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Isolation of high-CO_2 requiring mutants through the systematic gene disruption system in a cyanobacterium, *Synechocystis* sp. PCC6803

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Introduction

Aquatic photosynthetic organisms, such as a cyanobacterium, *Synechocystis* sp. PCC6803, induce a set of genes for a carbon concentrating mechanism (CCM) to acclimate to CO_2-limiting stress (Price 1998, Kaplan 1999). This mechanism elevates the CO_2 concentration around the active site of ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco) and thereby enables efficient CO_2 fixation despite the low affinity and selectivity of their Rubisco for CO_2. Analyses of high-CO_2 requiring mutants and low-CO_2 inducible genes have shown that several genes such as *icfA* coding for carbonic anhydrase (Fukuzawa 1992), *ccmKLM* for carboxysome components (Price 1993), *ictB* for putative regulator (Bonfil 1998), *cmpABCD* for ABC-type bicarbonate transporter (Omata 1999) and *ndhD* for NADH dehydrogenase subunit (Ohkawa 2000), play roles in the CCM. Nevertheless no high-CO_2 requiring mutants defective in essential Ci transporters have been identified to date. Taken with the fact that the profound effect of the *ictB* mutation on HCO_3^--transporting activity is incompatible with the presumed occurrence of multiple HCO_3^- transporters and that the disruptant of *cmpABCD* did not show high-CO_2 requiring phenotype, it is assumed that other transporters are operating in the Ci uptake processes.

To identify Ci-transporters and components of CO_2-signalling, we have isolated high-CO_2 requiring mutants through a systematic gene disruption system in cyanobacterium, *Synechocystis* sp. PCC6803.

Materials and methods

Strains and culture conditions.

Wild type and mutant cells of *Synechocystis* sp. PCC6803 were grown at 28˚C in BG-11 medium supplemented with Hepes-NaOH pH 7.8 (Stanier 1971) under continuous illumination with white fluorescent lamps.

Construction of the W6-lux strain.

In the *cmp* operon, *cmpA* (slr0040) encoding a component of the low-CO_2 inducible bicarbonate-transporter was replaced with a *lux* gene encoding luciferace to generate a mutant strain. This *cmpA*-disruptantion mutant, designated W6-lux, was used as a host for isolation of high-CO_2 requiring mutants after gene-tagging mutagenesis.

Random mutagenesis using transposon.

Systematic gene disruption was performed as described (Fukuzawa submitted). A transposon containing a gene encoding chloramphenicol acetyltransferase conferring chloramphenicol resistance (Cm^R) was randomly inserted into the 106 different cosmids DNA covering 90% of total genome of *Synechocystis* sp. PCC6803 (Kaneko 1996) by using a Genomic Priming System (New England Biolabs. Inc.). The W6-lux strain was transformed with transposon inactivation library and Cm^R mutants unable to grow in air containing 0.01% CO_2 were selected on BG-11 agar plates.

Determination of the insertional sites.

Total genomic DNA samples isolated from high-CO_2 reqiring mutants were digested with *Hha*I which does not cut the transposon internally. After self ligation of the DNA, the generated samples were used as templates for inverse PCR with the primers complementary to N- and C- terminal regions of the Cm^R cassette. The exact position of the Cm^R cassette in the mutant genome was determined by sequencing the PCR product.

Results and Discussion

By means of *in vitro* transposon tagging mutagenesis, DNA pools of insertionally inactivated cosmid DNAs were transformed into the host strain, W6-lux, in which a *cmpA* gene was substituted by a *lux* gene encoding luciferase in *Synechocystis* sp. PCC6803. Randomly selected 20,352 Cm^R transformants were screened for high-CO_2 requirement in the liquid culture medium under 0.01% CO_2 conditions. Then 10 mutants were confirmed to be high-CO_2 requiring as shown in Fig. 1. While the photosyntheticaly wild type strain, W6-lux, grew under both 3% CO_2 and 0.01% CO_2 conditions, growth of these mutants were highly dependent on the supplied CO_2 level.

