Carotenoids of *Gemmatimonas aurantiaca* (*Gemmatimonadetes*): identification of a novel carotenoid, deoxyoscillol 2-rhamnoside, and proposed biosynthetic pathway of oscillol 2,2'-dirhamnoside

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Gemmatimonas aurantiaca strain T-27^T is an orange-coloured, Gram-negative, facultatively aerobic, polyphosphate-accumulating bacterium belonging to a recently proposed phylum, *Gemmatimonadetes*. We purified its pigments and identified them as carotenoids and their glycoside derivatives using spectral data. The major carotenoid was (2*S*,2'*S*)-oscillol 2,2'-di-(α -L-rhamnoside), and the minor carotenoids were (2*S*)-deoxyoscillol 2-(α -L-rhamnoside) and didemethylspirilloxanthin. Deoxyoscillol 2-rhamnoside is a novel carotenoid. Oscillol 2,2'-diglycosides have hitherto only been reported in a limited number of cyanobacteria, and this is believed to be the first finding of such carotenoids in another bacterial phylum. Based on the identification of the carotenoids and the completion of the entire nucleotide sequence, we propose a biosynthetic pathway for the carotenoids and the corresponding genes and enzymes. We propose the involvement of geranylgeranyl pyrophosphate synthase (CrtE), phytoene synthase (CrtB) and phytoene desaturase (CrtI) for lycopene synthesis; and of carotenoid 1,2-hydratase (CruF) and carotenoid 2-O-rhamnosyltransferase (CruG) for oscillol 2,2'-dirhamnoside synthesis. Further, isopentenyl pyrophosphate could be synthesized by a non-mevalonate pathway (DXP pathway).

Received 1 September 2009
Revised 2 December 2009
Accepted 2 December 2009

INTRODUCTION

The Gram-negative bacterium *Gemmatimonas aurantiaca* strain T-27^T is representative of a recently proposed bacterial phylum, *Gemmatimonadetes* (Zhang *et al.*, 2003). Within this phylum, *G. aurantiaca* is the only cultured organism to be validly described, and it has occupied a solitary position among the huge phylogenetic diversity of bacteria. The distinct phylogenetic traits of this bacterium suggest that its metabolic pathways and enzymes are also unique.

G. aurantiaca was isolated from activated sludge formed in an aerobic/anaerobic sequential batch reactor for wastewater treatment (Zhang *et al.*, 2003). It is able to grow not only by anaerobic respiration under anaerobic conditions

but also by oxygen respiration under aerobic conditions. In the wastewater treatment system, *G. aurantiaca* is often exposed to a high concentration of oxygen due to vigorous aeration, and it has several defence mechanisms against reactive oxygen species, such as the production of catalase, peroxidase and superoxide dismutase. The antioxidant defence by such enzymes is quite effective in eliminating reactive oxygen species. As an additional defence, *G. aurantiaca* produces and stores carotenoid pigments that function as anti-oxidants.

Carotenoids are found in many organisms, and bacteria produce a very wide variety of them (Britton *et al.*, 2004). All photosynthetic bacteria contain a large amount of carotenoids for use in light-harvesting for photosynthesis and as antioxidants for protection from photo-oxidation (Takaichi, 2009). The oxygenic photosynthetic bacteria, cyanobacteria, also produce carotenoids, such as β -

Abbreviations: CD, circular dichroism; IPP, isopentenyl pyrophosphate.

carotene, echinenone and myxol glycoside (Takaichi & Mochimaru, 2007, 2009). However, carotenoids are not limited to photosynthetic bacteria, and are widely detected even in non-photosynthetic bacteria that exhibit facultatively aerobic growth. *G. aurantiaca* is a facultative aerobe and possesses carotenoids, and both its liquid culture and colonies appear orange. These carotenoids are useful in preventing harmful oxidation when *G. aurantiaca* is exposed to oxygen.

Preliminary studies based on spectroscopic observation indicated that the carotenoids in *G. aurantiaca* were different from those commonly found in non-photosynthetic facultative bacteria, and promised the discovery of new carotenoids. In the present study, we analysed and identified all the carotenoids in *G. aurantiaca* using spectroscopic methods, and found both new and rarely detected carotenoids. We propose a biosynthetic pathway of these carotenoids and the corresponding enzymes and genes based on genomic findings.

