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Evaluation of colors in green mutants isolated from purple bacteria as a host for colorimetric whole-cell biosensors

Kazuyuki Yoshida · Daiki Yoshioka · Koichi Inoue · Shinichi Takaichi · Isamu Maeda

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Abstract The change in carotenoid-based bacterial color from yellow to red can be applied to whole-cell biosensors. We generated several green mutants to emphasize the color change in such biosensors. The blue-green *crtI*-deleted mutant, *Rhodopseudomonas palustris* no.711, accumulated the colorless carotenoid precursor, phytoene. Green *Rhodovulum sulfidophilum* M31 accumulated neurosporene, a downstream product of phytoene. Another green mutant, *Rhodobacter sphaeroides* Ga, accumulated neurosporene and chloroxanthin, which are both downstream products of phytoene. All green mutants accumulated bacteriochlorophyll *a*. Photosynthetic membrane obtained from the green mutants all exhibited decreased absorption of wavelength range at 510–570 nm. Therefore, these indicate that the greenish bacterial colors were mainly caused by the existence of bacteriochlorophyll *a* and the changes in carotenoid composition in photosynthetic membrane. The colors of the green mutants and their wild-type strains were plotted in the CIE-L*a*b* color space, and the color difference (ΔE*ab) values between a green mutant and its wild type were calculated. ΔE*ab values were higher in the green mutants than in *Rdv. sulfidophilum* CDM2, the yellowish host strain of reported biosensors. These data indicate that change in bacterial color from green to red is more distinguishable than that from yellow to red as a reporter signal of carotenoid-based whole-cell biosensors.

K. Yoshida · D. Yoshioka · K. Inoue · I. Maeda (✉)
Faculty of Agriculture, Utsunomiya University,
Minemachi,
Utsunomiya 321-8505, Japan
e-mail: i-maeda@cc.utsunomiya-u.ac.jp

S. Takaichi
Department of Biology, Nippon Medical School,
Nakahara,
Kawasaki 211-0063, Japan

Introduction

To sense specific target molecules using simple and cost-effective procedures, various types of whole-cell bacterial biosensors have been established using recombinant DNA technology (Belkin 2003; Yagi 2007). These biosensors elicit detectable signals by expressing reporter genes such as green fluorescent protein (Tsien 1998; Stocker et al. 2003), luciferase (Wilson and Hastings 1998; Stocker et al. 2003; Trang et al. 2005), or β-galactosidase (Silhavy and Beckwith 1985; Stocker et al. 2003) in response to target molecules. Reporter genes are usually fused with a DNA response element that is specifically induced by target molecules. These genetically manipulated bacteria have become a useful tool for monitoring environmental pollutants such as heavy metals.

A novel type of colorimetric whole-cell biosensor has recently been established based on using the photosynthetic bacterium, *Rhodovulum sulfidophilum*, as a sensor strain and its carotenoid synthetic gene, *crtA*, as a reporter (Maeda et al. 2006; Fujimoto et al. 2006). Spheroidene monooxygenase, CrtA, catalyzes conversion of the yellow carotenoids, demethylspheroidene and spheroidene, to the red carotenoids, demethylspheroidenone and spheroidenone, in the terminal step of the spheroidene pathway (Yeliseev et al. 1996; Takaichi 1999). The *crtA*-deleted *Rdv. sulfidophilum* mutant, CDM2, is yellow in vitro due to the accumulation of demethylspheroidene and spheroidene, whereas *Rdv. sulfidophilum* W-1S is red in vitro due to spheroidenone accumulation (Maeda et al. 2005). Introducing a sensor plasmid composed of the *crtA* gene and a DNA response element into the CDM2 has resulted in the establishment of carotenoid-based whole-cell biosensors. The reporter gene, *crtA*, is expressed in the presence of pollutants such as arsenite, and biosensors change the carotenoid-based color

from yellow to red. This change is obvious to the naked eye without additional reagents and equipment (Maeda et al. 2006; Fujimoto et al. 2006).

