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A cotransformation system of the unicellular red alga *Cyanidioschyzon merolae* with blasticidin S deaminase and chloramphenicol acetyltransferase selectable markers

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Abstract

Background: The unicellular red alga *Cyanidioschyzon merolae* exhibits a very simple cellular and genomic architecture. In addition, procedures for genetic modifications, such as gene targeting by homologous recombination and inducible/repressible gene expression, have been developed. However, only two markers for selecting transformants, uracil synthase (*URA*) and chloramphenicol acetyltransferase (*CAT*), are available in this alga. Therefore, manipulation of two or more different chromosomal loci in the same strain in *C. merolae* is limited.

Results: This study developed a nuclear targeting and transformant selection system using an antibiotics blasticidin S (BS) and the BS deaminase (*BSD*) selectable marker by homologous recombination in *C. merolae*. In addition, this study has succeeded in simultaneously modifying two different chromosomal loci by a single-step cotransformation based on the combination of *BSD* and *CAT* selectable markers. A *C. merolae* strain that expresses mitochondrion-targeted *mSCARLET* (with the *BSD* marker) and *mVENUS* (with the *CAT* marker) from different chromosomal loci was generated with this procedure.

Conclusions: The newly developed *BSD* selectable marker enables an additional genetic modification to the already generated *C. merolae* transformants based on the *URA* or *CAT* system. Furthermore, the cotransformation system facilitates multiple genetic modifications. These methods and the simple nature of the *C. merolae* cellular and genomic architecture will facilitate studies on several phenomena common to photosynthetic eukaryotes.

Keywords: Cotransformation, *Cyanidioschyzon merolae*, Genetic modification, Photosynthetic eukaryote, Unicellular red alga

Background

The unicellular red alga *Cyanidioschyzon merolae* is an emerging model organism for studies on phenomena common to photosynthetic eukaryotes [1, 2]. The *C. merolae* nuclear and organellar genomes have been fully sequenced [3, 4]. The nuclear genome composition is very simple with very little genetic redundancy (16.5 Mbp; 4775 protein-coding genes). The cellular

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organization is also very simple; it contains a single nucleus, mitochondrion, chloroplast, and a minimal number of other membranous organelles [1, 2]. These organelles divide in a cell cycle-dependent manner, and cell cycle progression can be highly synchronized to a 12 h light/12 h dark cycle [1, 2, 5]. In addition, a genetic modification procedure via homologous recombination has been established. Based on this procedure, various genetic techniques, such as gene knockout [6], gene knock-in [7], stable expression of a transgene without any silencing activity [8], and inducible/repressible expression of an endogenous gene or transgene [9–11], have been developed. The combination of these genomic and cytological features and molecular genetic techniques in *C. merolae* has facilitated analyses on a variety of cellular phenomena in photosynthetic eukaryotes, such as cell cycle progression [7, 12], organelle division and inheritance [13–18], circadian rhythms [19], metabolism [20–22], photosynthetic apparatus [23–26], splicing [27], and epigenetics [28].

To manipulate the *C. merolae* nuclear genome, two kinds of selectable markers, uracil synthase (*URA*) gene [6, 8] and chloramphenicol (CP) acetyltransferase (*CAT*) gene [29, 30], have been developed. When multiple modifications on different chromosomal loci are required in a single strain, they can be achieved by a two-step transformation with *URA* and *CAT* markers [30] or by a marker recycling system, in which the *URA* marker is designed to be eliminated after the selection of transformants via intrachromosomal homologous recombination [31]. However, these sequential genetic modification methods are practically time-consuming (it takes ≥ 4 weeks for one round of genetic modification from transformation to obtain a liquid culture of a transformant before another round of genetic modification). To apply a *URA* selectable marker for genetic modification, the parental strain should be a uracil auxotrophic strain [32, 33]. Thus, a transformant generated using the *CAT* marker and wild-type (WT) cells as a parental strain can no longer be genetically modified. To overcome these limitations, this study developed a new drug-resistant selection system of *C. merolae* transformants that can work solely or in combination with the *CAT* marker system.

Blasticidin S (BS) was initially identified as an antibiotic inhibiting growth of the fungus *Piricularia oryzae*, a cause of rice plant disease [34]. The antibiotic is a nucleoside analogue (molecular weight 422) originally isolated from the bacterium *Streptomyces griseochromogenes* [34]. BS binds to the peptidyl transferase center of the large ribosomal subunit (50S and 60S in bacteria and eukaryotes, respectively) and thereby inhibits protein synthesis in eukaryotes and prokaryotes by interfering with elongation and termination step [35, 36].