METHODS

Cultivation of bacterium. *Gemmatimonas aurantiaca* strain T-27^T (=JCM 11422^T, DSM 14586^T, NBRC 100505^T) was cultivated aerobically in liquid NM-1 medium at 30 °C, as described previously (Zhang *et al.*, 2003). The grown cells were collected by centrifugation and stored at –80 °C.

Extraction and purification of carotenoids. All the carotenoids were isolated and purified as follows. We extracted the carotenoids from the wet cells with methanol using an ultrasonicator several times, and removed the cell debris by centrifugation. The solvent was then evaporated. The extracted carotenoids were applied to C₁₈-TLC plates (Whatman) developed with methanol, and a broad red band was collected. It was then applied to silica gel TLC plates (Merck) developed with dichloromethane/ethyl acetate/acetone/methanol (2:4:2:1, by vol.). Three red bands were collected: a major highly polar carotenoid ($R_f=0.1$), a polar carotenoid ($R_f=0.5$) and a less polar carotenoid ($R_f=0.9$). The highly polar carotenoid (carotenoid-1) corresponded to peak 1 of C₁₈-HPLC (Fig. 1). It was applied to silica gel TLC plates developed with dichloromethane/ethyl acetate/acetone/methanol (2:4:2:4, by vol.). A red band was collected, and then purified using C₁₈-HPLC eluted with methanol/water (9:1, v/v). Finally, it was loaded on a column of Toyopearl DEAE-650M (Tosoh), and the carotenoid was eluted with acetone, although polar lipids remained on the column. The polar carotenoid (carotenoid-2) corresponded to peak 2 (Fig. 1). It was applied to silica gel TLC plates developed with dichloromethane/ethyl acetate/acetone/methanol (2:4:2:1, by vol.). A red band was then purified using C₁₈-HPLC eluted with methanol/water (93:7, v/v). Finally, it was loaded on a column of Toyopearl DEAE-650M, and the carotenoid was eluted with acetone. The less-polar carotenoid (carotenoid-3) corresponded to peak 3 (Fig. 1). It was applied to silica gel TLC plates developed with dichloromethane/ethyl acetate (2:1, v/v). A red band was then purified using C₁₈-HPLC eluted with methanol/water (97:3, v/v). All processes were carried out under dim light.

HPLC system. The HPLC system was equipped with a µBondapak C₁₈ column (8 × 100 mm, RCM type; Waters). Elution was performed with methanol/water (9:1, v/v) for the first 20 min, and then 100% methanol at a constant flow rate of 2.0 ml min⁻¹ (Mochimaru *et al.*, 2008).

Fig. 1. HPLC elution profile of pigments extracted from *G. aurantiaca*. Elution was performed with methanol/water (9:1, v/v) for the first 20 min, and then with 100% methanol (2.0 ml min⁻¹). Peaks are referred to in the text and figures.

Spectroscopic analysis. We measured the absorption spectra of the carotenoids using an MCPD-3600 photodiode array detector (Otsuka Electronics) attached to the HPLC apparatus described above (Takaichi & Shimada, 1992). The circular dichroism (CD) spectra of the acetylated carotenoid in diethyl ether were measured using a J-820 spectropolarimeter (JASCO) at room temperature. The relative molecular masses of the carotenoids were measured using an FD-MS M-2500 double-focusing gas chromatograph-mass spectrometer (Hitachi) equipped with a field-desorption apparatus. The ¹H-NMR (500 MHz) spectra of the acetylated carotenoids in CDCl₃ and the carotenoids in *d*₆-pyridine at 24 °C were measured using the UNITY INOVA-500 system (Varian).

Sequence analysis. The amino acid sequences of enzymes in *G. aurantiaca* are listed in the DOGAN database (<http://www.bio.nite.go.jp/dogan/Top>). Database searches were carried out with BLASTP against protein sequences from the UniProt Knowledgebase (<http://www.expasy.ch/>). The best-matched genes for carotenogenesis were searched.

RESULTS

Carotenoids in *G. aurantiaca*

Fig. 1 shows the C₁₈-HPLC elution profile of the carotenoids extracted from *G. aurantiaca*, with three carotenoid peaks. Compounds eluted at around 2–5 min and around 31 min were not carotenoids based on their absorption spectra. Carotenoids-1, -2, and -3 constituted 88%, 9% and 3%, respectively, of the total carotenoids.