However, the reporter gene for carotenoid-based bacterial biosensors can undergo further improvement. Because CrtA catalyzes the ketolation reaction, the change from yellow to red is very sensitive to dissolved oxygen (Maeda et al. 2006). Furthermore, because yellow and red belong to a warm color group, distinguishing a reporter event from a yellow background might be difficult under some conditions. Taking these features into consideration, *crtI* is a candidate gene for the construction of an ideal reporter system. In carotenogenic pathways, phytoene desaturase CrtI catalyzes several desaturation steps and converts the colorless carotenoid, phytoene, into colored carotenoids without a requirement for molecular oxygen. The *crtI* mutant cannot synthesize all colored carotenoids and confers a blue-green color instead of red upon several photosynthetic bacteria (Giuliano et al. 1986; Coomber et al. 1990). If green mutants with a mutation in the carotenogenic pathways can be isolated, the change from a cool background color to a warm color upon completion of a reporter event will be more obvious in whole-cell biosensors, regardless of the dissolved oxygen concentration.

To establish a biosensor eliciting such a color change, we initially generated green mutants of *Rhodopseudomonas palustris* and *Rdv. sulfidophilum*. The differences in color between a corresponding wild-type strain and these two mutants as well as the green mutant, *Rhodobacter sphaeroides* Ga (Cogdell et al. 1976), were calculated using CIE-L*a*b* color space, respectively, and compared with the *crtA*-deleted mutant, the host strain of currently available carotenoid-based biosensors. This is the first evaluation of the colors of bacteria that have accumulated various carotenoids and bacteriochlorophyll *a* (Bchl) in vitro using color coordinates.

Materials and methods

Bacterial strains and growth conditions

Table 1 describes the bacterial strains and plasmids used in this study. *Rdv. sulfidophilum* W-1S (FERM P-15320,

Table 1 Bacterial strains and plasmids used in this study

Strains/plasmids	Relevant characteristics	Source
Strains		
E. coli JM109	Used for cloning and plasmid propagation	Takara
E. coli S17-1 (λ*pir*)	Used for conjugal transfer of pSM3065IUKD	Penfold and Pemberton (1992)
Rps. palustris no.7	Wild type; culture color is red	Fujii et al. (1983)
Rps. palustris no.711	A green mutant of *Rps. palustris* no.7; has a substitution of the *crtI* gene for a kanamycin-resistance cassette. Culture color is blue-green	This study
Rdv. sulfidophilum W-1S	Wild type; culture color is red	Maeda et al. (2005)
Rdv. sulfidophilum CDM2	A yellow mutant of *Rdv. sulfidophilum* W-1S; has a substitution of the *crtA* gene for a kanamycin-resistance cassette. Culture color is yellow	Maeda et al. (2005)
Rdv. sulfidophilum M31	A green mutant of *Rdv. sulfidophilum* W-1S; obtained by random mutagenesis with EMS. Culture color is green	This study
Rba. sphaeroides ATCC17023	Wild type; culture color is red	ATCC
Rba. sphaeroides Ga	A green mutant of *Rba. sphaeroides*; culture color is green	Cogdell et al. (1976)
Plasmids		
pGEM-T	ampR; used for cloning PCR products	Promega
pUC4K	ampR, kanR; used as a source of the kanamycin-resistance cassette	GE Healthcare
pGEMIUKD	pGEM-T carrying a fragment consisting of the upstream DNA fragment of *crtI* gene, a kanamycin-resistance cassette, and the downstream DNA fragment of *crtI* gene	This study
pSM3065	TcR; a R6K-based suicide vector	Penfold and Pemberton (1992)
pSM3065IUKD	pSM3065 carrying a fragment consisting of the upstream DNA fragment of *crtI* gene, a kanamycin-resistance cassette, and the downstream DNA fragment of *crtI* gene	This study