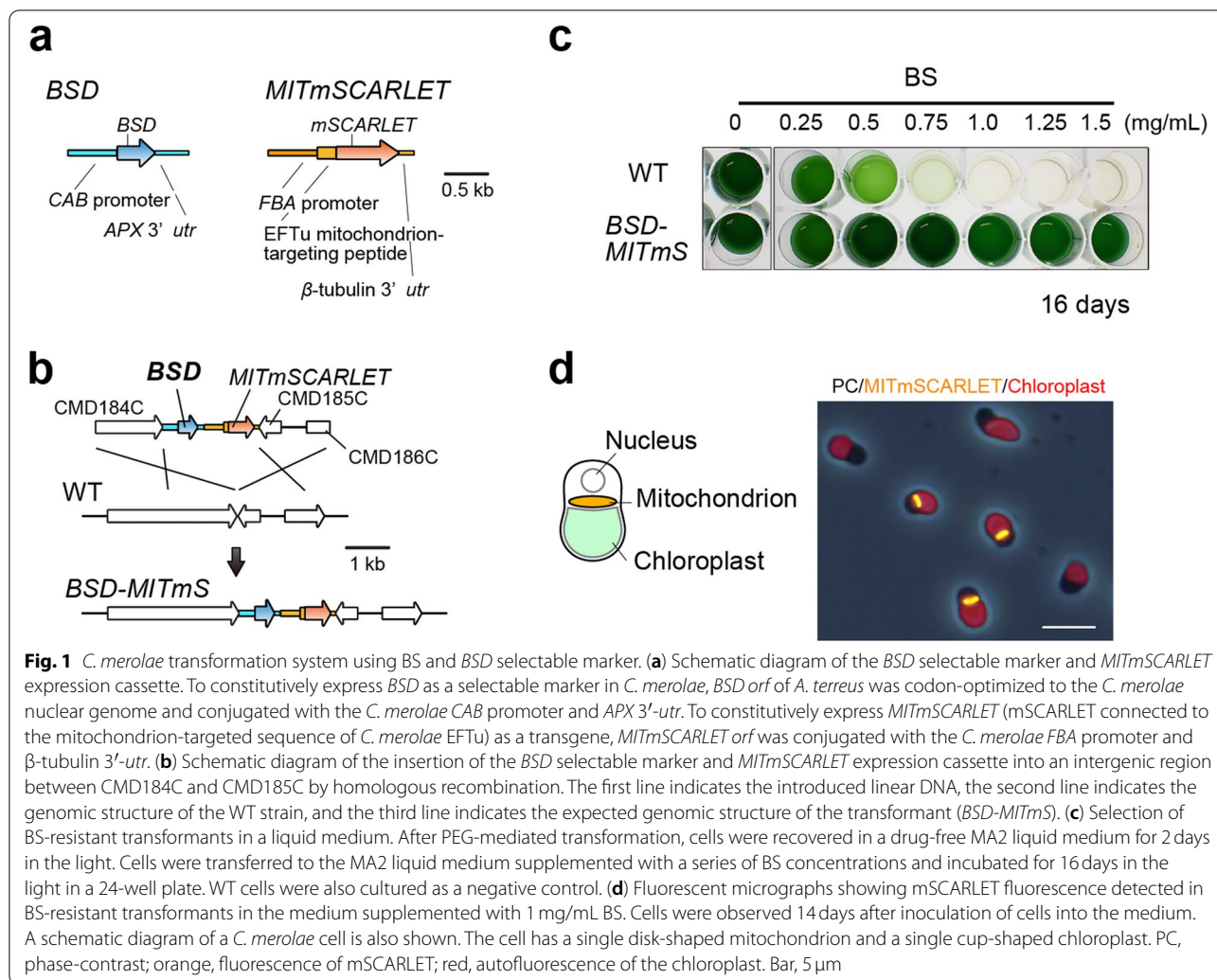
BS deaminase (BSD) detoxifies BS by catalyzing the deamination of a cytosine moiety in BS [37–39]. BSD belongs to the deaminase superfamily that are involved in nucleotide and ADP-ribose metabolism, and distributed through in eukaryotes, bacteria, and phases [37]. The *BSD* gene was found only in the phyla Firmicutes and Actinobacteria in bacteria and fungi in eukaryotes [37], and cloned from the bacterium *Bacillus cereus* [40] and the fungi *Aspergillus terreus* [41] and has been used as a marker for selecting genetically manipulated strains in a variety of organisms, such as mammals [42], plants [40], yeasts [43], and the green alga *C. reinhardtii* [44], which do not possess *BSD* gene.

This study developed a transformant selection system using BS and *BSD* of *A. terreus* in *C. merolae*. In addition, the *BSD* selectable marker can be used in combination with the already developed *CAT* marker system to simultaneously modify two different chromosomal loci by a single-step cotransformation. These procedures will facilitate studies using multiple genetic modifications in *C. merolae*.