Identification of carotenoid-1

The absorption maxima of carotenoid-1 in peak 1 of C₁₈-HPLC (Fig. 1) in the eluent of methanol/water (9:1, v/v) were 318, 387, 469, 494 and 528 nm (Fig. 2), and the spectral fine structure of %III/II was 43; that is the ratio of the peak heights of the longest and the middle wavelength absorption bands, as measured from the trough between the two peaks (Takaichi & Shimada, 1992). These results

Fig. 2. Absorption spectrum of carotenoid-1 in the HPLC eluent of methanol/water (9 : 1, v/v). AU, arbitrary units.

indicated that carotenoid-1 was an acyclic carotenoid with 13 conjugated double bonds (Takaichi & Shimada, 1992). The CD spectrum of acetylated carotenoid-1 in diethyl ether was compatible with that of (2*S*,2'*S*)-oscillol 2,2'-difucoside from the cyanobacterium *Gloeobacter violaceus* (Tsuchiya *et al.*, 2005). [Note that the CD spectrum of (2*R*,2'*R*)-oscillol shows the mirror CD spectrum of (2*S*,2'*S*)-oscillol.] Therefore, the stereochemistry at the C-2,2' hydroxyl groups was (2*S*,2'*S*). The relative molecular mass was 892, which was compatible with that of oscillol di-methylpentoside. For ¹H-NMR measurement, carotenoid-1 was acetylated due to low solubility in CDCl₃ and to prevent overlap with the signals in the glycoside region. Its ¹H-NMR analysis, including the chemical shifts (δ), multiplicity, and coupling constants (*J*), indicated that it was a symmetrical carotenoid (Table 1), and was compatible with the structure of the right half of acetylated myxol 2'-rhamnoside from the *wcaG* mutant of *Anabaena* sp. strain PCC 7120 (Mochimaru *et al.*, 2008). The glycoside moiety was α -L-rhamnoside, compatible with that of acetylated myxol 2'-rhamnoside, but not with those of fucoside and chinovoside (Mochimaru *et al.*, 2008; Takaichi *et al.*, 2005). Based on these results, the structure of carotenoid-1 was identified as (2*S*,2'*S*)-oscillol 2,2'-dirhamnoside (Fig. 3). The IUPAC-IUBMB semi-system-

Table 1. ¹H NMR spectral data for acetylated carotenoid-1 in CDCl₃

Protons	Acetylated carotenoid-1*	Acetylated myxol rhamnoside*†
Carotenoid moiety		
H ₃ -16	1.129 s	1.211 s
H ₃ -17	1.142 s	1.211 s
H ₃ -18	1.923 s	1.933 s
H ₃ -19	1.992 s	1.971 s
H ₃ -20	1.990 s	1.990 s
H-2	3.85 d (8.5)	3.85 d (9)
H-3	5.69 dd (8.5, 15.5)	5.69 dd (9, 15.5)
H-4	6.30 d (15.5)	6.30 d (15.5)
H-6	6.21 d (11)	6.20 d (11.5)
H-7	6.56 dd (11.5, 15.5)	6.56 dd (11.5, 15.5)
H-8	6.41 d (15.5)	6.40 d (15.5)
H-10	6.27 d (12)	6.27 d (11.5)
H-11	6.64 dd (12, 15.5)	6.64 dd (11.5, 15.5)
H-12	6.31 d (15)	6.31 d (15.5)
H-14	6.25 m	6.25 m
H-15	6.63 m	6.63 m
Sugar moiety		
H-1''	4.66 br s	4.66 br s
H-2''	5.50 br	5.50 d (3)
H-3''	5.01 dd (3, 10)	5.01 dd (3, 10)
H-4''	5.05 dd (10, 10)	5.06 dd (10, 10)
H-5''	3.47 dq (6.5, 10)	3.47 dq (6.5, 10)
H ₃ -6''	1.221 d (6.5)	1.22 d (6.5)
Acetyl	2.17 s, 2.19 s, 2.21 s	2.05 s, 2.17 s, 2.19 s, 2.21 s

* δ (p.p.m.), multiplicity (s: singlet, d: doublet, q: quartet, m: multiplet, br: broad) and coupling constants (Hz).

