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Title	Heterologous complementation systems verify the mosaic distribution of three distinct protoporphyrinogen IX oxidase in the cyanobacterial phylum
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Title:

Heterologous complementation systems verify the mosaic distribution of three distinct protoporphyrinogen IX oxidase in the cyanobacterium phylum.

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Corresponding author: Ryouichi Tanaka Institute of Low Temperature Science, Hokkaido University Kita-ku, N19W8, Sapporo 060-0819, JAPAN rtanaka@lowtem.hokudai.ac.jp a Expression cassette for a PPOX homologue



b hemJ knock-out cassette



C Verification of *psbA2*-*Gv_hemY/∆hemJ* transformant





Fig. S1. Disruption of the *hemJ* genein the *psbA2-Gv_hemY/\DemJ* and *psbA2-Te_hemY/\DemJ* transformants. First, a cassette comprising the *psbA2* promoter, a PPOX homologue, the kanamycin resistance gene and the *psbA2* terminator (a) were introduced into the Synechocystis genome. Subsequently, the endogenous *HemJ* gene of these transformants were disrupted by inserting the chloramphenicol marker using the cassette shown in (b). Approximate positions of the primers used in panels (c) and (d) are shown with arrows in (b), which span the disrupted region of the *hemJ* sequence. Verification of segregation of the disrupted *hemJ* gene was assessed in the *psbA2-Gv_hemY/\DemJ* transformant (c) or the *psbA2-Te_hemY/\DhemJ* transformant (d) by PCR using the primer set shown in B. The primer set gave a 250-bp product which amplified a part of the intact *hemJ* gene (lane WT of panels c and d). In contrast, the same primer set gave a 1.6-kbp product when the chloramphenicol marker was inserted into the *hemJ* locus (lane T of panels c and d). M: 1-kb DNA ladder from Watson BRG, WT: wild-type Synechocystis cells. N: no template control, T: *psbA2-Gv_hemY/\DhemJ* transformant cells (d) or *psbA2-Te_hemY/\DhemJ* transformant cells (d). It should be noted that a single gel image was cropped to show only relevant lanes in panels c and d. The original images are shown at the right side of cropped images to indicate no signs of inapprpriate image manipulations.



Fig. S2. Verification of the *hemJ* replacement with the cassette of PPOX homologue and the kanamycin (Kn) resistance marker. (a) Verification of the replacement of HemJ with Gv hemG. M: Marker (lambda DNA/Styl), W: wild type DNA, P: Gv hemG-Km plasmid (positive control), N: No template (negative control), T: Synechocystis Kn resistant transformant. The original picture which is shown on the right side contained extra lanes which are not relevant to the analysis mentioned here. Therefore, only relevant lanes are shown in this panel. (b) Verification of the replacement of hemJ with Pm hemG. M: Marker (lambda DNA/Styl), W: wild type DNA, P: *Pm hemG-Km* plasmid (positive control), N: No template (negative control), T: Synechocystis Kn resistant transformant.

а



Fig. S3. Effects of acifluorfen on the growth of psbA2- $Gv_hemY/\Delta hemJ$. The cultures of WT and psbA2- $Gv_hemY/\Delta hemJ$ cells were divided into four groups and diluted to $OD_{750} = 0.1$. Acifluorfen dissolved in dimethylformamide was added to WT and psbA2- $Gv_hemY/\Delta hemJ$ at the final concentration of 1 mM, which groups were denoted as WT+A and Gv_hemY+A , respectively. As a control, dimethylformamide was added to the other groups, which were denoted as WT and Gv_hemY , respectively. n =3. Error bars indicate standard deviation.