Results and discussion

Nuclear transformation system with the *BSD* selectable marker in *C. merolae*

To validate the application of BS and *BSD* to the nuclear transformation of *C. merolae*, a selectable marker consisting of the promoter of *C. merolae* *CAB* gene (chlorophyll a/b binding protein; CMN234C), *BSD* *orf* of *A. terreus* (UniProtKB/Swiss-Prot ID of the amino acid sequence, P0C2P0.1; the nucleotide sequence was codon-optimized to the *C. merolae* nuclear genome), and 3'-*utr* of the *C. merolae* *APX* gene (ascorbate peroxidase; CMM158C) was constructed (Fig. 1a). In addition, an expression cassette of a transgene consisting of the promoter of the *C. merolae* *FBA* gene (fructose-1,6-biphosphate aldolase; CMM158C), sequence encoding mitochondrion-targeted peptide [N-terminal 77 amino acids [45]; of *C. merolae* *EFTu* (elongation factor thermo unstable; CMS502C)], *mSCARLET* (a monomeric red fluorescent protein) *orf*, and 3'-*utr* of the *C. merolae* β -tubulin gene (CMN263C) was constructed to fluorescently label the mitochondrion of transformant cells (Fig. 1a). The *CAB* and *FBA* promoters were chosen because a previous RNA-seq analysis showed that the mRNA abundance of these genes was relatively high in logarithmically growing *C. merolae* cells in the light [10]. The *BSD* selectable marker and the mitochondrion-targeted *mSCARLET* (*MITmSCARLET*) expression cassette were combined and sandwiched between *C. merolae* chromosomal sequences around CMD184C and CMD185C loci. This was done to integrate the *BSD* selectable marker and *MITmSCARLET* expression cassette into a chromosomal neutral locus



between CMD184C and CMD185C [8] by homologous recombination (Fig. 1b). The resultant *C. merolae* transformant was named BSD-MITmS.

After introducing DNA to *C. merolae* WT cells by the polyethylene glycol (PEG)-mediated method [2, 46], cells were recovered for 2 days in an inorganic MA2 liquid medium without any drug in the light and transferred to the liquid medium with BS. To determine an appropriate BS concentration, WT cells and the BSD-MITmS transformant were cultured with a series of BS concentrations (0, 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mg/mL) in the light for 16 days (Fig. 1c). As a result, WT cells died with BS in a dose-dependent manner, whereas the transformant grew in MA2 liquid medium supplemented with 1.0, 1.25, and 1.5 mg/mL BS (Fig. 1c). Fluorescence microscopy showed that mSCARLET fluorescence in the mitochondrion in most algal cells in the medium with 1.0 mg/mL BS, indicating that the transgene (MITmSCARLET) was successfully transformed into cells (Fig. 1d). However, some

cells in the medium with 1.0 mg/mL BS lacked mSCARLET, suggesting that an unexpected integration of the BSD marker, such as truncation of the MITmSCARLET expression cassette, likely occurred in these nonfluorescent cells. Thus, we then tried to isolate clones with an expected integration of the selectable marker and the transgene.

Selection and isolation of BS-resistant transformant clones on a gellan gum-solidified medium

A gellan gum-solidified MA2 medium supplemented with BS was prepared. The medium was covered with a cornstarch bed (Fig. 2a) to facilitate the colony formation of *C. merolae* [6, 8]. After the PEG-mediated introduction of DNA into cells, cells were recovered in MA2 liquid medium in the light for 2 days. Then, 50 μ L of the culture, which was diluted to give a concentration of OD₇₅₀ = 0.01, were inoculated onto the cornstarch bed with BS and incubated in a CO₂ (3%) incubator at 42 °C in

