Review

New and Rare Carotenoids Isolated from Marine Bacteria and Their Antioxidant Activities

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Abstract: Marine bacteria have not been examined as extensively as land bacteria. We screened carotenoids from orange or red pigments-producing marine bacteria belonging to rare or novel species. The new acyclic carotenoids with a C30 aglycone, diapolycopenedioc acid xylosylesters A–C and methyl 5-glucosyl-5,6-dihydro-apo-4,4′-lycopenoate, were isolated from the novel Gram-negative bacterium Rubritalea squalenifaciens, which belongs to phylum Verrucomicrobia, as well as the low-GC Gram-positive bacterium Planococcus maritimus strain iso-3 belonging to the class Bacilli, phylum Firmicutes, respectively. The rare monocyclic C40 carotenoids, (3R)-saproxanthin and (3R,2′S)-myxol, were isolated from novel species of Gram-negative bacteria belonging to the family Flavobacteriaceae, phylum Bacteroidetes. In this review, we report the structures and antioxidant activities of these carotenoids, and consider relationships between bacterial phyla and carotenoid structures.

Keywords: diapolycopenedioc acid xylosylesters A–C; methyl 5-glucosyl-5, 6-dihydro-apo-4,4′-lycopenoate; (3R)-saproxanthin; (3R,2′S)-myxol; antioxidant activity
1. Introduction

Some species of bacteria, yeast, and fungi, as well as algae and higher plants, synthesize a large number of carotenoids with different molecular structures, and more than 750 carotenoids with different structures have been isolated from natural sources [1]. Many beneficial pharmaceutical effects of carotenoids have recently been reported. Therefore, evaluating the pharmaceutical potentials of various carotenoids may represent an interesting field in medical research. However, the number of carotenoid species that have been examined for this purpose has been limited, and has included \( C_{40} \) carotenoids possessing skeletons composed of 40 carbon atoms, such as dicyclic carotenoids, e.g., \( \beta \)-carotene, \( \alpha \)-carotene, \( \beta \)-cryptoxanthin, zeaxanthin, lutein, canthaxanthin, astaxanthin, and fucoxanthin, and the acyclic carotenoid lycopene [2–8]. Difficulties have been associated with identifying natural sources to supply sufficient amounts of new or rare carotenoids, with the exception of carotenoids that can be isolated from a species of higher plants or algae or chemically synthesized. It has therefore been desirable to find cultivable bacteria that produce new or rare carotenoids, since they can easily be reproduced.

Marine bacteria have not been examined as extensively as land bacteria. Thus, the Marine Biotechnology Institute Co., Ltd. (MBI, Kamaishi, Japan) was established in December, 1988, and continued to isolate novel or rare marine bacteria until March, 2008, the number of which reached more than ten thousand [9–12]. Many bacteria have been shown to produce dicyclic or monocyclic \( C_{40} \) carotenoids, in addition to some acyclic \( C_{30} \) carotenoids with a 30 carbon skeleton [1,13]. The MBI isolated new or rare dicyclic \( C_{40} \) carotenoids with the \( \beta \)-carotene (\( \beta,\beta \)-carotene) skeleton from Gram-negative marine bacteria belonging to the class \( \alpha \)-Proteobacteria, phylum Proteobacteria, e.g., astaxanthin glucoside from Paracoccus sp. strain N81106 (re-classified from Agrobacterium aurantiacum) [14], 2-hydroxyastaxanthin from Brevundimonas sp. strain SD212 [15], and 4-ketonostoxanthin 3′-sulfate from Erythrobacter sp. strain. PC6 (re-classified from Flavobacterium sp. PC-6; MBIC02351) [16]. These marine bacteria were also able to produce astaxanthin [17]. The carotenoid biosynthesis gene clusters of these marine bacteria have been elucidated in detail [17–19].

The generation of free radicals has been suggested to play a major role in the progression of a wide range of pathological disturbances, including myocardial and cerebral ischemia [20], atherosclerosis [21], renal failure [22], inflammation [23], and rheumatoid arthritis [24]. The subsequent peroxidative disintegration of cells and organelle membranes has also been implicated in various pathological processes [25]. Carotenoid pigments, which have been shown to possess strong antioxidant activities, have been attracting increasing attention due to their beneficial effects on human health, e.g., their potential to prevent diseases such as cancer and cardiovascular diseases [26].