†Data for the right half of myxol and rhamnoside moieties are shown (Mochimaru *et al.*, 2008).

Fig. 3. Structure of selected carotenoids. Numbers in parentheses indicate the peak numbers in Fig. 1.

atic name is (2S,2'S)-2,2'-di-(α -L-rhamnosyloxy)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro- ψ,ψ -carotene-1,1'-diol.

In the HPLC elution profile (Fig. 1), there were two shoulders of peak 1, designated peak 1' and peak 1''. Since their absorption spectra were similar to that of carotenoid-1 but the *cis*-peak at 388 nm was high, they were considered as *cis*-forms of carotenoid-1.

Identification of carotenoid-2

The absorption spectrum of carotenoid-2 in peak 2 of C_{18} -HPLC (Fig. 1) in the eluent of methanol/water (9:1, v/v) was compatible with that of carotenoid-1. The CD spectrum of acetylated carotenoid-2 was also compatible with that of acetylated carotenoid-1. The relative molecular mass was 730. The $^1\text{H-NMR}$ spectrum of carotenoid-2 in d_6 -pyridine indicated that one half of the structure was compatible with the structure of carotenoid-1, whose signals of H_3 -16, -17, -18, -19 and -20 in d_6 -pyridine were found at 1.489, 1.452, 1.952, 2.011 and 2.011 p.p.m. (singlet), respectively; the other half of the structure was compatible with the structure of didemethylspirilloxanthin, whose signals of H_3 -16', -17', -18', -19' and -20' were found at 1.306, 1.306, 1.952, 2.011 and 2.011 p.p.m. (singlet), respectively, and those of H_2 -2' and H -3' were found at 2.55 p.p.m. (doublet, $J_{2'-3'}=7.5$ Hz) and 6.16 p.p.m. (doublet doublet, $J_{2'-3'}=7.5$ and $J_{3'-4'}=15$ Hz), respectively; further, the glycoside moiety was compatible with that of carotenoid-1. Consequently, the structure of carotenoid-2 was identified as (2S)-deoxyoscillol 2-rhamnoside (Fig. 3). The IUPAC-IUBMB semi-systematic name is (2S)-2-(α -L-rhamnosyloxy)-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro- ψ,ψ -carotene-1,1'-diol. This is a novel carotenoid (Britton *et al.*, 2004).

Identification of carotenoid-3

The absorption spectrum of carotenoid-3 in peak 3 of C_{18} -HPLC (Fig. 1) in the eluent of methanol was compatible with that of carotenoid-1. Based on the relative molecular mass of 568, the structure of carotenoid-3 was identified as didemethylspirilloxanthin (dideoxyoscillol) (Fig. 3). The IUPAC-IUBMB semi-systematic name is 3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro- ψ,ψ -carotene-1,1'-diol.

Since the absorption spectrum of peak 3', eluted just before peak 3 (Fig. 3), was similar to that of carotenoid-3 but the *cis*-peak at 388 nm was high, it was considered to be a *cis*-form of carotenoid-3.

Genes involved in synthesis of carotenoids and isopentenyl pyrophosphate

The entire nucleotide sequence of the genome in *G. aurantiaca* has been completed, and the ORFs have been completely annotated (DOGAN database, <http://www.bio.nite.go.jp/dogan/Top>). The annotated ORFs with the most related homologues for carotenogenesis genes and enzymes are listed in Table 2. The predicted genes for geranylgeranyl pyrophosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI), carotenoid 1,2-hydrotase (CruF) and carotenoid 2-O-rhamnosyltransferase (CruG) were found in the genome of *G. aurantiaca*, while the genes for carotenoid 3,4-desaturase and carotenoid 2-hydroxylase were not found. Isopentenyl pyrophosphate (IPP) may be synthesized by a non-mevalonate pathway (DXP pathway), since all of the homologous genes for this pathway – *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG* and *ispH* – which were compared with the well-known enzymes of *Escherichia coli*, were found, while those for the mevalonate pathway were not found.