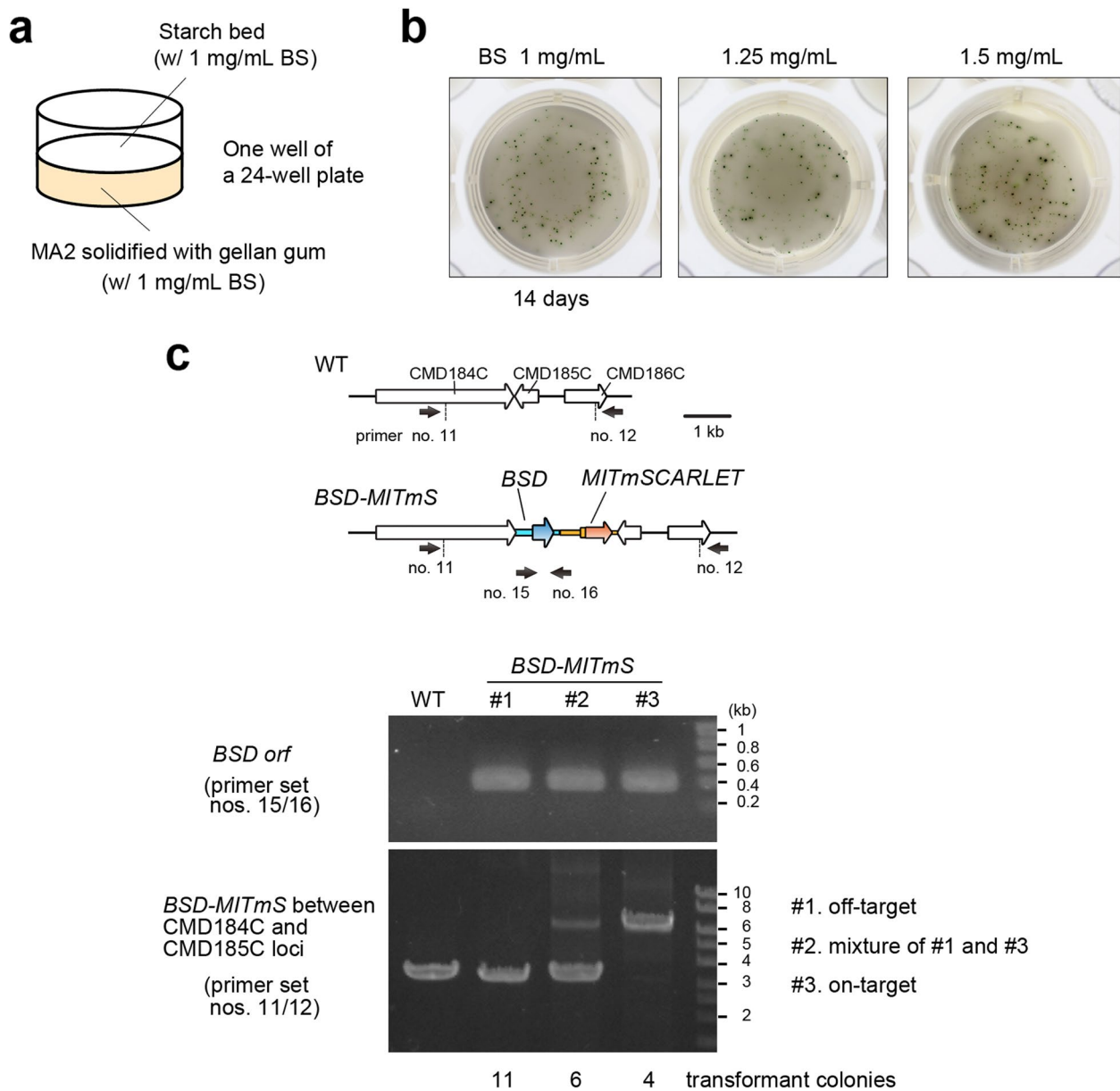


Fig. 2 Selection and isolation of BS-resistant transformant clones on a gellan gum-solidified medium supplemented with BS. **(a)** Schematic diagram of the MA2 gellan gum plate supplemented with 1 mg/mL BS. MA2 gellan gum supplemented with 1 mg/mL BS was solidified in one well of a 24-well plate. The medium was covered with a cornstarch bed to facilitate the colony formation of *C. merolae*. **(b)** Colonies of BS-resistant clones on MA2 gellan gum plate supplemented with 1.0, 1.25 and 1.5 mg/mL BS. After PEG-mediated transformation, cells were recovered in a drug-free MA2 liquid medium for 2 days in the light. Cells were spread on MA2 gellan gum plate supplemented with BS and incubated for 14 days in the light. **(c)** Colony PCR analyses of BS-resistant clones. The positions of the primers are indicated as arrows below the chromosomal structure of the WT and *BSD-MITmS* transformant. For detection of the *BSD* marker insertion either by off-target and on-target insertion (the primer set, nos. 15/16), the predicted size of the PCR product was 0.4 kb. In the PCR amplifying CMD184C-CMD186C loci (the primer set, nos. 11/12), the predicted size of the PCR product of on-target insertion of the *BSD-MITmS* construct was 6.3 kb and the size for off-target insertion or the WT chromosome was 3.3 kb. The exact positions and sequences of the primers are indicated in Supplementary Table S1. Full length unprocessed gel image of Fig. 2c is shown in Supplementary Fig. S1a.

the light. After incubation for 14 days, colonies appeared on the BS gellan gum plate (Fig. 2b).

To verify the targeted insertion of the *BSD* marker and *MITmSCARLET* into the intergenic region between *CMD184C* and *CMD185C*, polymerase chain reaction (PCR) analyses of the colonies were performed. Typical examples of electrophoretic patterns of the PCR products are shown in Fig. 2c and Supplementary Fig. S1a. The PCR analyses showed that the *BSD* marker was integrated into the genome of all transformant colonies examined (21/21 colonies; #1-#3 on the upper gel in Fig. 2c and Supplementary Fig. 1a). In contrast, the PCR analysis amplifying the *CMD184C*-*CMD186C* loci showed three patterns depending on the transformant colony; a single band showing off-target insertion of the transgene (11/21 colonies; #1 on the lower gel in Fig. 2c and Supplementary Fig. S1a), two bands showing on-target and off-target insertions of the transgene (6/21 colonies; #2 on the lower gel in Fig. 2c and Supplementary Fig. S1a), and a single band showing on-target insertion of the transgene (4/21 colonies; #3 on the lower gel in Fig. 2c and Supplementary Fig. S1a).

