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Marine bacterium *Seonamhaeicola algicola* strain CC1 as a potential source for the antioxidant carotenoid, zeaxanthin

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Abstract

Currently, there are only six species in the genus Seonamhaeicola, i.e., Seonamhaeicola aphaedonensis, S. algicola, S. marinus, S. acroporae, S. maritimus, and S. sediminis. These bacteria have typical yellow or orange color. Among the identified strains, only S. marinus that had been reported to have a yellow polyene flexirubin pigment. However, the presence of carotenoid pigments has not been reported in this genus. Recently, we successfully isolated a new strain, S. algicola strain CC1, bacterium that was found in association with a red seaweed, Halymenia sp., collected from the coast of South Malang, Indonesia. The strain was grown well in the Zobell marine agar 2216E producing yellowish pigments. According to the 16S rRNA sequencing analysis and BLAST search, the strain is closely related to S. algicola strain Gy8, with 99.78% identity. The pigment composition was separated and analyzed by a high-performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS) and the strain was found to produce zeaxanthin as the major component, which appeared at a retention time (t_R) of 28.89 min, showing a typical mass spectrum with a molecular ion at m/z 568.5 $[M]^+$ and four product ions at m/z 261.4 $[M-307]^+$, 476.6 $[M-92]^+$, 429.3 $[M-139]^+$, and 536.5 $[M-32]^+$. Other carotenoids, including zeaxanthin cis isomers, β-cryptoxanthin, β-carotene cis isomer, and β-carotene, are as minor components. The novel and noteworthy finding of this report is the identification of a Seonamhaeicola species that produces carotenoids and can be used as a source of zeaxanthin.

Keywords: *HPLC, marine bacterium, mass spectrometry, Seonamhaeicola, Zeaxanthin*

Introduction

Zeaxanthin [(3R,3'R)-dihydroxy-β-carotene] is a fat-soluble antioxidant belonging to the xanthophyll class of carotenoids. It is composed of 40 carbon atoms and contains two β-rings that are substituted at the 3 and 3' carbons. Zeaxanthin contributes to the yellow color of paprika fruit, corn, saffron, wolfberries, and some microorganisms (Pérez-Gálvez *et al.*, 2003; Sajilata *et al.*, 2008; Perry *et al.,* 2009). In plants and algae, zeaxanthin plays a photoprotective role, involves the prevention of singlet oxygen generation due to excess of excitation energy under strong light conditions. Zeaxanthin may be produced in response to long-term environmental stress and for the photoprotection of the photosynthetic apparatus, however, this photoprotection function varies from species to species in plants and algae (Mozaffarieh *et al.*, 2003; Galasso *et al.*, 2017). Zeaxanthin is also called a macular pigment, highly concentrated in the macula region of the retina and is responsible for our fine-feature vision. In the food industry, zeaxanthin, known as E161h food dyes, has considered as a functional food ingredient, that contribute to prevent the progression of age-related macular degeneration (AMD) (Asker *et al.*, 2018).

Microbial pigments have recently garnered an increasing attention, especially that they can be controlled in the culture for high productivity in a limited space and in a short time (Zhang *et al.*, 2018; Ramesh *et al.*, 2019). Several marine bacterial cultures have been reported to produce zeaxanthin (Zhang *et al.,* 2018). At the time of writing this report, 22 marine bacteria have been identified to have zeaxanthin. The majority (82%) of zeaxanthin-producing species are from the family Flavobacteriaceae (Miki *et al.,* 1996; Asker *et al.,* 2007a; Asker *et al.,* 2007b; Kahng *et al.,* 2010; Yoon *et al.,* 2010; Hameed *et al.,* 2012; Hameed *et al.,* 2013; Prabhu *et al.,* 2013; Subhash *et al.,* 2013; Hameed *et al.,* 2014a; Hameed *et al.,* 2014b; Lee *et al.,* 2014; Shahina *et al.,* 2014; Takatani *et al.,*

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2014; Hameed *et al.,* 2015; Hu *et al.,* 2015; Lee *et al.,* 2016; Motone *et al.,* 2020). The other four species are including the families Rhodobacteraceae (Berry *et al.,* 2003), Cytophagaceae (Thawornwiriyanun *et al.,* 2012), Sphingomonadaceae (Shindo *et al.,* 2007), and Erythrobacteraceae (Choi *et al.,* 2018). Approximately half of these taxa were obtained from seawater. The remaining bacteria were isolated from other sources, such as sea sediment, sea soil, sea sand, penguin feces, marine invertebrates (sponge and coral), and seaweed.