National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan) and its green mutant, M31, were incubated in modified Okamoto medium (MOM; Maeda et al. 2003) containing 3% NaCl. Wild-type and *crtI*-deleted mutant strains of *Rps. palustris* no.7 (Fujii et al. 1983) and no.711, as well as wild-type and green mutant strains of *Rba. sphaeroides*, ATCC17023 and Ga, were grown in MOM containing 0.02% NaCl. All photosynthetic bacterial strains were incubated in glass tubes or rectangular glass bottles at 30°C under an incandescent lamp at a photon flux intensity of 40–45 μmol s^{-1} m^{-2}. Semiaerobic-light conditions were achieved by covering the cultivation vessels with screw caps or silicone caps without agitation. *Escherichia coli* strains were cultivated in Luria–Bertani medium. Antibiotics were added to cultures as required at a final concentration of 50 μg/ml ampicillin or 30 μg/ml kanamycin.

Construction of a *crtI*-deleted mutant of *Rps. palustris*

The isolation of DNA, enzymatic treatments for plasmid construction, polymerase chain reaction (PCR), nucleotide sequencing, and *E. coli* transformation proceeded according to standard protocols (Sambrook and Russell 2001). Two sets of PCR primers were designed based on the upstream and downstream nucleotide sequences of *Rps. palustris* CGA 009 *crtI* (EMBL/GenBank/DDBJ accession number; NC005296; Larimer et al. 2004) with appropriate restriction sites. PCR with crtIUS (5′-*ACTAGT*AGCCTCAATGGC GCGTTTCT, the introduced *Spe*I site is italicized) and crtIUA (5′-A*CTCGAG*GGCAATCTGTAAGCAGATCC, the introduced *Xho*I site is italicized) or with crtIDS (5′-*GCATGC*GTCAAACGCCTTTATCTCG, the introduced *Sph*I site is italicized) and crtIDA (5′-*GGGCCCACTAGT* CCATCACCAGCGTCATCATC, the introduced *Apa*I and *Spe*I sites are italicized), together with *Rps. palustris* no.7 genomic DNA as a template, resulted in amplification of a 538-bp upstream (IU) or a 562-bp downstream (ID) DNA fragment, respectively. Both upstream and downstream fragments were subcloned into the pGEM-T vector (Promega, Tokyo, Japan), yielding pGEMIU and pGEMID, respectively. A *Sal*I-digested kanamycin-resistance cassette (Maeda et al. 2005), derived from pUC4K (GE Healthcare UK, Buckinghamshire HP7 9NA, UK), was inserted at the *Xho*I site of pGEMIU to generate pGEMIUK. The ID fragment obtained by digestion of pGEMID with *Apa*I and *Sph*I was inserted into pGEMIUK digested with *Apa*I and *Sph*I, generating pGEMIUKD. The plasmid pGEMIUKD was digested with *Spe*I to generate a fragment consisting of IU, a kanamycin-resistance cassette, and ID was finally inserted at an *Xba*I site of the suicide vector, pSM3065, to yield pSM3065IUKD. The plasmid pSM3065IUKD was introduced into *E coli*. S17-1 (λ*pir*; Penfold and Pemberton 1992) cells by electroporation and then transferred into *Rps. palustris* no.7 by conjugation (Yun et al. 1990). The conjugants were spread onto MOM-agar plates and cultured for a few days under light conditions to select kanamycin-resistant colonies. Substitution of the *crtI* ORF region for the kanamycin-resistance cassette through a double crossover event was confirmed by PCR with several sets of primers.

Isolation of green mutant of *Rdv. sulfidophilum*

The wild-type strain, *Rdv. sulfidophilum* W-1S, underwent mutagenesis by exposure to ethyl methanesulfonate (EMS) as described previously (Shanmugam and Valentine 1980). Green colonies on MOM-agar plates were selected and inoculated into liquid MOM. A green phenotype was stably inherited after several generations.