Simultaneous modification of two different chromosomal loci by a single-step cotransformation with *BSD* and *CAT* selectable markers in *C. merolae*

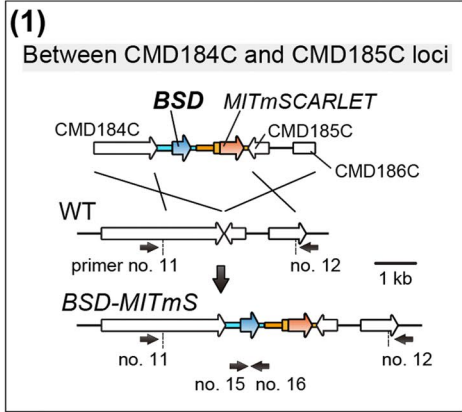
To develop an efficient and multiple genetic modification system in *C. merolae*, two different chromosomal loci were modified simultaneously using both *BSD* and *CAT* selectable markers. In addition to *BSD-MITmS* (Figs. 1b and 3a), another construct consisting of the *CAT* selectable marker and *mVENUS* transgene (*CAT-mV*) was prepared and designed to be

integrated into an upstream region of *CMK046C* locus as another chromosomal neutral locus [10] (Fig. 3a). *BSD-MITmS* and *CAT-mV* were simultaneously introduced to *C. merolae* WT cells by the PEG-mediated method (Fig. 3a). After recovery in drug-free MA2 liquid medium for 2 days, transformants were selected in MA2 liquid medium supplemented with both BS (1 mg/mL) and CP (0.2 mg/mL) rather than gellan gum-solidified medium. This was because, in a previous study, CP-resistant transformants could grow in MA2 liquid medium supplemented with CP but were not able to form colonies on MA2 gellan gum plate supplemented with CP [30]. After incubation in MA2 liquid medium supplemented with BS and CP in the light for 14 days, WT died, whereas the transformant survived (Fig. 3b). The transformant culture was spread and incubated on a drug-free MA2 gellan gum plate to generate single colonies. PCR analyses of the colonies showed that the *BSD* marker was integrated into the genome of all transformant colonies examined (21/21 colonies; #1-#3 on the upper gel in Fig. 3c and Supplementary Fig. S1b). *BSD-MITmSCARLET* was inserted into the intergenic region between *CMD184C* and *CMD185C* by on-target insertion in some colonies (7/21 colonies; #3 on the middle gel in Fig. 3c and Supplementary Fig. S1b) and the *CAT-mV* vector was inserted into the intergenic region of *CMK046C* upstream by on-target insertion in all colonies examined (21/21 colonies; #1, #2, and #3 on the lower gel in Fig. 3c and Supplementary Fig. S1b). In the transformant clones, in which both transgenes were inserted by on-target insertion (#3), fluorescence microscopy showed that the mitochondrion was labeled with mSCARLET fluorescence, and the cytosol

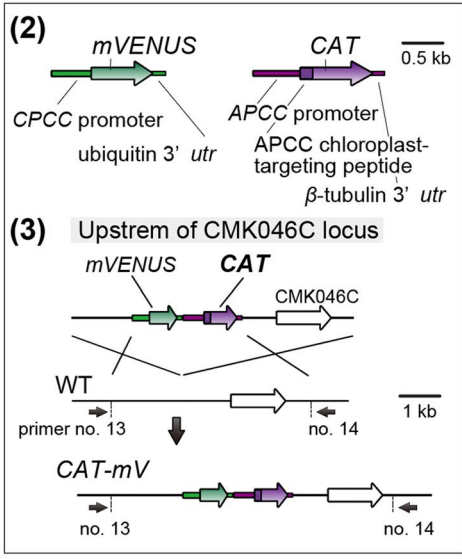
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Fig. 3 Simultaneous modification of two different chromosomal loci by a single-step cotransformation with *BSD* and *CAT* selectable markers in *C. merolae*. **(a)** Schematic diagram of the insertion of the *mVENUS* transgene with the *CAT* selectable marker and that of the *MITmSCARLET* transgene with the *BSD* selectable marker into two different chromosomal loci by homologous recombination. (1) Schematic diagram of the insertion of the *MITmSCARLET* transgene with the *BSD* selectable marker as in Fig. 1a. (2) Schematic diagram of the *mVENUS* expression cassette and the *CAT* selectable marker. To constitutively express *mVENUS*, *mVENUS* *orf* was conjugated with the *C. merolae* *CPCC* promoter and ubiquitin 3'-*utr*. To constitutively express the *CAT* selectable marker, *CAT* *orf* was conjugated with the *C. merolae* *APCC* promoter and β -tubulin 3'-*utr*. (3) Schematic diagram of the insertion of the *mVENUS* transgene with the *CAT* selectable marker into a chromosomal neutral locus. The *mVENUS* transgene was connected with the *CAT* selectable marker and integrated into the chromosomal region at *CMK046C* upstream. The first line indicates the introduced linear DNA, the second line indicates the genomic structure of the WT chromosome, and the third line indicates the expected genomic structure of the transformant (*CAT-mV*). **(b)** Selection of BS- and CP-resistant transformants in MA2 liquid medium supplemented with BS and CP. After PEG-mediated transformation of the two constructs, cells were recovered in a drug-free MA2 liquid medium for 2 days in the light. Cells were transferred to MA2 liquid medium supplemented with 1 mg/mL BS and 0.2 mg/mL CP and incubated for 14 days in the light in a 24-well plate. WT cells were also cultured as a negative control. **(c)** Colony PCR analyses of BS- and CP-resistant clones. The primer positions are indicated in **(a)**. The transformants in the liquid culture above were spread onto a drug-free MA2 gellan gum plate to generate colonies of transformant clones before the PCR analyses. For detection of the *BSD* marker insertion either by off-target and on-target insertion (the primer set, nos. 15/16), the predicted size of the PCR product was 0.4 kb. In the PCR amplifying *CMD184C*-*CMD186C* loci (the primer set, nos. 11/12), the predicted size of the PCR product of on-target insertion of the *BSD-MITmS* construct was 6.3 kb and the size for off-target insertion or the WT chromosome was 3.3 kb. In the PCR amplifying the *CMK046C* locus and its upstream (the primer set, nos. 13/14), the predicted size of the PCR product of on-target insertion of the *CAT-mV* construct was 7.5 kb and the size for off-target insertion or the WT chromosome was 4.3 kb. The exact positions and sequences of the primers are indicated in Supplementary Table S1. Full length unprocessed gel images of Fig. 3c are shown in Supplementary Fig. S1b. **(d)** Fluorescent micrographs showing mSCARLET fluorescence in the mitochondrion (*MITmSCARLET*, orange) and *mVENUS* (green) fluorescence in the cytosol in the *BSD-MITmS/CAT-mV* transformant clone. PC, phase-contrast; red, autofluorescence of the chloroplast. Bar, 5 μ m