The genus *Seonamhaeicola* (S.) belongs to the family Flavobacteriaceae (Park *et al.,* 2014). Currently, only six species are known, *i.e., S. aphaedonensis, S. algicola, S. marinus, S. acroporae, S. maritimus, and S. sediminis* (Park *et al.,* 2014; Zhou *et al.,* 2016; Fang *et al.,* 2017; Yoon *et al.,* 2018; Yu *et al.,* 2020; Cao *et al.,* 2020). These six species have been isolated from different media and locations. The main phenotypic characteristic of this genus is its yellow or orange color. Flexirubin-type pigments were detected in *S. marinus* strain B011, while the visible absorption spectrum of pigments did not show the present carotenoid (Fang *et al.,* 2017). At the time this article was written, no additional reports corresponding to carotenoid pigments produced by *Seonamhaeicola* were available.

Materials and Methods

Bacterial isolation and purification

The bacterium was isolated from Kondang Merak, South Malang, East Java. Sample of *Halymenia* sp. was stored in sterile plastic bags and then kept in a cold box for temporary storage. The sample was brought to the laboratory for immediate isolation of the symbiotic bacteria. The isolation of symbiont bacteria was carried out by cleaning with sterile seawater and cutting out *Halymenia* sp. into small pieces using sterile knife, to prevent contamination of other microorganisms. The sample was then put into a test tube containing sterile seawater for dilutions ranging from 10^{-1} to 10^{-6}. Afterward, the purification was carried out by taking each colony resulting from morphological identification and then was cultured using the scratch method. To do this, each dilution (35 µL) was taken and spread on the surface of Zobell marine agar 2216E (Difco, USA) media using a spreader and incubated for 3 days at 32°C. The yellow and orange colonies were selected and purified.

DNA extraction

Extraction of bacterial symbiont DNA was done by Chelex 100 method (Walsh *et al.,* 2013). This metod was carried out by inserting a bacterial culture (24 h) into an Eppendorf tube containing 50-100 µL sterile ddH_2O and then adding 1 mL 0.5% saponins in 1x PBS, stored at -20°C overnight. The sample was centrifuged at 8,000 ×g for 10 minutes, then the supernatant was discarded. The precipitate was re-suspended in 1 mL of PBS once, then centrifuged at 12,000 rpm for 5 min and then the supernatant was discarded. Afterward, the precipitate was re-suspended in 100 µL sterile ddH_2O and 50 µL 20% Chelex 100 and shaked well to ensure that Chelex granules enter the sample. The final solution was boiled for 10 min and vortexed for 5 min. The sample was then centrifuged at 12,000 rpm for 10 min. The supernatant (DNA extract) was stored at -20°C.

PCR amplification of the 16S rRNA gene sequence

Denaturation was carried out at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The next step was the final extension at 72°C for 7 min. The primers used for the 16S rRNA were universal primers, namely 27F (5'AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'TACGGTTAACCTTGTTACGACTT-3') primers (Lee *et al.,* 2006). The PCR mixture consisted of Promega GoTaq®Green Master Mix (25 µL), primer 27F (1 µM), primer 1492R (1 µM), DNA extract (2 µL, 50-100 ng), and nuclease-free water (50 µL). These materials were put into Eppendorf tubes then run according to the above temperature treatment. Mixing all ingredient was done in a coolbox filled with ice to prevent damage.

DNA sequencing of the 16S rRNA gene and BLAST search

DNA sequencing was performed at PT Genetika Science, Jakarta and 1st Base, Malaysia, using Big Dye Terminator v.3.1. and a DNA sequencer (ABI 3130XL instrument, Applied Biosystems, Carlsbad, California, USA). Database searches were carried out using the internet through the Basic Local Alignment Search Tool (BLAST) database tracking program in the GenBank database of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov). The 16S rRNA option was selected on the BLAST nucleotide selection.