HPLC analysis of pigments

When the OD_{600} reached 1.0 cm^{-1}, cells were collected by centrifugation, stored at −80°C overnight, and freeze-dried. A mixture of acetone and methanol (7:2 *v*/*v*) was added to the lyophilized cells and vortex mixed for 2 min. The supernatants collected after centrifugation were analyzed using a high-performance liquid chromatography system (HPLC) equipped with a μBondpack C18 column (3.9× 300 mm, 125-Å pore size, Nihon Waters, Tokyo, Japan) and monitored with a photodiode array detector (type 2996, Nihon Waters), as described previously (Takaichi et al. 2001). All pigments were identified based on absorption spectra and specific retention times.

Preparation of photosynthetic membranes and absorption spectroscopy

All strains were grown semiaerobically in the light and harvested in late logarithmic phase. The cells were suspended in 10-mM MOPS–NaOH buffer (pH 7.0) and disrupted in a French pressure cell (Maki et al. 2003) or by sonication. After removal of cellular debris, photosynthetic membranes were pelleted by ultracentrifugation and suspended in 10-mM MOPS–NaOH buffer. Absorption spectra of photosynthetic membranes were measured using a UV–vis recording spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan).

Colorimetric analysis

When the OD_{600} reached 1.0 cm^{-1}, cells were concentrated to OD_{600} of 5.0 cm^{-1}. Samples were colorimetrically analyzed by spectrophotometry (CM-3500d, Konica Minolta Sensing, Tokyo, Japan) as described previously (Maeda et al. 2006). Colors based on light spectra transmitted through the

cultures were plotted in the CIE-L*a*b* color space (CIE 1976). The CIE-L*a*b* color space is a general system that measures the colors of objects and can thus evaluate differences in color between objects (Wiegand et al. 2005; Tsubura and Yamaguchi 2005). In the CIE-L*a*b* color space, the L* and a*b* coordinates are expressed as values of lightness and chroma. Increases in an a* value from the center of the color space toward the plus and minus directions mean increases in red and green chromas, respectively. Increases in a b* value toward the plus and minus directions mean increases in yellow and blue chromas, respectively. ΔE*ab, which indicates the color difference between objects, was used to compare color changes between mutants and their wild types. ΔE*ab is defined by the following equation, $\Delta E^*ab = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$, where ΔL*, Δa*, and Δb* are differences in L*, a*, and b* values between culture colors, respectively.

Results

Construction of green mutants

The ORF region of *crtI* in the *Rps. palustris* no.7 chromosomal DNA was replaced with a kanamycin-resistance cassette, which caused *Rps. palustris* no.711 to become weakly blue-green (Fig. 1). The mutants *Rdv. sulfidophilum* M31 and *Rba. sphaeroides* Ga were bright green and yellowish green, respectively (Fig. 1). Wild-type strains and *crtA*-deleted *Rdv. sulfidophilum* mutant, CDM2, are also indicated in Fig. 1.

Accumulation of pigments in green mutants

Bchl was detected in all three of the green mutants. HPLC analysis monitored at A_{450} revealed that *Rps. palustris* no.7 had accumulated five carotenoids that were identified as rhodovibrin, rhodopin, spirilloxanthin, anhydrorhodovibrin, and lycopene (Fig. 2a). However, *Rps. palustris* no.711 lacked all of these carotenoids. Analysis by HPLC monitored at A_{280} showed that phytoene accumulated in *Rps. palustris* no.711 but not in *Rps. palustris* no.7 (Fig. 2b). The polyene composition in no.7 corresponded to that predicted in the *crtI*-deleted mutant. These results suggested that the blue-green color of *Rps. palustris* no.711 was derived from Bchl.

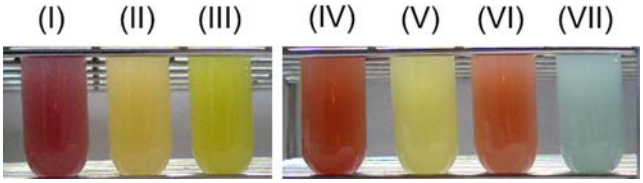

Fig. 1 Culture colors of wild types and their mutants. *(I) Rdv. sulfidophilum* W-1S, wild type; *(II) Rdv. sulfidophilum* CDM2, a *crtA*-disrupted mutant; *(III) Rdv. sulfidophilum* M31, a green mutant; *(IV) Rba. sphaeroides* ATCC17023, wild type; *(V) Rba. sphaeroides* Ga, a green mutant; *(VI) Rps. palustris* no.7, wild type; *(VII) Rps. palustris* no.711, a *crtI*-disrupted mutant