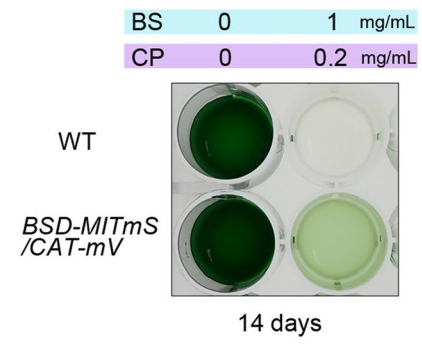
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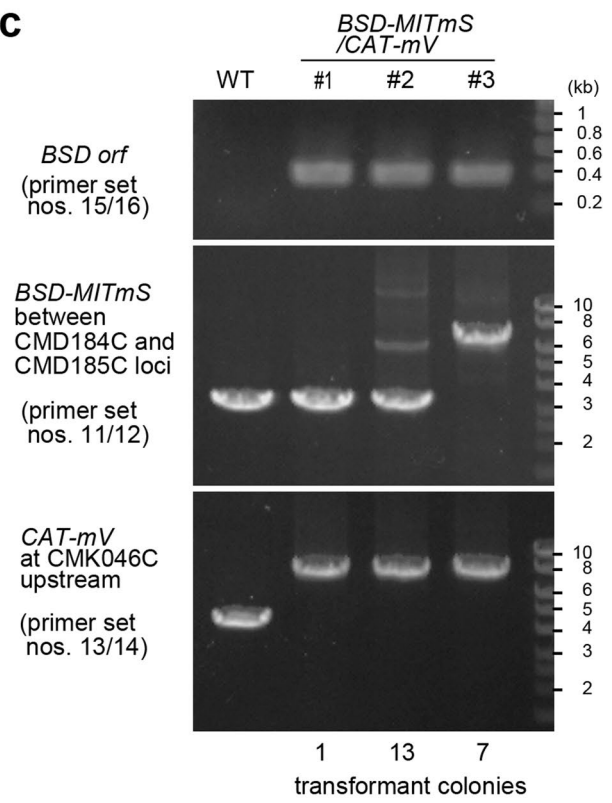
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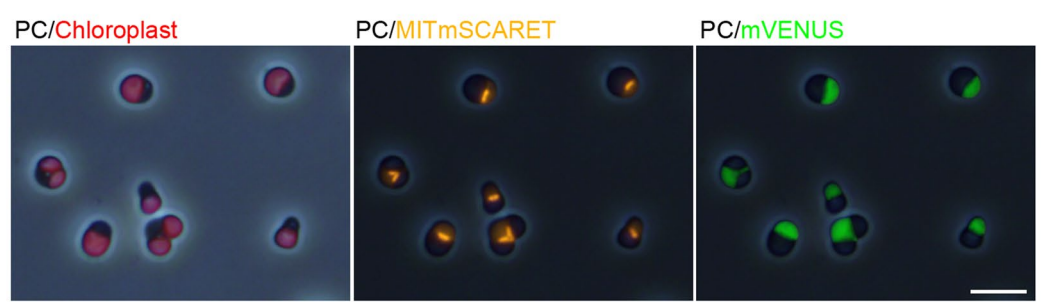


Fig. 3 (See legend on previous page.)

emitted mVENUS fluorescence (Fig. 3d). These results demonstrated that cotransformation is feasible in *C. merolae*.

