Identification of flexixanthin and its derivatives in *Algoriphagus* bacteria and evaluation of their antioxidant and anti-inflammatory effects

Naoki Takatani[1,] *, Yuki Sato-Takabe[2], Misaki Aoike[1], Rika Sekine[3], Takashi Maoka[4], Tomoo Sawabe[1], Fumiaki Beppu[1], Kazutoshi Shindo[3], Masashi Hosokawa[1]

[1] Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan

[2] School of Economics, Senshu University, 2-1-1 Higashi-Mita, Tama-Ku, Kawasaki-Shi, Kanagawa, 214-8580, Japan

[3] Department of Food and Nutrition, Japan Women's University, 2-8-1 Mejirodai, Bunkyo-ku, Tokyo 112-8681, Japan

[4] Research Institute for Production Development, 15 Shimogamo-morimoto-cho, Sakyo-ku, Kyoto 606-0805, Japan

*Corresponding author

Naoki Takatani

E-mail: n-takatani@fish.hokudai.ac.jp

Tel & Fax: +81-138-40-8892

Abstract

The genus *Algoriphagus* comprises a group of red-colored, Gram-negative, aerobic bacteria that inhabit various marine environments. Several species of *Algoriphagus* produce the keto-monocyclic carotenoid flexixanthin; however, information on its derivatives and their biological activities is limited. In this study, *Algoriphagus* sp. strain Fs4 was isolated from the surface of seaweed collected at Nanaehama, Hokkaido, Japan. Based on structural analysis via nuclear magnetic resonance spectroscopy, of the carotenoid produced by the strain, it was identified as 2'-hydroxyflexixanthin, a new carotenoid in the genus *Algoriphagus*. A comparison of the carotenoid profiles of 25 species of *Algoriphagus* bacteria revealed that flexixanthin was present in all strains, and some strains also produced 2- or 2'-hydroxyflexixanthin. The biological activities of flexixanthin and its derivatives were evaluated in terms of their antioxidant and anti-inflammatory effects against macrophages. The degree of inhibitory effect on lipid peroxidation was in the order: 2-hydroxyflexixanthin > 2'-hydroxyflexixanthin > flexixanthin. Conversely, flexixanthin significantly inhibited lipopolysaccharide-induced interleukin-1β and -6, and nitric oxide synthase 2 mRNA expression, demonstrating a stronger inhibitory effect than that of 2'-hydroxyflexixanthin; 2-hydroxyflexixanthin showed no significant inhibitory effects. These results indicate that the introduction of hydroxyl groups at specific sites affects the antioxidant and anti-inflammatory activities of flexixanthin. The findings of this study highlights the potential of the genus *Algoriphagus* as a source of flexixanthin and its derivatives and provides a basis for the future application of these monocyclic carotenoids in the production of nutraceuticals and pharmaceuticals.

Keywords

Monocyclic carotenoids; *Algoriphagus*; Flexixanthin; 2'-Hydroxyflexixanthin; 2-Hydroxyflexixanthin; Antioxidant activity; Anti-inflammatory activity

1. Introduction

The health benefits of carotenoids—natural fat-soluble pigments synthesized by plants and some microorganisms—have attracted attention in recent years. The carotenoid structure is a polyene linear chain with a long conjugated double bond and terminal groups. Dicyclic carotenoids with both end groups cyclized, include β-carotene and zeaxanthin, are abundant in nature. In contrast, marine bacteria produce monocyclic carotenoids with only one cyclized end group, such as myxol (Yokoyama & Miki, 1995; Takatani et al., 2014; Takatani et al., 2015). However, these monocyclic carotenoids are rarer than the dicyclic types, and information on their sources and biological activity is limited.

