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 polyeone 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 (tR) 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−H]+, 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; Hamed 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., 2021).
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 symbiotic 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 and then run according to the above temperature treatment. Mixing all ingredient was done in a coolbox filled with ice to prevent damage.

**DNA extraction**

Extraction of bacterial symbiont DNA was done by Chelex 100 method (Walsh et al., 2013). This method was carried out by inserting a bacterial culture (24 h) into an Eppendorf tube containing 50-100 µL sterile ddH₂O and then adding 1 mL 0.5% saponins in 1x PBS, stored at -20°C overnight. The sample was centrifuged at 8,000 xg 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 minutes. The supernatant was stored at -20°C.

**PCR amplification of the 16S rRNA gene sequence**

Denaturation was carried out at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The next step was the final extension at 72°C for 7 minutes. The primers used for the 16S rRNA were universal primers, namely 27F (5'AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'TACGCTTAACCTGTAGACT3') 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 CaCO3 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. 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 wavelength 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 unshii).

**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 perform 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. 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 $\lambda_{\text{max}}$ at 450 and 470 nm and a shoulder with $\lambda_{\text{max}}$ 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://ddeb.nig.ac.jp) under accession number LC435076.

![Figure 1. HPLC elution profile and the co-chromatography analysis](image)

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, $\beta$-cryptoxanthin, and $\beta$-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.
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 (Ussov, 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 $\lambda_{\text{max}}$ at 453 and 480 nm. Compounds 4 and 6 had similar $\lambda_{\text{max}}$ values as 1 and were eluted at 33.66 and 37.88 min, respectively, showing $\lambda_{\text{max}}$ 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 $\lambda_{\text{max}}$ 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 carotenoids. 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'-dil (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 λmax 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 **algicola** strain CC1 using ESI-MS are presented in Table 1.

**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 **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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Identification</th>
<th>Compound formula</th>
<th><em>t</em> (min)</th>
<th><em>λ</em>&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>Molecular ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>CE (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zeaxanthin</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>28.89</td>
<td>(426), 453, 480</td>
<td>568.5 [M]+</td>
<td>476.6 [M − 92]+</td>
<td>−10</td>
</tr>
<tr>
<td>2</td>
<td>Zeaxanthin cis isomer</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>30.36</td>
<td>340, (426), 447, 472</td>
<td>568.5 [M]+</td>
<td>476.1 [M − 92]+</td>
<td>−10</td>
</tr>
<tr>
<td>3</td>
<td>Zeaxanthin cis isomer</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>30.83</td>
<td>337, (426), 448, 474</td>
<td>568.5 [M]+</td>
<td>430.1 [M − 129]+</td>
<td>−10</td>
</tr>
<tr>
<td>4</td>
<td>β-cryptoxanthin</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>33.66</td>
<td>(426), 453, 479</td>
<td>552.3 [M]+</td>
<td>460.4 [M − 92]+</td>
<td>−10</td>
</tr>
<tr>
<td>5</td>
<td>β-carotene cis isomer</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>37.28</td>
<td>337, (424), 449, 474</td>
<td>536.5 [M]+</td>
<td>444.2 [M − 92]+</td>
<td>−10</td>
</tr>
<tr>
<td>6</td>
<td>β-carotene</td>
<td>C&lt;sub&gt;40&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>37.88</td>
<td>(426), 452, 479</td>
<td>536.5 [M]+</td>
<td>444.5 [M − 92]+</td>
<td>−10</td>
</tr>
</tbody>
</table>

marine bacteria that have been reported to produce zeaxanthin include from the genera *Zeaxanthinibacter*, *Mesoflavibacter*, *Hyunsoonlyella*, *Muriola*, *Siansivirga*, *Kordia*, *Aquiabacter*, *Gramella*, *Hantruepera*, *Paracoccus*, *Jejuia*, *Flexibacter*, *Sphingomonas*, *Gaetbulimicrobium*, *Muricauda*, *Erythrobacter*, and *Flavobacterium* (Table 1). Zeaxanthin is known to accumulate via the xanthophyl cycle in response to photodamage by 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 photosphere 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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