We have attempted to identify novel or rare types of carotenoids from yellow or red pigment-producing marine bacteria that were classified to belong to rare or novel species by 16S rRNA analyses since 2002. The results of this screening led to the isolation of diapolydihydrodiacids xylosylesters A–C (new carotenoids) from Rubritalea squalenifaciens [27,28], methyl 5-glucosyl-5,6-dihydro-apo-4,4′-lycopenoate (a new carotenoid) from Planococcus maritimus [29], and (3R)-saproxanthin and (3R,2′S)-myxol (rare carotenoids) from a novel species belonging to the family
Flavobacteriaceae [30]. In this review, we report the structures and antioxidant activities of these carotenoids, and consider relationships between bacterial phyla and carotenoid structures.

2. Results

2.1. Diapolydopenedioc Acid Xylosylesters A–C from Rubritalea Squalenifaciens [27,28]

A yellow pigment-producing bacterium (strain HOact23\textsuperscript{T}) that was found to produce squalene was isolated from the homogenate of the marine sponge Halicondria okadai, which had been collected from the Miura peninsula (Kanagawa, Japan), and was subsequently classified as a novel species in the genus Rubritalea, belonging to phylum Verrucomicrobia, based on 16S rRNA gene sequence data. The name proposed for the new taxon was Rubritalea squalenifaciens [31], with the type strain HOact23\textsuperscript{T} (=MBIC08254\textsuperscript{T} = DSM 18772\textsuperscript{T}).

R. squalenifaciens was cultured in 100 mL of medium (1.0% starch, 0.4% yeast extract, and 0.2% peptone in seawater) in a 500 mL Erlenmeyer flask at 30 °C on a rotary shaker at 120 rpm for 2 days, and the carotenoids produced were purified from the cells using chromatographic methods (EtOAc/H\textsubscript{2}O partition → silica gel column chromatography CH\textsubscript{2}Cl\textsubscript{2}–MeOH (20:1) → preparative silica gel HPLC CH\textsubscript{2}Cl\textsubscript{2}–MeOH (15:1) → preparative ODS HPLC (MeOH)). Three carotenoids were purified from cells in the 42-liter culture (diapolydopenedioc acids xylosylesters A (1) 10.2 mg, B (2) 3.0 mg, and C (3) 2.2 mg, respectively). The structures of compounds 1–3 were determined by HRESI-MS and spectroscopic (UV-Vis, NMR (1D and 2D investigations on \textsuperscript{1}H and \textsuperscript{13}C nuclei), and \([\alpha]_D\) analyses as shown in Figure 1. Compounds 1–3 were all new carotenoids.

Compounds 1–3 possessed diapolydopenedioc acid (C\textsubscript{30} carotenoid) [32,33] as their aglycone. Diapolydopenedioic acid glucosyl ester and diapolydopenedioic acid diglucosyl were previously shown to be carotenoids that possessed diapolydopenedioic acid as the aglycone [32]. Compounds 1–3 were the first carotenoids to include 2-acyl-D-xylene in their structures.

The antioxidant activity of compound 1 was evaluated using \(^{18}O_2\) suppression activity. Its IC\textsubscript{50} was 5.1 \(\mu\text{M}\) (the IC\textsubscript{50} values of astaxanthin and \(\beta\)-carotene were 8.9 \(\mu\text{M}\) and >100 \(\mu\text{M}\), respectively).

Figure 1. The structures of diapolydopenedioic acids A (1), B (2) and C (3).

2.2. Methyl 5-Glucosyl-5,6-Dihydro-Apo-4,4′-Lycopenoate from Planococcus Maritimus [29]

A yellow pigment-producing bacterium (strain iso-3), which was found to be solvent-tolerant, was isolated as an orange-pigmented colony from the microbial analysis of a sample derived from an
The result obtained revealed that the two bacterial strains should be classified as novel species of the family Flavobacteriaceae.
Both 04OKA-13-27 and YM6-073 were cultured in 100 mL of medium (Marine Broth 2216, Difco) in a 500 mL Sakaguchi flask at 30 °C on a rotary shaker at 100 rpm for 1 day, and the carotenoids produced were each purified from the cells using chromatographic methods (EtOAc/H2O partition → silica gel column chromatography hexane–ethyl acetate (2:1) → preparative silica gel high performance thin layer chromatography (HPTLC; Merck, Darmstadt, Germany) CH2Cl2–MeOH (10:1) → preparative ODS HPLC (MeOH)). A total of 0.3 mg (04OKA-13-27) and 0.5 mg (YM6-073) of pure carotenoids were obtained from the cells of each 2 liter culture, and the carotenoids were identified as (3R)-saproxanthin (04OKA-13-27) (5) and (3R,2'S)-myxol (YM6-073) (6) by MS, 1H-NMR, and CD analyses, respectively (Figure 3).