Genomic DNA isolated from each mutants was used as a template for inverse PCR. Different length of PCR products were obtained (Fig. 2), suggesting that the insertion of the transposons inserted at different loci in respective mutants. Since PCR products

except for those derived from M2 and M4 strains showed only single major bands, the single insertion events of the chloramphenicol cassette have occurred in eight mutants. On the other hand, two strains, M2 and M4, seem to have two insertion events of the transposons on their genomes. Further analyses of the mutants would reveal which coding regions are inactivated by the transposons.

Determination of the exact position of the insertion sites in the mutants by sequencing indicated that the known CCM-related genes described above were not disrupted (data not shown). It is likely that these genes, which are inserted by transposons, play roles for the CCM function. Further work is required for elucidation of the biochemical function of the products of those genes.

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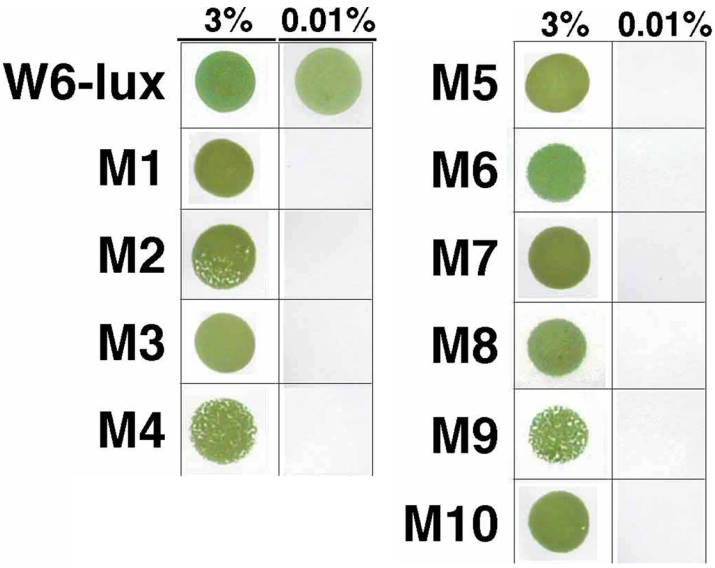

Fig. 1. Screening of high-CO_2 requiring mutants on agar plates. The W6-lux strain and newly isolated mutants were cultured until OD_{730} values of 1.0. These cells were diluted to the OD_{730} values of 0.3, and 10 microliter droplets of the cell suspensions, were spotted on BG-11 agar plates. The cells were incubated under 3% CO_2 conditions or under 0.01% CO_2 conditions for 5 days at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$.

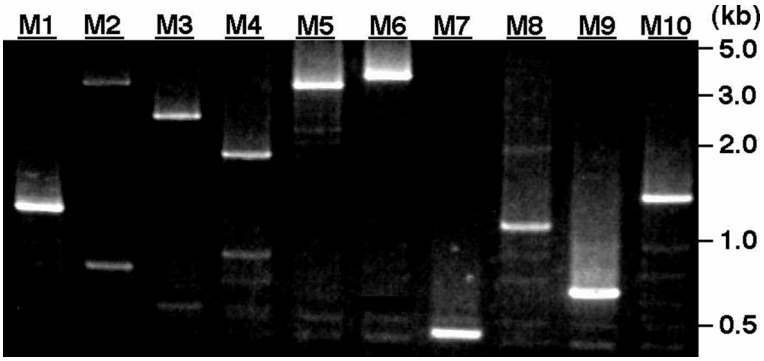

Fig. 2. Agarose electrophoresis analyses of the PCR products. Genomic DNA isolated from each mutant was digested with *Hha*I, self-ligated, and then used as a template for inverse PCR with the primers complementary to the N- and C- terminal region of the Cm^R cassette.