Phylogenetic analysis

The ClustalX program was used to conduct alignment and MEGA 6 software was used for

phylogenetic analysis. The Kimura's two-parameters analysis was implemented. A bootstrap analysis was then performed to evaluate the result of tree topology. This analysis was using the neighbor-joining method based on 1,000 resamplings (Kamei and Isnansetyo, 2002).

Carotenoid extraction

A total of 0.1 g dry weight bacterial cells was mixed with an extraction solution, composed of 0.7 mL of methanol and 0.3 mL of acetone. Sodium ascorbate and $CaCO_3$ were added to prevent carotenoid degradation from oxidation. The mixture was then homogenized 5 times by vortexing for 1 min and 1 min on ice. Afterward, sonication (QSonica, Newtown, Connecticut, USA) was used to break the cell using in pulse mode with a 60% amplitude and 10 s on/30 s off for 10 min. After cell disruption, the carotenoid extracts were separated by centrifugation (Kubota 6500, Tokyo, Japan) at 19,230×g for 5 min at 4°C. The supernatants were taken and immediately dried in a rotary evaporator (Heidolph Laborota 4010 digital, Schwabach, Germany) at speed of 100 rpm and 35°C. The dried carotenoid extract was kept at a temperature of −30°C

Spectrophotometric analysis

The dried carotenoid extract was diluted in 1 mL of methanol, and the absorbance was measured using a Shimadzu UV−Vis 1700 spectrophotometer (Shimadzu, Kyoto, Japan) at wavelengths (λ) of 200–1100 nm. The spectrum data was processed using OriginPro 8.5.1 software (OriginLab, Northampton, Massachusetts, USA). The carotenoid spectra and the maximum wavelength were then compared with several references (Zapata *et al.,* 2000; Britton *et al.,* 2004; Setiyono *et al.,* 2019; Setiyono *et al.,* 2020).

HPLC separation and purification of carotenoids

The crude carotenoid was separated and analyzed using an analytical-HPLC (Shimadzu analytical-UFLC) through a Symmetry C8 column (150 x 4.6 mm, 3.5 μm particle size, 100 Å pore size) (Waters, Milford, MA, USA) (Setiyono *et al.,* 2019). The mobile phase used two eluents: eluent A contained methanol:acetonitrile:pyridine solution (0.25 M, pH 5) = 50:25:25, v/v/v) and eluent B contained methanol:acetonitrile:acetone (20:60:20, v/v/v). The gradient elution program setting was 100% eluent A (0 to 22 min), 60% eluent A (22 to 28 min), 5% eluent A (28 to 38 min), and 100% eluent A (40 to 50 min). The flow rate was 1 $mL.min^{-1}$. The column was kept at a constant temperature at 30 °C. A diode array detector (Shimadzu SPD M20A, 190–800 nm) was activated to detect carotenoid at wavelenght 370, 450, and 530 nm. At each peak identified as carotenoid was based on spectral and chromatographic properties. The purified carotenoids were stored in a deep freezer at −85°C until use. Standard carotenoids, β-carotene and zeaxanthin were obtained from NATChrom (Malang, East Java, Indonesia). While the standard β-cryptoxanthin was obtained from the purification from the peels of the Japanese citrus unshu mikan (Citrus unshiu).

MS/MS analysis

An electron spray ionization-mass spectrometer with a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu) was used to determine the molecular ion mass of the carotenoid. To performe analysis, the highly purified carotenoid was injected into an HPLC system using an isocratic elution of 0.1% formic acid in a mixture of methanol (90%) and water (10%) for 2 min at a flow rate of 0.3 $mL.min^{-1}$. Column was always kept at temperature of 30°C. While the MS parameters included DL temperature of 250°C, nebulizing gas flow rate of 3 $L.min^{-1}$, heat block temperature of 400°C, drying gas flow rate of 15 $L.min^{-1}$, and cooler temperature of 5 °C. First, Q1Q3 scans in the positive and negative modes was conducted with a scan speed of 6000 u/s. After the precursor ion was found, the product ion scan was performed, and subsequently MRM analysis was carried out to optimize the collision energy (CE). The chemical structures of the identified compounds were drawn using ChemDraw software version 12.0.2 (PerkinElmer, Inc., Massachusetts, USA). The identification process was done by comparing chromatographic and spectral data recorded with the standard carotenoids stored that was already in the library using LabSolution LCMS Ver. 5.4 (Shimadzu). This software automatically aligned the retention times and MS spectrum, and then calculate a match factor and the degree of similarity between spectra (Setiyono *et al.,* 2019; Setiyono *et al.,* 2020).