In the preset study, the efficiency of on-target insertion of the *BSD-MITmS* construct was lower than that of the *CAT-mV* construct. Because the *BSD* marker was inserted into the genome by off-target insertion in some transformant clones, further optimization of the homology arms in the *BSD-MITmSCARLET* construct would be required to improve the targeting efficiency.

In the cotransformation system described above, it took 2 weeks to select BS and CP-resistant cells in a liquid medium after transformation and another 2 weeks to obtain single colonies of intended transformants on a gelatin gum plate, which was faster than the previous procedures for a two-step sequential transformation [30, 31]. In addition, the newly developed *BSD* selectable marker will enable an additional genetic modification to the already generated *C. merolae* transformants based on the *URA* or *CAT* system. Besides these advantages, a large-sized DNA will likely be introduced into a chromosome by conjugating *BSD* and *CAT* markers to the respective borders of DNA. Multiple genetic modifications will also be useful for metabolic engineering because such modifications often require the inactivation of two or more enzymatic genes [47].

Conclusions

This study has developed a transformant selection method using BS and *BSD* in *C. merolae*. In addition, a cotransformation system has been developed to simultaneously modify two different chromosomal loci by a combination of *BSD* and *CAT* selectable markers. These methods and the simple nature of *C. merolae* cellular and genomic architecture will facilitate studies on several phenomena in photosynthetic eukaryotes.

Methods

Algal culture

C. merolae 10D WT (NIES-3377), *BSD-MITmS*, and *BSD-MITmS/CAT-mV* strains were maintained in MA2 liquid medium [2] [(NH₄)₂SO₄ 40 mM, KH₂PO₄ 8 mM, MgSO₄ 4 mM, CaCl₂ 1 mM, FeCl₃ 100 μM, EDTA-2Na 72 μM, ZnCl₂ 2.8 μM, MnCl₂ 16 μM, Na₂MoO₄ 7.2 μM, CuCl₂ 1.3 μM, and CoCl₂ 0.7 μM; the pH was adjusted to 2.3 with H₂SO₄] in 60 mL tissue culture flasks (TPP Techno Plastic Products AG), with agitation at 120 rpm under continuous white light (30 μmol·m⁻²·s⁻¹) at 42 °C.

Preparation of linear DNA for *C. merolae* transformation

The sequences and primers used in this study are listed in Supplementary Table S1. Linear DNA for the transformation of *C. merolae* was prepared as described below.

To generate the *C. merolae BSD-MITmS* strain, DNA that contained the *BSD* selectable marker and *MITmSCARLET* expression cassette was prepared. For the homologous recombination at an intergenic region between CMD184C and CMD185C loci in the nuclear genome, the *BSD* selectable marker and *MITmSCARLET* expression cassette were tandemly connected and sandwiched between sequences identical to CMD184C and CMD185C loci, respectively (Fig. 1b), as described below. DNA fragment I [a portion of CMD184C *orf* (773rd nucleotide to the stop codon) flanked with the 25bp downstream sequence] was amplified by PCR with the primer set #1/2 using *C. merolae* WT genomic DNA as a template. DNA fragment II consisting of the *CAB* (CMN234C) promoter, *BSD orf*, and *APX* (CMM158C) 3'-*utr* and DNA fragment III consisting of the *FBA* (CMI049C) promoter, sequence encoding the mitochondrion-targeting peptide of EFTu (CMS502C), *mVENUS orf* (codon-optimized to *C. merolae* nuclear genome), and β-tubulin (CMN263C) 3'-*utr* were commercially synthesized. DNA fragment IV (sequence from the 28th to 1880th nucleotide downstream of CMD184C *orf*; this sequence corresponded to CMD185 and a portion of CMD186) was amplified by PCR with the primer set #3/4 using *C. merolae* WT genomic DNA as a template. DNA fragments I to IV were assembled and cloned into the plasmid pUC19 vector (TAKARA, Japan) using the In-Fusion HD cloning kit (TAKARA) for amplification in *Escherichia coli*. The assembled DNA fragments I to IV were amplified by PCR with the primer set #5/6 to prepare linear DNA for *C. merolae* transformation. The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen).