Algoriphagus species are Gram-negative, aerobic bacteria belonging to the family *Cyclobacteriaceae*. They have been isolated from various marine environments, including Antarctic ice, seawater, seaweeds, and brackish lakes (Alegado et al., 2013; Nedashkovskaya et al., 2004; Bowman et al., 2003; Maejima et al., 2019; Lee et al., 2012). Notably, *A. machipongonensis* PR1 induces the formation of multicellular colonies in the choanoflagellate *Salpingoeca rosetta* (Woznica et al., 2016). Recently, the structure of the extracellular contractile injection system required for cell–cell interactions in *A. machipongonensis* was elucidated (Xu et al. 2022). *Algoriphagus* bacteria appear red to pink in color (Alegado et al., 2013; Nedashkovskaya et al. 2004; Bowman et al., 2003; Lee et al., 2012). One of the pigment components, the monocyclic carotenoid flexixanthin, has been identified in *Algoriphagus* sp. KK10202C (Tao et al., 2006). Recently, we identified 2-hydroxyflexixanthin, a novel monocyclic carotenoid, in *Algoriphagus* sp. strain oki45 (Takatani et al., 2024a). Thus, *Algoriphagus* species may have the ability to synthesize several derivatives in addition to flexixanthin. However, the carotenoid compositions of many other species and strains of *Algoriphagus* is currently unknown.

Several monocyclic carotenoids have been evaluated for their biological activity. In our previous study we showed that myxol and saproxanthin, isolated from marine bacteria, have stronger antioxidant and neuroprotective activities than β-carotene and zeaxanthin (Shindo et al., 2007). In addition, C30 monocyclic carotenoids exhibit higher neurogenic activity compared to that of acyclic and dicyclic carotenoids (Kim et al., 2016). These findings suggest that monocyclic structures yield high biological activity. However,

the biological activities of flexixanthin and its derivatives have never been investigated, and there is insufficient knowledge regarding the effects of structural differences among monocyclic carotenoids on biological functions.

In this study, we focused on the bacterial genus *Algoriphagus*, which produces flexixanthin and its derivatives. We isolated a novel *Algoriphagus* strain from the surface of seaweed collected at Nanaehama, Hokkaido, Japan, and the carotenoid profiles of 25 species of *Algoriphagus*, including this strain, were investigated. Additionally, the antioxidant and anti-inflammatory activities of flexixanthin, its derivatives, and myxol, which have similar structures, were evaluated. The results demonstrate the potential of *Algoriphagus* as a source of flexixanthin and its derivatives and provides a basis for the future application of these monocyclic carotenoids in the development of nutraceuticals and pharmaceuticals.

2. Materials and Methods

2.1. Bacterial strains

The bacterial strains used in this study were obtained from RIKEN BioResource Center, Japan Collection of Microorganisms (JCM), National Institute of Technology and Evaluation, Biological Resource Center (NBRC), and DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (DSM). The strains were as follows: *Nonlabens ulvanivorans* strain NR17 (JCM 19296), *Algoriphagus* sp. strain oki45 (JCM 19877), *A. taiwanensis* JCM 19755^T, *A. confluentis* NBRC 111222^T, *A. aquatilis* NBRC 104237^T, *A. mannitolivorans* DSM 15301^T, *A. marincola* JCM 12319^T, *A. zhangzhouensis* DSM 25035^T, *A. vanfongensis* DSM 17529^T, *A. ornithinivorans* DSM 15282^T, *A. hitonicola* DSM 19315^T, *A. faecimaris* JCM 16561^T, *A. boritolerans* NBRC 101277^T, *A. trabzonensis* DSM 24576^T, *A. jejuensis* JCM 16112^T, *A. terrigena* DSM 22685^T, *A. taeanensis* NBRC 105728^T, *A. halophilus* DSM 15292^T, *A. aestuarii* NBRC 110552^T, *A. machipongonensis* DSM 24695^T, *A. aquimarinus* DSM 23399^T, *A. winogradskyi* JCM 13505^T, *A. yeomjeoni* JCM 12598^T, *A. locisalis* JCM 12597^T, and *A. chordae* DSM 19830^T.

2.2. Isolation and bacterial species estimation of strain Fs4

Unidentified seaweed collected at Nanaehama (Hakodate, Hokkaido, Japan) in 2022 was soaked in artificial seawater and seeded on Marine Agar 2216 (Difco) at 25 °C. After several days of incubation, red colonies were selected and plated on fresh Marine Agar 2216. This process was repeated until only a single colony remained. The strain Fs4 was deposited at the RIKEN BRC (JCM 36816). The 16S ribosomal RNA (rRNA) gene sequence obtained from the genomic DNA of strain Fs4 and the bacterial species identification was performed based on the nucleotide sequence using a homology search service of Hokkaido System Science Co., Ltd. (Sapporo, Japan). Briefly, the bacterial genomic DNA was extracted by PrepMan^{TM} Ultra Reagent (Applied Biosystems Japan Ltd.). The 16S rRNA gene was amplified from the DNA using 6F (5'-GRAGAGTTTGATCMTGGC-3') and 519R (5'-ATTACCGCGGCKGCTG-3') universal primers. Polymerase chain reaction (PCR) was performed using TaKaRa Ex Taq® Hot Start Version (Takara Bio Inc., Japan) according to the manufacturer's protocol. A single