HPLC analysis monitored at A_{450} revealed the accumulation of spheroidenone in *Rdv. sulfidophilum* W-1S and of spheroidenone and spheroidene in *Rba. sphaeroides* ATCC 17023 (Fig. 2c, d). On the other hand, the major accumulated carotenoid in *Rdv. sulfidophilum* M31 was neurosporene instead of spheroidenone (Fig. 2c), and in *Rba. sphaeroides* Ga, neurosporene and chloroxanthin instead of spheroidenone and spheroidene (Fig. 2d). The carotenoid composition in Ga was consistent with published findings (Cogdell et al. 1976). We considered that the carotenoid composition in *Rdv. sulfidophilum* M31 and *Rba. sphaeroides* Ga was caused by a dysfunction of 1,2-hydratase (CrtC) and of 3,4-desaturase (CrtD) in the spheroidene pathway (Lang et al. 1995). Neurosporene and chloroxanthin were separated by HPLC, and those colors were evaluated using the CIE-L*a*b* color space. The colors were plotted along the +b* axis (data not shown), indicating that neurosporene and chloroxanthin are yellow. These results suggested that the green colors of *Rdv. sulfidophilum* M31 and *Rba. sphaeroides* Ga were derived from Bchl in combination with the yellow carotenoid(s).

Spectroscopic characterization of photosynthetic membranes

Compared to the wild types, no significant differences were observed for absorption peaks at 800 and 855 nm, corresponding to the light-harvesting 2 (LH2) complex, in *Rdv. sulfidophilum* CDM2, M31, and *Rba. sphaeroides* Ga, regardless of differences in the carotenoid composition (Fig. 3b, c). In contrast, *Rps. palustris* no.711 lacked the absorption peaks at 800 and 855 nm, compared to no.7, and exhibited single absorption peak at 875 nm corresponding to LH1 (Fig. 3a). These results were consistent with published findings (Bartley and Scolnik 1989; Lang and Hunter 1994).

The wild-type strains, *Rps. palustris* no.7, *Rdv. sulfidophilum* W-1S, and *Rba. sphaeroides* ATCC17023, exhibited the absorption of wavelength range at 450–570 nm, corresponding to the absorbance peaks for carotenoids in the photosynthetic complexes (Fig. 3a–c). In contrast, green mutant strains, no. 711, M31, and Ga, lacked the absorption of wavelength range at 510–570 nm (Fig. 3a–c), and these findings suggested that phytoene, neurosporene, and chloroxanthin, accumulated in the green mutants in the photosynthetic membrane, could not absorb a wavelength range of green light. The yellow mutant strain, *Rdv. sulfidophilum* CDM2, exhibited increased absorption of wavelength range at 510–540 nm