DISCUSSION

G. aurantiaca strain T-27^T is an orange-coloured, Gram-negative, facultatively aerobic, polyphosphate-accumulating bacterium, belonging to a recently proposed phylum (Zhang *et al.*, 2003). In the present study, we identified all carotenoids in *G. aurantiaca*, including their stereochemical structures (Fig. 3). The major carotenoid was (2S,2'S)-oscillol 2,2'-di-(α -L-rhamnoside), and the minor carotenoids were (2S)-deoxyoscillol 2-(α -L-rhamnoside) and didemethylspirilloxanthin (dideoxyoscillol). Didemethylspirilloxanthin has hitherto only been found in *Polyangium fumosum* (Proteobacteria), which also contains didemethylspirilloxanthin glucoside fatty acid esters (Jansen *et al.*, 1995). Spirilloxanthin, which is the dimethylated form of didemethylspirilloxanthin, is a major carotenoid in purple photosynthetic bacteria (Proteobacteria) (Takaichi, 2009). Deoxyoscillol 2-rhamnoside is a novel carotenoid (Britton *et al.*, 2004).

The free form of oscillol is not found in any natural sources (Britton *et al.*, 2004), while oscillol 2,2'-di-methylpento-

Table 2. ORFs in *G. aurantiaca* homologous to genes encoding enzymes of isopentenyl pyrophosphate and carotenoid biosynthesis

ORF ID	Description	Gene	BLAST hits	Identity (%) / e-value
GAU_1866	1-Deoxy-D-xylulose-5-phosphate synthase	<i>dxs</i>	(DXS_ECOLI) <i>E. coli</i> K12	40/e-138
GAU_1770	1-Deoxy-D-xylulose-5-phosphate reductoisomerase	<i>dxr</i>	(DXR_ECOLI) <i>E. coli</i> K12	39/1e-72
GAU_1684	2-C-Methyl-D-erythritol-4-phosphate cytidyltransferase	<i>ispD</i>	(ISPD_ECOLI) <i>E. coli</i> K12	35/1e-25
GAU_1244	4-Diphosphocytidyl-2-C-methyl-D-erythritol kinase	<i>ispE</i>	(ISPE_ECOLI) <i>E. coli</i> K12	34/1e-12
GAU_1631	2-C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase	<i>ispF</i>	(ISPF_ECOLI) <i>E. coli</i> K12	40/3e-27
GAU_0148	4-Hydroxy-3-methyl-2-butenyl-1-diphosphate synthase	<i>ispG</i>	(ISPG_ECOLI) <i>E. coli</i> K12	36/6e-49
GAU_2758	4-Hydroxy-3-methyl-2-butenyl 2-diphosphate reductase	<i>ispH</i>	(ISPH_ECOLI) <i>E. coli</i> K12	30/9e-11
GAU_1867	Geranylgeranyl pyrophosphate synthase	<i>crtE</i>	(CRTE_RHOCA) <i>Rhodobacter capsulatus</i>	35/2e-26
GAU_2406	Phytoene synthase	<i>crtB</i>	(CRTB_SYNE7) <i>Synechococcus elongatus</i> PCC 7942	37/3e-43
GAU_2408	Phytoene desaturase	<i>crtI</i>	(CRTI_RHOCA) <i>Rhodobacter capsulatus</i>	41/e-106
GAU_2407	Carotenoid 1,2-hydratase	<i>cruF</i>	(B1XHV0_SYNP2) <i>Synechococcus</i> sp. PCC 7002	34/5e-029
GAU_1378	Carotenoid 2-O-glycosyltransferase	<i>cruG</i>	(B1XHU9_SYNP2) <i>Synechococcus</i> sp. PCC 7002	48/5e-090