PCR product was purified using the QIAquick PCR purification kit (Qiagen), and the sequences were determined by the BigDye Terminator v3.1 Cycle Sequencing Kit using 3730x1 DNA Analyzer (Applied Biosystems Japan Ltd.). A homology search for the obtained sequence was performed by BLASTn (Mount 2007) in the National Center for Biotechnology Information (NCBI) 16S rRNA nucleotide database. The 16S rRNA sequence of the strain Fs4 was deposited in GenBank/EMBL/DDBJ under the accession number of LC829652.

2.3. Chromatography analysis of bacterial carotenoids

The bacteria were cultured on Marine Agar 2216 (Difco) at 25 °C. After 3 days of culture, the cells were harvested and suspended in distilled water. Total lipids were then extracted by the Folch method (Folch et al., 1957). The total lipids were dissolved in a *n*-hexane/acetone (70/30, v/v). The carotenoid profile of each strain was analyzed using a Shimadzu HPLC system under the following conditions: column, Mightysil Si 60 column (250 × 4.6 mm, Kanto Chemical Co., Inc.); column temperature, 30 °C; solvent, *n*-hexane/acetone (70/30, v/v); flow rate, 1.0 mL/min; Photodiode array, 250–750 nm.

2.4. Monocyclic carotenoid preparation

The strain Fs4 for 2'-hydroxyflexixanthin and *N. ulvanivorans* strain NR17 for myxol (Nakanishi et al., 2014) were incubated for one week in 300 mL Marine Broth 2216 (Difco) in 500-mL Sakaguchi flasks at 110 rpm and 25 °C on a rotary shaker. Each cell pellet was collected by centrifugation at 12,000 g and 25 °C for 15 min, and then the total lipid was extracted via the Folch method. Then, fractions including 2'-hydroxyflexixanthin (*Rf* 0.31) or myxol (*Rf* 0.33) were separated on a silica gel 60 TLC plate (Merck Millipore) with *n*-hexane/acetone (60/40, v/v). Each fraction was scraped from silica gel and then eluted with acetone. Subsequently, 2'-hydroxyflexixanthin were purified using HPLC with a Mightysil Si 60 column (250 × 4.6 mm), eluting with *n*-hexane/acetone (70/30, v/v) at a flow rate of 1.0 mL/min. Myxol were purified using Develosil ODS column (250 × 4.6 mm; Nomura Chemical Co., Inc.) eluted by methanol/acetonitrile (70/30, v/v) at a 1.0-mL/min flow rate. Carotenoids were detected using a UV–Vis detector (Hitachi L-2400) at 470 nm. Flexixanthin and 2-

hydroxyflexixanthin were obtained from *Algoriphagus* sp. strain oki45 according to previously reported methods (Takatani et al., 2024a). Each carotenoid was isolated until the purity was >99% (**Fig. S1**).

2.5. Spectroscopy

^{1}H NMR and ^{13}C NMR spectra were acquired in $CDCl_3$ using a UNITY INOVA-500 (Varian) system and tetramethylsilane as an internal standard. The peak assignments of the homo and hetero two-dimensional NMR spectra were determined by comparing the data to those previously reported (Andrewes et al., 1984; Takaichi et al., 2001). The positive ion mode electrospray ionization mass spectrometry (ESI-MS) spectra data were obtained using an Acquity LC Xevo G2-S Q time-of-flight (TOF) MS spectrometer (Waters). The ESI TOF MS spectra were acquired by scanning from *m/z* 100 to 1500 with a capillary voltage of 3.2 kV, a cone voltage of 40 eV, and a source temperature of 120 °C. Nitrogen was used as a nebulizing gas at a flow rate of 30 L/h.