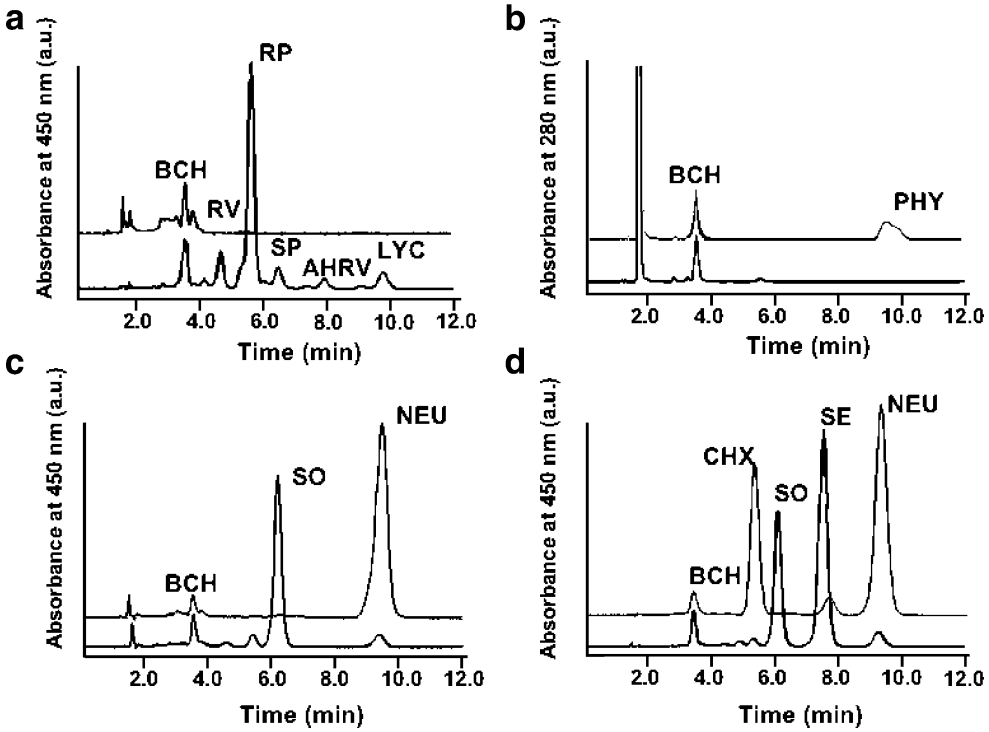

Fig. 2 Comparison of pigment composition by HPLC. **a**, **b** Chromatograms of *Rps. palustris* no.7 and no.711; **c** chromatograms of *Rdv. sulfidophilum* W-1S and M31; **d** chromatograms of *Rba. sphaeroides* ATCC17023 and Ga. Chromatograms of wild type (*lower*) and its mutant (*upper*) are placed in parallel with retention time. All pigments were identified based on the absorption spectrum and specific retention time. *AHRV* Anhydrorhodovibrin, *BCH* bacteriochlorophyll *a*, *CHX* chloroxanthin, *LYC* lycopene, *NEU* neurosporene, *PHY* phytoene, *RP* rhodopin, *RV* rhodovibrin, *SE* spheroidene, *SO* spheroidenone, *SP* spirilloxanthin

compared to M31 (Fig. 3b), and this finding suggested that spheroidene and demethylspheroidene in the photosynthetic membrane of CDM2 would absorb green light with a shorter wavelength range.

These results indicated that the bacterial colors recognized by naked eye were mainly caused by the existence of Bchl and the differences in carotenoid composition in the photosynthetic membranes.

Colorimetric analysis using the CIE-L*a*b* color space

The L*, a*, b*, and ΔE*ab values of culture colors were calculated after adjustment for cell mass concentrations (Table 2). In comparison with *Rps. palustris* no.7, the a* value obviously decreased from 18.27 to −2.22 as a consequence of the defect in the carotenoids (Fig. 3a), and the L* value increased from 75.08 to 89.40 in no.711.