sides are found only in some cyanobacteria (Hirschberg & Chamovitz, 1994; Takaichi & Mochimaru, 2007, 2009), for example oscillol 2,2'-difucoside and oscillol 2,2'-dirhamnoside in *Oscillatoria agardhii* (Foss *et al.*, 1986), and (2S,2'S)-oscillol 2,2'-di-(α -L-fucoside) in *Gloeobacter violaceus* (Tsuchiya *et al.*, 2005). The present identification of oscillol 2,2'-dirhamnoside in *G. aurantiaca* is the first in a bacterial phylum other than in cyanobacteria. The structure of oscillol (Fig. 3) is symmetrical and compatible with that of the right half of myxol. The presence of myxol 2'-methylpentoside and its derivatives is also limited to cyanobacteria, but they are not present in all cyanobacteria (Takaichi & Mochimaru, 2007, 2009). The presence of the free form of myxol (not glycosides) has only been reported in two species of the family *Flavobacteriaceae* (*Bacteroidetes*): in the marine bacterium strain P99-3 (MBIC 03313) (Yokoyama & Miki, 1995) and in the marine bacterium *Robiginitalea myxolifaciens* (Shindo *et al.* 2007; Manh *et al.*, 2008). The stereochemistry of the C-2 hydroxyl group in myxol (2'S) and oscillol (2S,2'S) is the same, and therefore the biosynthetic pathway of these structures would be similar. One cyanobacterial strain, *Anabaena variabilis* ATCC 29413, is reported to contain free myxol but to lack myxol 2'-methylpentoside; this could be due to loss of glycosyltransferase activity (Takaichi *et al.*, 2006). A similar carotenoid, (2S)-spirilloxanthin-2-ol, whose stereochemistry at the C-2 hydroxyl group is the same as that of oscillol, is found in a purple sulfur bacterium, *Thiocapsa* sp. strain BH-1 (*Proteobacteria*) (Herbert *et al.*, 2008). The relationships among the similar end-groups of these carotenoids and their distribution in bacteria are an interesting subject.

The glycoside moieties of oscillol 2,2'-diglycosides and myxol 2'-glycosides in cyanobacteria usually comprise one

of three methylpentoses: rhamnose, fucose or chinovose (Takaichi & Mochimaru, 2007, 2009). *G. aurantiaca* also contains carotenoids with a glycoside moiety, oscillol 2,2'-dirhamnoside. On the other hand, the glycoside moieties in carotenoid glycosides in other bacterial phyla, such as *Chlorobi*, *Chloroflexi* and *Proteobacteria*, are usually glucosides (Britton *et al.*, 2004; Takaichi, 2009). Methylpentosides are found only in *Cyanobacteria* and *Gemmatimonadetes*. As for the carotenoid synthesis, rhamnose-adding enzymes, e.g. rhamnosyltransferase, are hardly found in bacteria, unlike glucose-adding enzymes, which are commonly found in several bacterial phyla.

Our identification of the carotenoids here, and the completion of the entire nucleotide sequence of the genome in *G. aurantiaca* (<http://www.bio.nite.go.jp/dogan/Top>) will help elucidate its biosynthetic pathways and the corresponding enzymes and genes (Table 2). Fig. 4 presents a proposed biosynthetic pathway of the carotenoids in *G. aurantiaca*. The carotenogenesis genes seemed to be separated and distributed all over the genome, although a small cluster of *crtB*, *crtI* and *cruF* was found (Table 2). Such a distribution of carotenogenesis genes is also found in cyanobacteria (Takaichi & Mochimaru, 2007, 2009), while in purple photosynthetic bacteria they are generally located in one cluster (Takaichi, 2009).

IPP, from which carotenoids are synthesized via farnesyl pyrophosphate (Fig. 4), is likely to be synthesized by a non-mevalonate pathway (DXP pathway), since all of the homologous genes for the pathway have been identified (Table 2). However, the homologous genes for the mevalonate pathway were not found in the genome. Non-mevalonate pathways are found in many bacteria, such as purple photosynthetic bacteria, green sulfur

Fig. 4. Biosynthetic pathways of carotenoids and their enzymes in *G. aurantiaca*. Numbers in parentheses indicate the peak numbers in Fig. 1. See the text for further details.

bacteria (*Chlorobaculum* species), *Deinococcus* and cyanobacteria (Eisenreich *et al.*, 2004; Maresca *et al.*, 2008). We are only beginning to understand the relationship between the phylogeny and distribution of both pathways among bacteria (Eisenreich *et al.*, 2004). The pathway of the two *Flavobacteriaceae* species that produce the myxol described above was not investigated.

Our findings indicate that *G. aurantiaca* contains geranylgeranyl pyrophosphate synthase (CrtE), phytoene synthase (CrtB) and phytoene desaturase (CrtI) (Table 2, Fig. 4), by which lycopene is produced from IPP. The presence of didemethylspirilloxanthin suggested that both ends of lycopene were hydrated by carotenoid 1,2-hydratase and desaturated by carotenoid 3,4-desaturase. Further, for oscillol 2,2'-dirhamnoside synthesis, carotenoid 2-hydroxylase and carotenoid 2-O-rhamnosyltransferase should be present. Since the final product was oscillol 2,2'-dirhamnoside, and since deoxyoscillol 2-rhamnoside was an intermediate product, it is likely that, after hydroxylation at C-2 on one end-group by 2-hydroxylase, carotenoid 2-O-rhamnosyltransferase immediately produces rhamnoside at C-2, and then the other end group is similarly modified. Thus, it is likely that these two enzymes might be one complex, or that the activity of carotenoid 2-O-rhamnosyltransferase might be very high.

