Electronic supplementary materials

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

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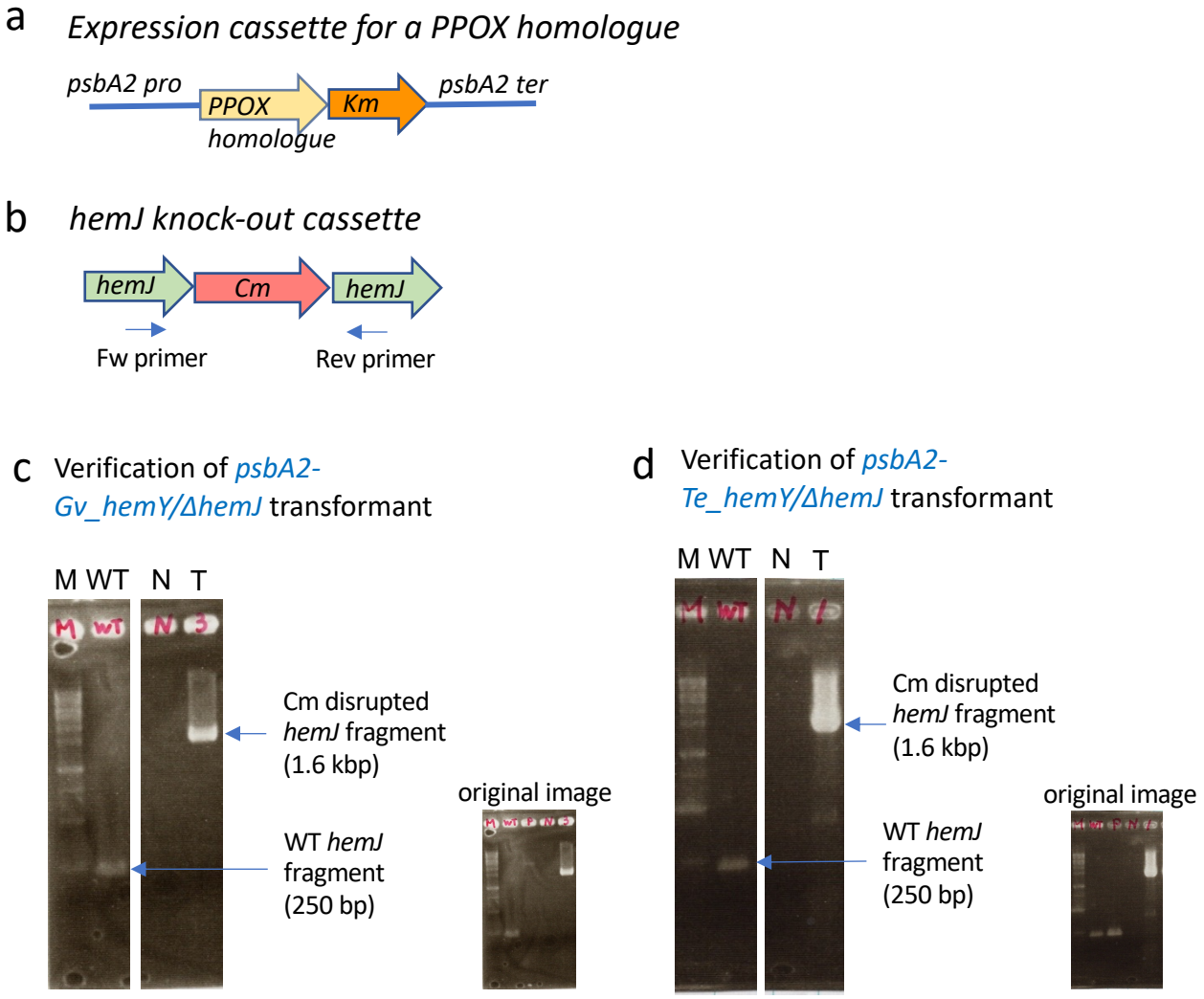

Fig. S1. Disruption of the *hemJ* genein the *psbA2-Gv_hemY/ΔhemJ* and *psbA2-Te_hemY/ΔhemJ* transformants. First, a cassette comprising the *psbA2* promoter, a PPOX homologue, the kanamycin resistance gene and the *psbA2* terminator (a) were introduced into the Synechocystis genome. Subsequently, the 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/ΔhemJ* transformant (c) or the *psbA2-Te_hemY/ΔhemJ* 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/ΔhemJ* transformant cells (d) or *psbA2-Te_hemY/ΔhemJ* 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 inapprppriate image manipulations.