Result and Discussion

Exploration of new microbial sources to produce carotenoids is important as an alternative to synthetic carotenoids. To explore the potential of marine bacteria to this end, we successfully isolated *S. algicola* strain CC1, a bacterium that is associated with a red seaweed, which was collected from the coast of Kondang Merak, South Malang, East Java, Indonesia. The bacterium was successfully cultivated and consistently produced yellowish pigments. The pigment from cells of *S. algicola* strain CC1 was then extracted using a methanol:acetone (7:3, v/v) mixture, and its UV-Vis absorption spectrum was recorded. The absorption

spectrum exhibited a typical absorption spectrum of carotenoids, i.e., two prominent absorption bands with λ_{max} at 450 and 470 nm and a shoulder with λ_{max} at 426 nm (Figure 1.).

PCR amplification of 16S rRNA gene with the universal primers and genomic DNA of *S. algicola* strain CC1 yielded 1367 bp fragments (Figure 2a.). The similarity of the 16S rRNA gene of strain CC1 was determined according to the NCBI nucleotide BLAST program using the MegaBLAST program that selects highly similar nucleotide sequences. Based on the BLAST results of nucleotide sequence similarity analyses with other marine bacteria, CC1 was affiliated with the genus *Seonamhaeicola* and presented 99.78%, 96.94%, and 95.84% identity to *S. algicola* strain Gy8, *S. marinus* strain B011, and *S. aphaedonensis* strain AH-M5, respectively. The phylogenetic tree constructed with the neighbor-joining method showed that strain CC1 and *S. algicola* strain Gy8 established a strong phyletic clade with a bootstrap value of 100%. The result was also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms, and the recovered nodes were marked with an asterisk, indicating that strain CC1 belongs to the genus *Seonamhaeicola* (Figure 2b.). In addition, based on the results of the BLAST search and phylogenetic positions of their 16S rRNA sequences, CC1 was designated *S. algicola* strain CC1. The nucleotide data from S. algicola strain CC1 have been deposited in the DNA Data Bank of Japan (DDBJ, https://ddbj.nig.ac.jp) under accession number LC435076.