The antioxidative activities of compounds 5 and 6 were examined using rat brain homogenate model. Compounds 5 and 6 showed potent antioxidant activities (IC50 2.1 μM (5) and 6.2 μM (6)) (IC50 10.9 μM (β-carotene)).

**Figure 3.** The structures of (3R)-saproxanthin (5) and (3R,2'S)-myxol (6).

3. Discussion

The MBI has isolated approximately 1000 pigment-producing marine bacteria. We selected 10 strains, which were identified as rare or novel species by 16S rRNA, including strain HOact23T (Rubritalea squalenifaciens sp. nov., phylum Verrucomicrobia), strain iso-3 (Planococcus maritimus, the class Bacilli, phylum Firmicutes), strain 04OKA-13-27 (novel species of the family Flavobacteriaceae), and strain YM6-073 (novel specie of the family Flavobacteriaceae) from these isolated bacteria.

We found two-type new C30 carotenoids diapolycondioc acid xylosylesters (compound 1–3) from HOact23T and methyl 5-glucosyl-5,6-dihydro-apo-4,4′-lycopenoate (compound 4) from iso-3 through the isolation and structural analyses of carotenoids produced by these strains. Acyclic C30 carotenoids were previously shown to be contained in land bacteria including Staphylococcus aureus, belonging to the class Bacilli, and the methanotrophs Methylobacterium rhodium (formerly Pseudomonas rhodos), belonging to the class α-Proteobacteria, and Methylomonas sp., belonging to the class γ-Proteobacteria [17,34]. Thus, acyclic C30 carotenoids are likely to widely exist in domain bacteria (prokaryotes), i.e., they are present not only in some low-GC Gram-positive bacteria, but also in some phyla in Gram-negative bacteria. The strong singlet-oxygen-quenching activities of our C30...
carotenoids also indicated that such C₃₀ carotenoids are promising as functional carotenoids, although these in vivo functional analyses have not yet been conducted.

We isolated two rare monocyclic C₄₀ carotenoids with one 3-hydroxy-β-ring ((3R)-saproxanthin (compound 5) from 04OKA-13-27 and (3R,2'S)-myxol (compound 6) from YM6-073), which belong to the family Flavobacteriaceae, phylum Bacteroidetes. (3R)-Saproxanthin has only previously been detected from Saprospira grandis, which belongs to the family Saprospiraceae, phylum Bacteroidetes [35]. Hence, marine bacterial strain 04OKA-13-27 was the second species to produce saproxanthin. (3R,2'S)-Myxol has only previously been detected in marine bacterial strain P99-3 (MBIC03313), belonging to the family Flavobacteriaceae [15], and in the cyanobacterium Anabaena variabilis ATCC 29413, phylum Cyanobacteria [36]. Therefore, marine bacterial strain YM6-073 was the third species to produce myxol. Myxoxanthophyll (myxol 2'-fucoside), which is widely distributed in phylum Cyanobacteria, contains myxol as its aglycone. These findings indicated that such monocyclic C₄₀ carotenoids with one 3-hydroxy-β-ring exist in phylum Bacteroidetes as well as phylum Cyanobacteria.

The carotenoids produced by the six other strains isolated were all zeaxanthin, which is a common carotenoid in domain bacteria. Our study may be effective for identifying rare and new carotenoids based on its ratio (4/10). In addition, all the rare and new carotenoids (1–6) isolated possessed potent antioxidant activities.

4. Conclusions

Marine bacteria are likely to produce carotenoids to protect themselves from activated oxygen produced by sunlight (mainly ¹⁰⁻²); therefore, their potent antioxidant activities were expected and reasonable. Therefore, the techniques performed in our study effectively identified new antioxidant carotenoids.

Author Contributions

Kazutoshi Shindo performed the experiments and wrote the text; Norihiko Misawa supervised the project and corrected the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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