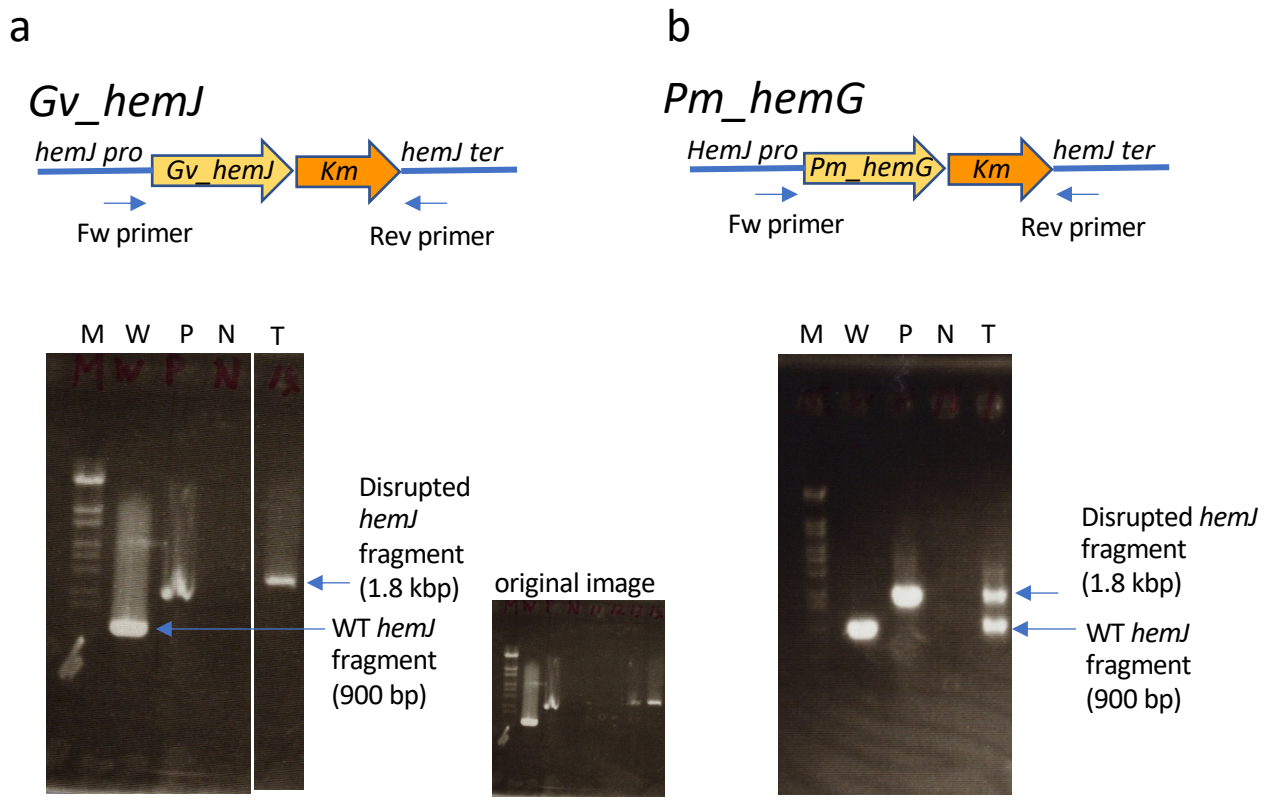

Fig. S2. Verification of the *hemJ* replacement with the cassette of PPOX homologue and the kanamycin (Kn) resistance marker. (a) Verification of the replacement of *HemJ* with *Gv_hemG*. M: Marker (lambda DNA/StyI), W: wild type DNA, P: *Gv_hemG-Km* plasmid (positive control), N: No template (negative 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/StyI), W: wild type DNA, P: *Pm_hemG-Km* plasmid (positive control), N: No template (negative control), T: Synechocystis Kn resistant transformant.

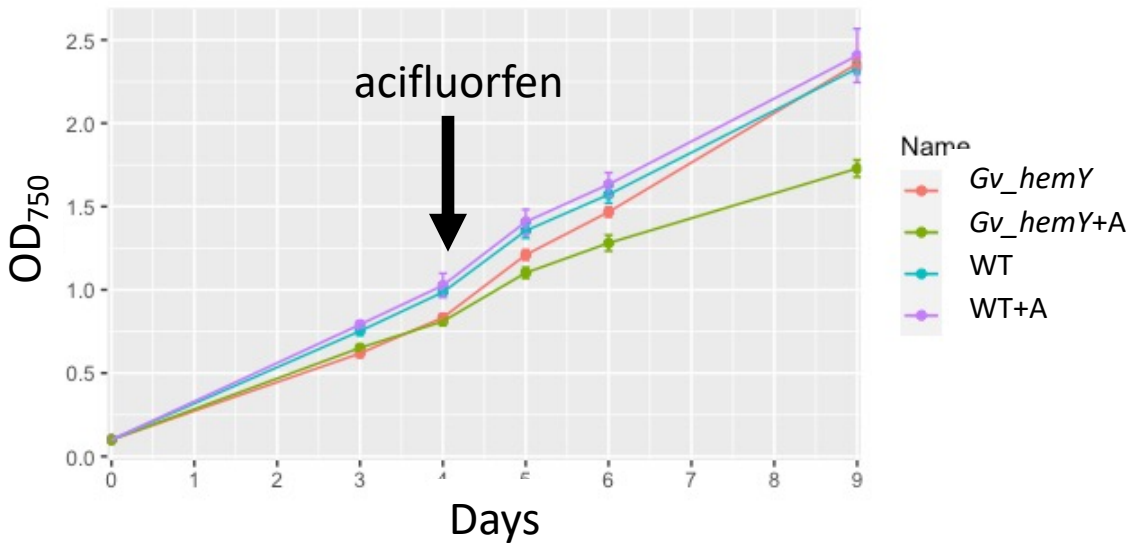

Fig. S3. Effects of acifluorfen on the growth of *psbA2-Gv_hemY/ΔhemJ.*
The cultures of WT and *psbA2-Gv_hemY/ΔhemJ* cells were divided into four groups and diluted to OD_{750} = 0.1. Acifluorfen dissolved in dimethylformamide was added to WT and *psbA2-Gv_hemY/ΔhemJ* at the final concentration of 1 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.