2.6. Lipid peroxidation inhibiting activity

Lipid peroxidation inhibiting activity was measured using brain homogenates, as described in a previous publication (Buchin et al., 2019). Briefly, frozen rat brains (Wistar, 8-week-old males), purchased from Funakoshi (Tokyo, Japan), were defrosted in ice-cold 0.1 M phosphate buffer at pH 7.2. Then, 0.8 g of each brain sample was mixed with 30 mL of ice-cold phosphate buffer for 2 min in a Teflon homogenizer. Two hundred microliters of the resulting homogenate, 0.65 mL of 0.1 M phosphate buffer at pH 7.2 with or without 0.15 mM $FeSO_4$, 0.1 mL of 1 mM sodium ascorbate, and 50 μL of the sample dissolved in acetone (final concentration, 0.1–100 μM) was added to small glass test tubes (5 mL), and mixed well. The tubes were incubated at 37°C for 1 h under reciprocal agitation.

Malondialdehyde (MDA) was produced in the reaction mixture in proportion to lipid peroxide concentration. Since MDA reacts with thiobarbituric acid and produces compounds with maximum absorption at 532 nm, the OD_{532} of the solution measured using a spectrophotometer was used to quantify MDA production. The percentage lipid peroxidation inhibition was calculated using the formula: $(1 - [T - B]/[C - B]) \times 100$ (%),

where *T*, *C*, and *B* are the OD_{532} readings of the tested compound with homogenate, the control (no compound with homogenate), and the zero control (tested compound without homogenate), respectively. The lipid peroxidation-inhibiting activity was determined as the IC_{50} value, representing the concentration at which 50 % inhibition was observed.

2.7. Animal cell culture

Murine macrophage-like RAW264.7 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells (passage numbers 13-14) were incubated in RPMI 1640 with 10% FBS containing 100 μg/mL streptomycin and 100 U/mL penicillin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After 24 hours of preincubation in a 24-well plate containing 40,000 cells per well, the cells were treated with 10 μM of each carotenoid dissolved in dimethyl sulfoxide (DMSO). The treatment of the control groups was limited to DMSO. 0.1% DMSO was added to each culture medium to prevent cytotoxicity. After 24 h of treatment, lipopolysaccharides (LPS) were added to the media at a final concentration of 10 ng/mL, and the cells were incubated for an additional 6 h.

2.8. Cell viability

RAW264.7 macrophages (20,000 cells per well) were seeded with 200 μL in 96-well culture plates and treated with 10 μM of each carotenoid for 24 h. WST-1 reagent (10 μL of each well) was then added and incubated for an additional 4 h. The absorbance at 450 nm of each well was determined using a microplate reader (Molecular Devices, CA, USA). Media samples containing each carotenoid without cells were used as blanks.

2.9. Gene expression analysis

Extraction of RNA from RAW264.7 cells and gene expression analysis of inflammatory factors were performed according to previous methods (Takatani et al., 2024b; Takatani et al., 2022; Takatani et al., 2021). Briefly, total RNA was extracted using the QIAzol lysis reagent (Qiagen) from RAW264.7 cells stimulated by LPS for 6 h. Extracted total RNA was reverse-transcribed to cDNA using ReverTra Ace (Toyobo) according to the manufacturer's protocol. The gene expression level was quantified using GeneAce Probe

qPCR Mix II (Nippon gene) on the StepOnePlus real-time polymerase chain reaction (PCR) system (Applied Biosystems Japan Ltd.). The PCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 30 s and 60 °C for 1 min. TaqMan Gene Expression Assays purchased from Thermo Fisher Scientific were as follows: *Nos2* (Mm00440502_m1), *Il6* (Mm00446190_m1), *Il1b* (Mm00434228_m1), and *Rps18* (Mm02601777_g1). Relative quantification was performed using the standard curve method (Čikoš et al., 2007). The target amount was divided by the endogenous (*Rps18*) amount to obtain a normalized target value.

2.10. Statistics

The results are represented as the mean ± standard error of the mean. One-way analysis of variance followed by Tukey's honest significant difference test evaluated the statistical differences ($p < 0.05$) using the software Statcel Ver.3.