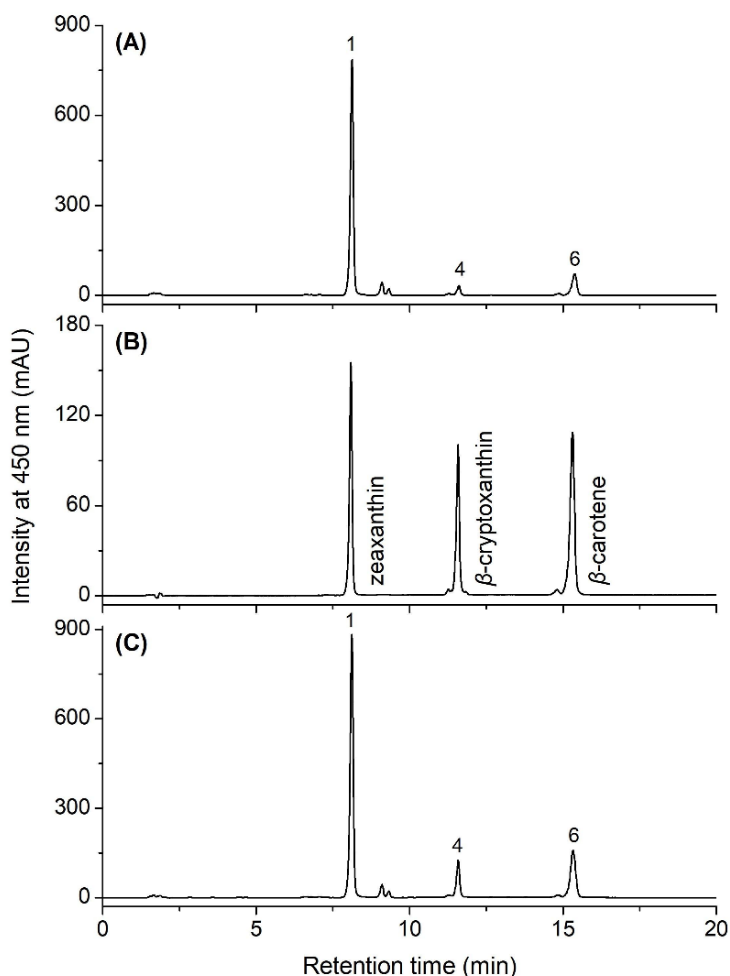

Figure 1. HPLC elution profile and the co-chromatography analysis. The column and eluent are same as those used in method of separation and purification of the carotenoids. However, the method used refers to the following different gradient, 60% eluent A (0 to 6 min), 5% eluent A (6 to 20 min), 60% eluent A (20 to 30 min). (A) HPLC chromatogram of carotenoids extract of *S. algicola* strain CC1, (B) HPLC chromatogram of standard zeaxanthin, β-cryptoxanthin, and β-carotene. (C) HPLC co-chromatogram using a mixture of standards and carotenoids extract of *S. algicola* strain CC1. Compounds 1, 4, 6 have same retention time to carotenoids standard. On the other hand, those compounds increase significantly after added with standard carotenoids.

