-50). IV: primary peak is slightly higher than that of WT. Category IV includes only 1111-41. V: primary peak is slightly lower than that of WT. Category V includes only 1111-54. (Code number of the mutants described here shows the DNA fragment for preparation in the former quadruple digits, and colony number in the last two digits.) These results indicate that among 72 gene disruptions, at least 5 categories of the chlorophyll fluorescence kinetics exist, although 134 mutants do not necessarily cover all of the 72 gene disruptions. Here, we demonstrate that

Table2 Partial genomic DNA fragments used to prepare mutants by transposon-mediated random insertion. Antibiotics markers were randomly inserted into these cosmid fragments by in vitro transposition and cyanobacterial mutants were prepared by genetic transformation.

Name of genomic DNA fragment	cs1418	cs1111
Length of fragment (base pairs)	34870	40119
ORFs included in the fragment	36	36
Number of mutants prepared from the fragment	85	49
Number of mutants showed difference from WT	3	6

classification of various mutants into multiple categories according to their fluorescence kinetics is possible by the 2-D imaging of chlorophyll fluorescence.

Taking into account the results in the experiments with targeted gene disruptants, these categories might reflect the function of disrupted genes. Using this system, it becomes possible to infer the function of novel genes from the information about known genes classified into the same category as the novel ones.

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References

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) *DNA Research* **3,** 109-136

Li XP, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S, Niyogi KK (2000) *Nature* **403,** 391-395

Ohkawa H, Price GD, Badger MR, Ogawa T (2000) *Journal of Bacteriology* **182,** 2591-2596