3. Results

3.1. Identification of 2'-hydroxyflexixanthin produced by strain Fs4

The 16S rRNA sequence of strain Fs4, isolated from the surface of seaweed at Nanaehama, showed homology with *A. winogradskyi* JCM 13505^T and *A. yeomjeoni* JCM 12598^T. These two strains also form red-colored colonies, but their pigments have not been identified. Spectroscopic analysis of the chromatogram of total lipids extracted from strain Fs4 revealed three peaks (**Fig. 1A**). The retention times and absorption spectra of peaks I and II were consistent with those of lycopene and flexixanthin, respectively (**Figs. 1B and C**). Peak III exhibited maximum absorption at 480 nm (**Fig. 1D**) with a broad curve, characteristic of a keto-type monocyclic carotenoid. ESI TOF-MS of the purified peak III detected a sodium adduct ion at *m/z* 621.3914 $[M+Na]^+$ (calculated for $C_{40}H_{54}O_4Na$, 621.3920) (**Fig. S2**). Based on the ^1H- and ^{13}C-NMR data (**Table 1 and Figs. S3–S6**) it was determined that peak III was 2'-hydroxyflexixanthin (**Fig. 2**).

Table 1 Nuclear magnetic resonance data of peak III

Position	δ_C	δ_H (*J* in Hz)
1	36.8	
2	45.3	1.81, dd (13, 13)
2		2.16, dd (13, 6)
3	69.2	4.33, ddd (14, 6, 1.5)
4	200.4	
4		
5	126.3	
6	162.3	
7	123.2	6.22, d (16)
8	142.5	6.43, d (16)
9	134.0	
10	135.3	6.30, d (11)
11	124.4	6.66, dd (15, 11)
12	139.8	6.45, d (15)
13	136.1	
14	133.0	~6.28
15	130.9	~6.67, m

16	26.1	1.32, s
17	30.7	1.21, s
18	14.0	1.95, s
19	12.8	2.00, s
20	12.8	1.99, s
1'	73.1	
2'	80.0	4.00, dd (8, 1)
3'	126.3	5.71, dd (15, 8)
4'	138.2	6.38, d (15)
5'	134.0	
6'	133.0	6.20, d (11)
7'	124.4	6.59, dd (15, 11)
8'	138.7	6.39, d (15)
9'	135.3	
10'	132.9	6.20, d (11)
11'	125.3	6.64, dd (15, 11)
12'	138.1	6.39, d (15)
13'	137.1	
14'	130.9	~6.28
15'	131.0	~6.67, m
16'	26.5	1.18, s
17'	23.9	1.24, s
18'	12.8	1.93, s
19'	12.6	1.99, s
20'	12.9	1.99, s

s, singlet; d, doublet; dd, doublet of doublets; ddd, a doublet of doublets of doublets; m, multiplet.

3.2. Carotenoid analysis of other *Algoriphagus* strains

To determine whether flexixanthin and its derivatives are also produced in other *Algoriphagus* strains, the carotenoid profiles of the total lipids extracted from the 24 strains were investigated (**Fig. 3**). A peak corresponding to flexixanthin was detected in all *Algoriphagus* strains. Eleven of the 24 strains produced only flexixanthin, whereas the remaining 13 strains also produced one of two types of flexixanthin derivatives: either 2'-

hydroxyflexixanthin or 2-hydroxyflexixanthin. The seven strains that produced 2'-hydroxyflexixanthin were *A. winogradskyi* JCM 13505^T, *A. yeomjeoni* JCM 12598^T, *A. locisalis* JCM 12597^T, *A. aquimarinus* DSM 23399^T, *A. faecimaris* JCM 16561^T, *A. hitonicola* DSM 19315^T, and *A. terrigena* DSM 22685^T. The six strains that produced 2-hydroxyflexixanthin were *A. trabzonensis* DSM 24576^T, *A. boritolerans* NBRC 101277^T, *A. aquatilis* NBRC 104237^T, *A. confluentis* NBRC 111222^T, *A. taiwanensis* JCM 19755^T, and *Algoriphagus* sp. oki45 JCM 19877.