To generate the *C. merolae BSD-MITmS/CAT-mV*, DNA containing the *CAT* selectable marker and *mVENUS* expression cassette was prepared. For homologous recombination at an intergenic region at CMK046C upstream, the tandemly connected *CAT* selectable marker and *mVENUS* expression cassette were sandwiched between two genomic sequences around the CMK046C locus as described below. DNA fragment V (a chromosomal sequence from 2300 to 898 bp upstream of CMK046C *orf*) was amplified by PCR with the primer set #7/8 using *C. merolae* WT genomic DNA as a template. DNA fragment VI consisting of *CPC* (CMP166C) promoter, *mVENUS orf*, and ubiquitin (CMK296C) 3'-*utr* and DNA fragment VII consisting of *APCC* (CMO250C) promoter, sequence encoding the chloroplast-targeting peptide of *APCC*, *CAT orf*, and β-tubulin 3'-*utr* were commercially synthesized. DNA fragment VIII (CMK046C *orf* flanked with 897 bp upstream and 471 bp downstream sequences) was amplified by PCR with the primer set #9/10 using *C.*

merolae WT genomic DNA as a template. DNA fragments V to VIII were assembled and cloned into the plasmid pUC19 vector using the In-Fusion HD cloning kit for amplification in *E. coli*. The assembled DNA fragments V to VIII were amplified by PCR with the primer set #5/6 to prepare linear DNA for *C. merolae* transformation. The PCR product was purified using the QIAquick PCR Purification Kit.

Transformation of *C. merolae*

The transformation of *C. merolae* was carried out by the PEG-mediated method [46], as described previously [2, 30], with minor modifications. To generate the *BSD-MITmS* strain, 4 µg of *BSD-MITmS* PCR product was introduced into *C. merolae* WT. To generate the *BSD-MITmS/CAT-mV* strain, 4 µg each of *BSD-MITmS* and *CAT-mV* PCR products were mixed (8 µg in total) and introduced into *C. merolae* WT.

The transformed cells were cultured for 2 days in 8 mL of MA2 liquid medium in one well of a 6-well plate (VIOLAMO) in a CO₂ (3%) incubator at 42 °C in the light (40 µE) for recovery and selected on an MA2 gellan gum plate supplemented with 1, 1.25, and 1.5 mg/mL BS (the preparation procedure is described below) or 2 mL MA2 liquid medium supplemented with both 1 mg/mL BS and 0.2 mg/mL CP (the concentration was defined previously [30]); in one well of a 24-well plate (TPP Techno Plastic Products). Colony PCR analysis was carried out to verify targeted insertions of constructs into the intergenic regions between CMD184C and CMD185C loci and upstream of the CMK046C locus using the primer sets #11/12 and #13/14, respectively. The *BSD* marker integration into the genome in transformants was verified by PCR using the primer set #15/16.

MA2 gellan gum plate supplemented with BS

The MA2 gellan gum plate was prepared, as described previously [8], with minor modifications. One milliliter of 1% gellan gum (FUJIFILM, Japan) solution (autoclaved to dissolve gellan gum in water) and 1 mL of 2× MA2 liquid medium were poured into one well of a 24-well plate. Immediately, a stock solution of BS (Cayman Chemical, USA; 50 mg/mL in distilled water) was added to the mixture at a final concentration of 1.0, 1.25, and 1.5 mg/mL and mixed. After gellan gum had solidified, 0.5 mL of 40% slurry of cornstarch, suspended in MA2 liquid medium with 1, 1.25, and 1.5 mg/mL BS, was placed on the solidified medium (Fig. 2a). After cornstarch had sedimented onto the gellan gum plate, excess suspension solution was removed, and cornstarch was dried up on a clean bench.

Microscopy

Images of cells were captured using a fluorescence microscope (BX51; Olympus) equipped with a three-charge-coupled device camera system (DP71; Olympus). To detect mVENUS and mSCARLET fluorescence, narrow-band filter sets (U-MNIBA3; Olympus and XF37; Omega, respectively) were used.

Abbreviations

BS: Blastidicin S; BSD: Blastidicin S deaminase; CAT: Chloramphenicol acetyltransferase; CP: Chloramphenicol; PEG: Polyethylene glycol.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-021-03365-z>.

Additional file 1: Supplementary Figure S1. Full length unprocessed gel images of Figs. 2c and 3c.

Additional file 2: Supplementary Table S1. List of nucleotide sequences of primers and synthetic DNA used in this study.

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Authors' contributions

Conceived and designed the experiments: TF, SH, and S-yM. Performed the experiments: TF and SH. Wrote the paper: TF and S-yM. The author(s) read and approved the final manuscript.

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Availability of data and materials

The plasmids and *C. merolae* transformant strains generated in the current study are available from the corresponding authors upon request. The nucleotide sequences of *C. merolae* genetic loci (ID: CMN234C/CAB, CMM158C/APX, CMI049C/FBA, CMS502C/EFTu, CMN263C/β-tubulin, CMP166C/CPCC, CMO250C/APCC, CMD184C, CMD185C, CMD186C and CMK046C) used during the current study are available in *Cyanidioschyzon merolae* Genome Project v3 (<http://czon.jp/>).

Declarations

Ethics approval and consent to participate
No applicable.

Consent for publication
No applicable.

Competing interests
The authors declare there are no competing interests.

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