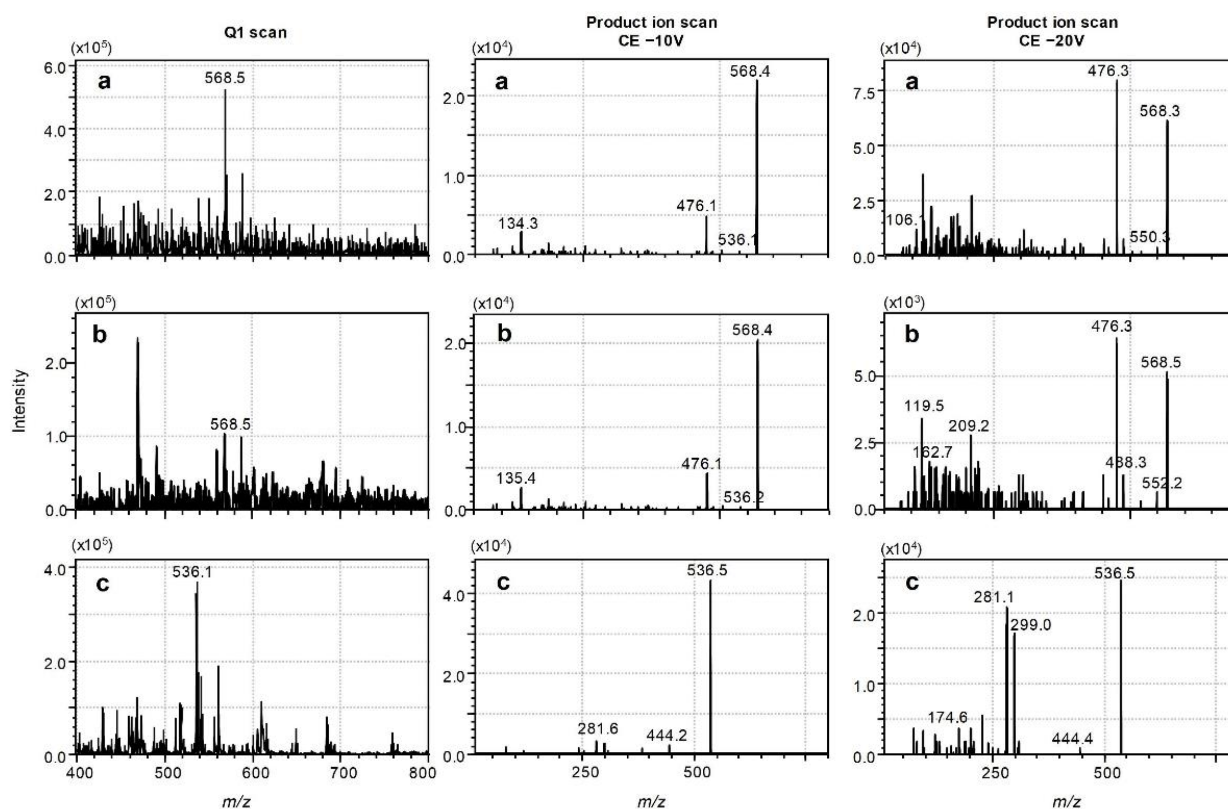

Figure 2. ESI mass spectra of the Q1 scan and product ion scan. The optimized CE was used at −10 V and −20 V from all-*trans*-carotenoids in *S. algicola* strain CC1. a Zeaxanthin, b *β*-cryptoxanthin, and c *β*-carotene

S. algicola strain Gy8 is a unique and rarely discussed marine bacterial species. It is a yellow, motile, rod-shaped, gram-negative marine bacterium. This species was associated with the red algae *Gracilaria blodgettii*, which was found in the Lian port of LingShui County, China (Zhou *et al.,* 2016). Red algae are known as sources of polysaccharides, including agar and alginate (Usov 2011), which can be hydrolyzed by *S. algicola* strain Gy8 into various carbon sources, such as maltose and lactose (Zhou *et al.,* 2016). In this report, we did not study whether *S. algicola* strain CC1 could perform this function; however, we cultivated the bacterium on marine agar 2216E and observed that the production of carotenoids could be maintained. *S. algicola* strain CC1 revealed only 96.94% similarity with *S. marinus* strain B011, which produced flexirubin pigments. *S. marinus* strain B011 was also originally isolated from a red alga. Thus, red algae might harbor a rich diversity of associated microorganisms, such as from the genus Seonamhaeicola, which interact with their hosts (Egan *et al.,* 2013; Lachnit *et al.,* 2016).

The carotenoids from *S. algicola* strain CC1 were separated and purified using a C8 reverse-phase column (Figure 1a.). HPLC chromatograms of *S. algicola* strain CC1 revealed an elution profile with six different compounds, 1, 2, 3, 4, 5 and 6, which exhibited the typical absorption bands of carotenoids such as zeaxanthin or β-carotene (Figure 3). The carotenoids included three all-trans carotenoids, namely, compounds 1, 4, and 6. The other three carotenoids, 2, 3, and 5, were cis isomers. The first compound, 1, was the major and most polar carotenoid eluted at a retention time (t_R) of 28.89 min, with λ_{max} at 453 and 480 nm. Compounds 4 and 6 had similar λ_{max} values as 1 and were eluted at 33.66 and 37.88 min, respectively, showing λmax at 453 and 479 nm. The minor compounds 2, 3, and 5 were identified as cis isomers of the carotenoids based on the additional absorption bands that appeared at a λ_{max} of approximately 337−340 nm. All compounds exhibited similar absorption spectra. Thus, all carotenoids produced by strain CC1 contained the same conjugated double bond. Previously it was reported that the difference in the number of hydroxyl compounds that attached at the end rings did not show significant alteration on the spectral properties of its carotenoids (Setiyono *et al.,* 2019), but the number of conjugated double bonds in the main polyene chain did. Three standard carotenoids, *i.e.,* zeaxanthin, β-cryptoxanthin, and β-carotene, were injected into HPLC using the same method for the separation of carotenoids from the extract of *S.*