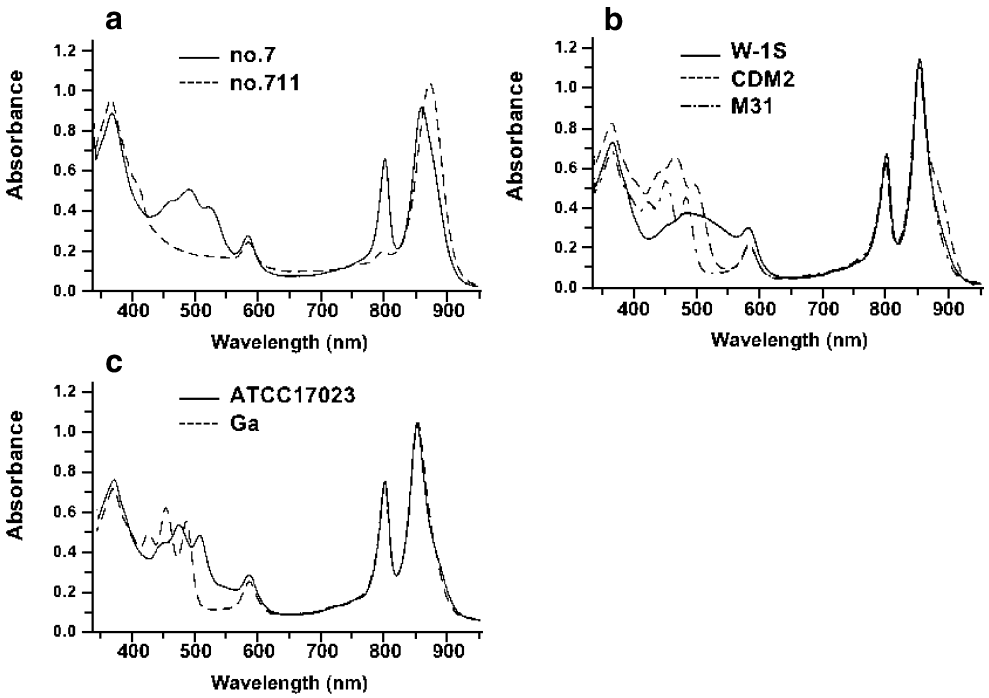

Fig. 3 Absorption spectra of the photosynthetic membranes of *Rps. palustris* (**a**), *Rdv. sulfidophilum* (**b**), and *Rba. sphaeroides* (**c**). Absorbance at 855 nm of each mutant was normalized against its wild type, respectively

These changes increased ΔE*ab in no.711 (25.52), compared with that in *Rdv. sulfidophilum* CDM2 (11.57).

In comparison with *Rba. sphaeroides* ATCC 17023, the a* value obviously decreased from 8.35 to −4.27 as a consequence of the defect in red carotenoid, spheroidenone, and the b* value also significantly increased from 14.78 to 36.46 as a consequence of accumulating the yellow carotenoids, neurosporene and chloroxanthin, in Ga (Table 2; Fig. 3d). The tendencies in the a* (from 8.42 to −1.32) and b* (from 6.22 to 19.79) values were the same in *Rdv. sulfidophilum* W-1S and M31 due to the defect in red carotenoid, spheroidenone, and accumulation of the yellow carotenoid, neurosporene (Table 2; Fig. 3c). Only the b* value increased from 6.22 to 16.57 in *Rdv. sulfidophilum* CDM2 as a consequence of accumulating the yellow carotenoids, spheroidene and demethylspheroidene, as reported previously (Maeda et al. 2005). The ΔE*ab values in *Rba. sphaeroides* Ga (25.13) and *Rdv. sulfidophilum* M31 (17.46) were also higher than that in *Rdv. sulfidophilum* CDM2 (11.57). The color differences in these green mutants were emphasized by Δa* in addition to Δb*.

These results demonstrated that the three green mutants caused the obvious increases in ΔE*ab in comparison with their wild-type strains and that signals caused by complementation of the mutation might be more distinguishable than those obtained from CDM2-based biosensors.

Discussion

An obvious change in color is a critical factor for detecting target molecules by the naked eye in carotenoid-based biosensors. Investigation of the correlation between carotenoid composition and color coordinates is helpful for establishing carotenoid-based biosensors that elicit a clear color change after a reporter event. The present study determined carotenoid composition and color coordinates of several green mutant bacteria and their wild-types in vitro using the CIE-L*a*b* color space.

Mutants of *Rhodobacter* species with a *crtI* deletion have already been constructed. Here, we generated the first *crtI*-deleted mutant of *Rps. palustris*, namely no.711, the phenotype of which was very similar to that of *crtI*-deleted *Rhodobacter* mutants (Giuliano et al. 1986; Coomber et al. 1990). The increased L* value in *Rps. palustris* no.711 was probably due to a low level of the LH2 complex. Colored carotenoids are essential for assembly of the LH2 complex (Lang and Hunter 1994), as no absorption peaks correspond to LH2 in the *crtI*-deleted mutant (Bartley and Scolnik 1989; Lang and Hunter 1994). Therefore, *Rps. palustris* no.711 could not absorb light energy through the LH2 complex. Furthermore, phototrophic growth of no.711 was partly impaired compared with other green mutants and wild types (data not shown), supporting our consideration concerning the ΔL*.