3.3. Inhibition of lipid peroxidation by flexixanthin and its derivatives

The antioxidant activity of flexixanthin and its derivatives was examined based on their inhibitory effects on lipid peroxidation. The IC_{50} values of the dicyclic carotenoids astaxanthin and β-carotene were both above 100 μM (data not shown). In contrast, all monocyclic carotenoids, except flexixanthin, showed strong inhibitory activity against lipid peroxidation (**Table 2**). The IC_{50} values of myxol and 2'-hydroxyflexixanthin were similar: 40 ± 0.32 and 33 ± 0.57 μM, respectively. 2-Hydroxyflexixanthin, a structural isomer of 2'-hydroxyflexixanthin, showed the highest inhibitory activity (IC_{50} 14 ± 1.19 μM).

Table 2 Lipid peroxidation inhibiting activity of carotenoids

Carotenoid	IC_{50} [μM]
Myxol	40 ± 0.32
2'-Hydroxyflexixanthin	33 ± 0.57
Flexixanthin	>100
2-Hydroxyflexixanthin	14 ± 1.19

Data are represented as the mean ± SEM (n = 3)

3.4. Inhibition of inflammatory cytokine and mediator expression in activated macrophages by flexixanthin and its derivatives

The anti-inflammatory effects of flexixanthin and its derivatives were evaluated in activated RAW264.7 macrophages. None of the carotenoids tested in this experiment were cytotoxic at 10 μM (**Fig. 4A**). After stimulating RAW264.7 macrophages with LPS,

283 the gene expression levels of the inflammatory factors such as interleukin 6 (gene name
284 *Il6*), interleukin-1β (*Il1b*), and inducible nitric oxide synthase (*Nos2*) were analyzed (**Figs.**
285 **4B–D**). Myxol significantly inhibited the expression of *Il1b*, whereas 2'-
286 hydroxyflexixanthin inhibited the expression of both *Il1b* and *Il6*. Flexixanthin
287 significantly suppressed the mRNA expression of *Il1b*, *Il6*, and *Nos2*. In contrast, no
288 inflammatory factors were significantly inhibited in the cells treated with 2-
289 hydroxyflexixanthin.
290
291

4. Discussion

Flexixanthin and 2-hydroxyflexixanthin have been identified in *Algoriphagus* KK10202C and oki45, respectively (Tao et al., 2006; Takatani et al., 2024a). However, the carotenoid compositions of other *Algoriphagus* bacteria have not been fully elucidated. The 16S rRNA sequence of the Fs4 strain isolated in this study showed high homology to that of *A. winogradskyi* (Nedashkovskaya et al., 2004) and *A. yemomjeoni* (Yoon et al., 2005). Structural analysis of the carotenoids produced by the strain Fs4 identified 2'-hydroxyflexixanthin (**Table 1 and Figs. S2–S6**). To our knowledge, this derivative, characterized by a hydroxy group at the C-2' position of flexixanthin, has been identified previously in bacteria of the genera *Hymenobacter* (Klassen et al., 2009) and *Flexibacter* (Andrewes et al., 1984), but this is the first time it has been identified in the genus *Algoriphagus*. Several flexixanthin biosynthetic genes have been cloned from *Algoriphagus* sp. KK10202C (Tao et al., 2006). More recently, in *Algoriphagus* sp. oki45, in addition to the eight genes required for flexixanthin biosynthesis (*crtE*, *crtB*, *crtI*, *cruF*, *crtD*, *crtYcd*, *crtW*, and *crtZ*), the *crtG* gene, homologous to 2,2'-β-hydroxylase, was discovered (Takatani et al., 2024a). In this study, carotenoid analysis of several *Algoriphagus* bacteria confirmed flexixanthin in all strains examined, and no species produced both 2- and 2'-hydroxyflexixanthin (**Fig. 3**). These results indicate that in *Algoriphagus* bacteria there exists a pathway to synthesize one or the other of two different flexixanthin derivatives, 2-hydroxyflexixanthin or 2'-hydroxyflexixanthin (**Fig. 2**), from the precursor flexixanthin. Genome comparisons between 2'-hydroxyflexixanthin-producing bacteria (such as the strain Fs4) and 2-hydroxyflexixanthin-producing bacteria (such as the strain oki45) may open the way to finding new types of carotenoid hydroxylases distinct from those associated with *crtG*. Recently, genome analysis of *Algoriphagus* sp. strain PAP.12, isolated from marine samples in Papua, Indonesia, has revealed a gene cluster involved in flexixanthin biosynthesis (Riyanti et al., 2023), although the carotenoids of this strain have not been identified. Furthermore, a whole genome analysis of *A. halophytocola* sp. nov., isolated from halophytes, has also been performed (Peng et al., 2024). While genomic information of the genus *Algoriphagus* tends to accumulate year by year, structural information on the produced carotenoids is lacking. Genome comparison is a great tool in discovering new

genes. However, structural information of the product is important in estimating whether the gene of interest actually functions. Clarifying the enzyme functions encoding genes involved in carotenoid biosynthesis through both genome and molecular structure analysis approaches will contribute to the development of nutraceuticals and pharmaceuticals through alternative production in different host organisms such as *Escherichia coli*.