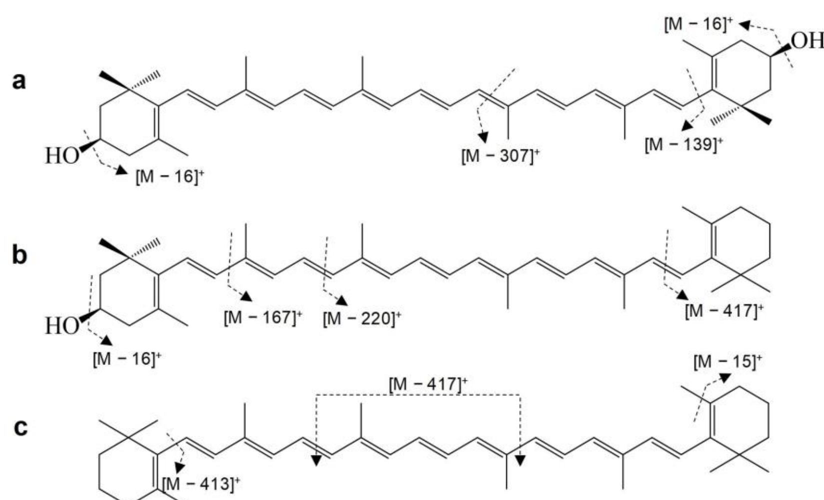

Figure 3. The chemical structures with possible fragments of identified carotenoid. Fragment analysis was based on the product ion spectral data shown in Table 1 for **a** zeaxanthin, **b** *β*-cryptoxanthin, and **c** *β*-carotene.

algicola strain CC1. Compounds 1, 4, and 6 displayed the same retention time (Figure 1a,b.) and the same UV-Vis spectrum (Figure 3.), indicating that these compounds are zeaxanthin, β-cryptoxanthin, and β-carotene, respectively. The linear relationship between the log capacity factor (k') of those compounds and the number of hydroxyl moieties of the carotenoid molecule instead of carbon atoms of the alcohol molecule (Figure 4.), as reported previously for esterified alcohols in chlorophylls and hydroxyl moieties in β-carotene congeners (Setiyono *et al.,* 2019) was used to identify and confirm the compounds 1, 4, and 6. All six compounds were purified to > 97% for identification using ESI-MS/MS. The carotenoids identified in this extract are listed in Table 1.

Trans and cis carotenoids were purified and isolated from the carotenoid extract using HPLC prior to analysis with ESI-MS to determine the molecular masses of the pigments. A fragmentation study to obtain the masses of product ions from a precursor ion was conducted using CE –10 and –20 V. The major carotenoid, 1, eluted at 28.89 min and presented a molecular ion at *m/z* 568.5 $[M]^+$ and four product ions at *m/z* 261.4 $[M−307]^+$, 476.6 $[M−92]^+$, 429.3 $[M−139]^+$, and 536.5 $[M−32]^+$ (Figure 2a.). The comparison of UV-Vis spectra (Figure 3.) along with the molecular and product ions detected corresponded to zeaxanthin, (3S,3'S)-β,β-carotene-3,3'-diol (Britton *et al.,* 2004). Therefore, compound 1 was identified as zeaxanthin. Carotenoid 4 (33.36 min) was consistent with those of β-cryptoxanthin (Britton *et al.,* 2004), determined to have a *m/z* of 552.3 $[M]^+$, and five product ions were detected at *m/z* 135.2 $[M−417]^+$, 460.4 $[M−92]^+$, 332.1 $[M−220]^+$, 385.2 $[M−167]^+$, and 536.0 $[M−16]^+$ (Figure 2b.). Therefore, compound 4 was identified as β-cryptoxanthin. The most nonpolar carotenoid, 6, exhibited a molecular ion at *m/z* 536.5 $[M]^+$ and four product ions at *m/z* 123.4 $[M−413]^+$, 444.5 $[M−92]^+$, 119.4 $[M−417]^+$, 521.8 $[M−15]^+$ (Figure 2c.) and corresponded to β-carotene (Britton *et al.,* 2004). Thus, compound 6 was identified as β-carotene. Based on the molecular and product ion data, we conducted a fragmentation study to determine the positions of lost ions in the chemical structure of carotenoids 1, 4, and 6, as shown in Figure 3.

The Q1 and product ion scans of three minor carotenoids, 2, 3, and 5, are shown in Figure 3. Compounds 2, 3, and 5 are cis isomers of carotenoids according to the additional λ_{max} at 337 and 340 nm. Carotenoids 2 and 3 had the same molecular ions as carotenoid 1, with *m/z* at 568.5 $[M]^+$. However, carotenoid 5 showed the same molecular weight as carotenoid 6 at *m/z* 536.5 $[M]^+$. Therefore, carotenoids 2 and 3 were identified as zeaxanthin *cis* isomers, and carotenoid 5 was identified as the β-carotene cis isomer based on the UV-Vis spectra (Figure 3.) and molecular mass (Figure 3.). To the best of our knowledge, this study is currently the first to report the carotenoids present in the genus *Seonamhaeicola*. The results of the identification of carotenoids from *S. algicola* strain CC1 using ESI-MS are presented in Table 1.