The shift of the a* value to minus in the CIE-L*a*b* color space (i.e., an emergence of greenish colors) was probably due to the lack of the absorption of wavelength range at 510–570 nm caused by the absence of the particular carotenoids and the presence of Bchl in the three green mutants. In *Rba. sphaeroides* Ga and *Rdv. sulfidophilum* M31, the b* values increased not only from those in their wild types but also from *Rdv. sulfidophilum* CDM2 despite that it looked more yellowish than Ga and M31 did by the naked eye. This phenomenon is essentially due to accumulation of the yellow carotenoids in the photosynthetic membranes of Ga and M31. Besides this, however, one of the important elements by which a culture color looks yellowish or greenish is likely whether photosynthetic membrane absorbs light with a wavelength range at 510–540 nm or not. In a case of the spheroidene pathway, this change in the absorption spectrum has been shown to be caused by the increase in the number of conjugated double bonds of carotenoid in the membrane from 9 to 10 after 3,4-desaturation with CrtD. The human optic nerve would have a

Table 2 CIE-L*a*b* color values for the bacterial cultures

	L*[a]	a*[a]	b*[a]	ΔL*	Δa*	Δb*	ΔE*ab
Rps. palustris							
no.7	75.08	18.27	10.60				
no.711	89.40	−2.22	5.46	14.32	−20.49	−5.14	25.52
Rba. sphaeroides							
ATCC 17023	82.77	8.35	14.78				
Ga	81.23	−4.27	36.46	−1.54	−12.62	21.68	25.13
Rdv. sulfidophilum							
W-1S	82.51	8.42	6.22				
CDM2	85.58	4.26	16.57	3.07	−4.16	10.35	11.57
M31	87.58	−1.32	19.79	5.07	−9.75	13.58	17.46

[a] Values are the means of three measurements.

superior sensitivity to light with the wavelength range, and if one senses it, a visual bacterial color in vitro might be recognized as a cool color even though the bacterium accumulates much yellowish carotenoid(s). In this study, therefore, it has been found as the least condition necessary to be a green mutant that photosynthetic membrane, regardless of whether it contains colored carotenoids or not, does not absorb green light at 510–540 nm. Increased L* and a* values in *Rps. palustris* no.711 and increased a* and b* values in *Rdv. sulfidophilum* M31 and *Rba. sphaeroides* Ga contributed the obvious increases in ΔE*ab. Thus, the green mutants will serve as a useful tool for inducing the obvious reporter signal in carotenoid-based whole-cell biosensors.

The three green mutants with different color coordinates have considerable potential as host strains for carotenoid-based biosensors, as they could improve the signal-to-noise ratio in a reporter event. Furthermore, the three green mutants are able to grow under aerobic conditions (data not shown). So it is assumed that green mutant-based biosensors would be functional irrespective of concentrations of molecular oxygen in contrast to CDM2-based biosensors. However, further studies to evaluate the susceptibility to molecular oxygen are required in the *Rps. palustris* no.711. Introducing a sensor plasmid composed of the reporter gene and a DNA response element into green mutants will generate biosensor strains that change color from green to red. Although the positions of the mutations in both *Rdv. sulfidophilum* M31 and *Rba. sphaeroides* Ga have not been elucidated, introducing *crtI* from *Rps. palustris* no.7 into *Rdv. sulfidophilum* M31 resulted in accumulation of the red carotenoid, lycopene (data not shown). Thus, our findings indicate that biosensors, which change color from green to red when *crtI* is the reporter gene, can be constructed based on *Rdv. sulfidophilum* M31 and probably on *Rba. sphaeroides* Ga, in addition to *Rps. palustris* no.711.

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