Reactive oxygen species (ROS), including hydroxyl radicals, oxidize biological molecules such as lipids, DNA, and proteins, causing cellular damage. Lipid peroxidation reflects the cumulative effect of ROS and deterioration of biological systems (Niki et al., 2005; Gianazza et al., 2021). In the present study, we investigated the lipid peroxidation inhibitory activity of several monocyclic carotenoids (**Table 2**). All monocyclic carotenoids, except flexixanthin, strongly inhibited lipid peroxidation. A common structural feature of them is the presence of two hydroxyl groups at the terminus. The lack of inhibitory effect by flexixanthin within the tested range suggests that the C-4 carbonyl group has little effect on the inhibitory activity against lipid peroxidation. Interestingly, when comparing the structural isomers 2'-hydroxyflexixanthin and 2-hydroxyflexixanthin, the latter showed stronger inhibition of lipid peroxidation. It has been reported that the lipid peroxidation inhibitory activity of C-2 hydroxylated canthaxanthin is stronger than that of canthaxanthin (Nishida et al., 2005). The C-2 hydroxylated monocyclic carotenoid deinoxanthin has strong scavenging activity against hydrogen peroxide and hydroxyl radicals (Tian et al., 2007). In this study, lipid peroxidation caused by hydroxyl radicals was evaluated, and the strongest inhibitory effect of 2-hydroxyflexixanthin supports the possibility that the ring hydroxy group at the C-2 position plays an important role in radical scavenging. In addition, it is possible that the adjacent diols on the cyclic terminus may contribute more strongly to the inhibition of lipid peroxidation than those on the acyclic terminus. The tendency of the inhibitory activity to be enhanced by the presence of adjacent diols on the ring, such as the catechol ring, has also been observed in other compounds (Buchin et al., 2019). The carbon (C-1') to which the hydroxyl group at the acyclic terminus is bonded also has a methyl group, and the stereochemical differences may affect the activity. Recently, it has been reported that treatment of the human foreskin fibroblast cell line HFF-1 with deinoxanthin

suppresses UV irradiation-induced intracellular ROS production and associated cell death (Kuzucu, 2023). Furthermore, deinoxanthin also inhibits hydrogen peroxide-induced ROS generation in NIH 3T3 cells (Yu et al., 2023). However, evaluation of the antioxidant capacity of monocyclic carotenoids, including this study, has been limited to cell-free and in vitro tests. Further research is needed to support the possibility that monocyclic carotenoids may be superior antioxidants.

Excessive inflammation contributes to the onset and progression of noncommunicable diseases (NCDs) such as obesity and nonalcoholic fatty liver disease (Furman et al., 2019; Shapouri-Moghaddam et al., 2018). This is associated with the exacerbation of tissue inflammation via the promotion of inflammatory cytokines and mediators by macrophages (Fujiwara, & Kobayashi, 2005; Oishi, & Manabe, 2018). Therefore, compounds that suppress inflammation may be useful in preventing NCDs. In the present study, several monocyclic carotenoids were found to inhibit the expression of inflammatory factors without exhibiting cytotoxicity **(Fig. 4)**. When comparing myxol and 2'-hydroxyflexixanthin, the latter tended to have stronger inhibitory activity. This suggests that the carbonyl group at the C-4 position of monocyclic carotenoids enhances their anti-inflammatory activity. In addition, flexixanthin inhibited the expression of more inflammatory cytokines than 2'- or 2-hydroxyflexixanthin, suggesting that the addition of a hydroxyl group may weaken the anti-inflammatory effect. 2-Hydroxyflexixanthin did not exhibit any inhibition at this concentration. Previous studies have reported that zeaxanthin, which contains a hydroxyl group attached to β-carotene, shows weaker anti-inflammatory activity than β-carotene (Konishi et al., 2008; Manabe et al., 2021), supporting our findings that the presence of a hydroxyl group at the cyclic terminus attenuates the anti-inflammatory activity. In the case of flavonoids as well, the anti-inflammatory activity is attenuated depending on the position of the hydroxyl groups (Chen et al., 2018). Our results suggest that, in monocyclic carotenoids, the number and position of hydroxyl groups, in addition to carbonyl groups, are important for modulating cellular inflammation. Previous studies have shown that monocyclization by cleavage of the polyene linear chain of dicyclic carotenoids enhanced their anti-inflammatory activity against immune cells (Takatani et al., 2024b; Takatani et al., 2022; Takatani et al., 2021). Interestingly, Manabe and colleagues (Manabe et al., 2021) reported that monocyclic