The functions of carotenoid 1,2-hydratase have been confirmed in two distinct enzymes: CrtC in both purple photosynthetic bacteria (*Proteobacteria*) (Takaichi, 2009) and *Chlorobaculum* (previously *Chlorobium*) *tepidum*

(*Chlorobi*) (Frigaard *et al.*, 2004); and CruF for myxol in *Synechococcus* sp. strain PCC 7002 (*Cyanobacteria*) (Graham & Bryant, 2009) and for deinoxanthin in *Deinococcus radiodurans* (*Deinococcus-Thermus*) (Sun *et al.*, 2009). The functions of carotenoid 3,4-desaturase have been confirmed: CrtD in purple photosynthetic bacteria (Takaichi, 2009), for myxol in the marine bacterium strain P99-3 (*Bacteroidetes*) (Teramoto *et al.*, 2004) and for deinoxanthin in *D. radiodurans* (Tian *et al.*, 2008). Although genes homologous to *crtD* are widely distributed in cyanobacteria, those of *Anabaena* sp. strain PCC 7120 and *Synechocystis* sp. strain PCC 6803 have not shown CrtD functions (Mochimaru *et al.*, 2008; Maresca *et al.*, 2008). The functions of carotenoid 2-hydroxylase have been confirmed in the CrtA-like enzyme (CrtA-OH) for myxol in the bacterium strain P99-3 (Rählert *et al.*, 2009), and those of carotenoid 2-O-glycosyltransferase (CruG) have been confirmed in *Synechococcus* sp. strain PCC 7002 (Graham & Bryant, 2009).

In the *G. aurantiaca* genome, genes homologous to *cruF* (carotenoid 1,2-hydratase) and *cruG* (carotenoid 2-O-glycosyltransferase) are found (Table 2); thus carotenoid 2-O-glycosyltransferase of *Synechococcus* sp. strain PCC 7002 might be carotenoid 2-O-rhamnosyltransferase in this bacterium. Homologies of the putative carotenoid 3,4-desaturase and carotenoid 2-hydroxylase genes are less than 30% to three functional *crtD* genes and to the functional *crtA-OH* gene described above, respectively (data not shown). Therefore, another type of carotenoid 3,4-desaturase and carotenoid 2-hydroxylase should exist in this bacterium. Thus, the carotenogenesis enzymes of oscillol 2,2'-dirhamnoside in *G. aurantiaca* are similar to those of myxol 2'-methylpentoside in cyanobacteria, but different from those of myxol in strain P99-3 (*Flavobacteria*). The enzymes for the biosynthesis of the end group of oscillol 2,2'-diglycosides have yet to be identified in cyanobacteria, except for two enzymes of *Synechococcus* sp. strain PCC 7002 (Graham & Bryant, 2009).

Oscillol 2,2'-dirhamnoside, observed in *G. aurantiaca*, was also found in the phylum *Cyanobacteria* (Britton *et al.*, 2004). The phylum *Gemmatimonadetes* is clearly distant from the phylum *Cyanobacteria* in the phylogenetic tree (Zhang *et al.*, 2003), and may have diverged at an early stage of microbial evolution (at least 3 billion years ago). Thus, it is a significant finding that oscillol is held in common between the different bacterial phyla. Our molecular evolutionary comparison of genes encoding enzymes related to oscillol synthesis between *G. aurantiaca* and certain species of *Cyanobacteria* revealed low sequence similarities among these enzymes (Table 2). Further, it strongly suggests that the origin of oscillol synthesis is quite old, and it is not due to a lateral transfer of these genes. In spite of a comprehensive survey of the many carotenoid-containing bacteria, oscillol has only been detected in the two phyla mentioned above. This suggests that the ability to synthesize oscillol was difficult to acquire, even given the long history of bacterial evolution.

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Edited by: M. Daffé