S. algicola strain CC1 produced zeaxanthin at high concentrations. Based on our HPLC analysis above, the relative content of zeaxanthin comprised more than 80% of the total carotenoid content in S. algicola strain CC1, while β-cryptoxanthin (7%) and β-carotene (5%) were minor carotenoids. Several

Table 1. Identification of carotenoids in *S. algicola* strain CC1

Compounds	Identification	Compound formula	t_R (min)	λ_{max} (nm)	Molecular ion (m/z)	Product ion (m/z)	CE (V)
1	Zeaxanthin	$C_{40}H_{56}O_2$	28.89	(426), 453, 480	568.5 $[M]^+$	476.6 $[M - 92]^+$	−10
						261.4 $[M - 307]^+$	−10
						429.3 $[M - 139]^+$	−20
						536.5 $[M - 32]^+$	−20
2	Zeaxanthin *cis* isomer	$C_{40}H_{56}O_2$	30.36	340, (426), 447, 472	568.5 $[M]^+$	476.1 $[M - 92]^+$	−10
						536.1 $[M - 32]^+$	−10
						119.5 $[M - 449]^+$	−20
						550.3 $[M - 18]^+$	−20
3	Zeaxanthin *cis* isomer	$C_{40}H_{56}O_2$	30.83	337, (426), 448, 474	568.5 $[M]^+$	476.1 $[M - 92]^+$	−10
						536.2 $[M - 32]^+$	−20
						430.1 $[M - 129]^+$	−20
						552.2 $[M - 16]^+$	−20
4	*β*-cryptoxanthin	$C_{40}H_{56}O$	33.66	(426), 453, 479	552.3 $[M]^+$	460.4 $[M - 92]^+$	−10
						135.2 $[M - 417]^+$	−10
						332.1 $[M - 220]^+$	−20
						385.2 $[M - 167]^+$	−20
						536.0 $[M - 16]^+$	−20
5	*β*-carotene *cis* isomer	$C_{40}H_{56}$	37.28	337, (424), 449, 474	536.5 $[M]^+$	444.2 $[M - 92]^+$	−10
						281.6 $[M - 252]^+$	−10
						174.6 $[M - 362]^+$	−20
						299.0 $[M - 237]^+$	−20
6	*β*-carotene	$C_{40}H_{56}$	37.88	(426), 452, 479	536.5 $[M]^+$	444.5 $[M - 92]^+$	−10
						123.4 $[M - 413]^+$	−10
						119.4 $[M - 417]^+$	−20
						521.8 $[M - 15]^+$	−20

marine bacteria that have been reported to produce zeaxanthin include from the genera *Zeaxanthinibacter, Mesoflavibacter, Hyunsoonleella, Muriicola, Siansivirga, Kordia, Aquibacter, Gramella, Hanstruepera, Paracoccus, Jejuia, Flexibacter, Sphingomonas, Gaetbulimicrobium, Muricauda, Erythrobacter,* and *Flavobacterium* (Table 1). Zeaxanthin is known to accumulate via the xanthophyl cycle in response to photooxidative damage under excessive light exposure and induces NPQ by dissipating excess excitation energy (Müller *et al.,* 2001; Kalituho *et al.,* 2007). Recently, a zeaxanthin-producing bacterium isolated from the algal phycosphere was reported to be able to protect coral endosymbionts from increased production of reactive oxygen species (ROS) under light stresses (Motone *et al.,* 2020). Further study to understand how this bacterium metabolizes zeaxanthin and its function is currently ongoing.

Conclusion

S. algicola strain CC1 had been successfully isolated and cultivated on marine agar 2216E. Based on the 16S rRNA sequencing analysis and BLAST search, the strain is closely related to *S. algicola* strain Gy8, with 99.78% identity. *S. algicola* strain CC1 produced zeaxanthin as the major carotenoid and other carotenoids, i.e., β-cryptoxanthin and β-carotene.

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