types metabolized intracellularly from dicyclic carotenoids may be the executors of anti-inflammatory action. These findings suggest the potential usefulness of monocyclic carotenoids as anti-inflammatory agents. On the other hand, there is a lack of knowledge about bioavailability, tissue accumulation, and metabolism of monocyclic carotenoids. Recently, when the monocyclic carotenoid neurosporaxanthin was orally administered to mice, it was found to accumulate in tissues and that the polyene chain was cleaved by the carotenoid metabolic enzymes including β-carotene-15,15'-dioxygenase and β-carotene-9',10'-dioxygenase (Miller et al., 2023). This suggests that the monocyclic carotenoids themselves, or their metabolites, may exert biological activities such as anti-inflammatory activity in the body. Further in vivo studies are needed to verify the beneficial properties of monocyclic carotenoids as anti-inflammatory agents.

5. Conclusion

In this study, we isolated the marine bacterium *Algoriphagus* sp. Fs4 and identified 2'-hydroxyflexixanthin, a first in this genus. Carotenoid analysis of a total of 25 *Algoriphagus* bacteria revealed species that produce 2'- or 2-hydroxyflexixanthin in addition to flexixanthin, demonstrating the diversity of the carotenoid biosynthetic pathway in this genus. Furthermore, investigation of the antioxidant and anti-inflammatory activities of flexixanthins and their derivatives revealed potential structure-activity relationships. This means two strengths when considering the food application of monocyclic carotenoids; the first is the possibility of using *Algoriphagus* bacteria as a source of monocyclic carotenoids, and the second is useful information for molecular design to enhance the activity of monocyclic carotenoids. On the other hand, a limitation of this study is that only cell-free or in vitro tests were performed. In order to discuss the health functionality of bioactives, in vivo tests including bioavailability are necessary. In this regard, the findings of the absorption and tissue accumulation of monocyclic carotenoids are noteworthy (Miller et al., 2023). However, there have been no reports on the in vivo efficacy of monocyclic carotenoids. In advancing future applications, sourcing monocyclic carotenoids could be a major hurdle. Therefore, it is significant that this study has found the potential of the genus *Algoriphagus* as a source of monocyclic carotenoids. In the future, it is hoped that research using each monocyclic carotenoid will progress.

CRediT authorship contribution statement

Naoki Takatani: Conceptualization, investigation, funding acquisition, visualization, and writing - original draft. **Yuki Sato-Takabe**: Conceptualization, investigation, visualization, and writing - review & editing. **Misaki Aoike**: Investigation. **Rika Sekine**: Investigation. **Takashi Maoka**: Investigation, visualization, and writing - original draft. **Tomoo Sawabe**: Resources and writing - review & editing. **Fumiaki Beppu**: Conceptualization and writing - review & editing. **Kazutoshi Shindo**: Conceptualization, supervision, and writing - review & editing. **Masashi Hosokawa**: Conceptualization, supervision, and writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Funding sources

This work was supported by JSPS KAKENHI (21H02276 and 23K14018) and J-PEAKS.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

HPLC chromatogram of purified monocyclic carotenoids (**Fig. S1**); The data of MS and NMR spectra of purified peak III (**Figs. S2–S6**), respectively.

441 **References**

442 Alegado, R. A., Grabenstatter, J. D., Zuzow, R., Morris, A., Huang, S. Y., Summons, R.
443 E., & King, N. (2013). *Algoriphagus machipongonensis* sp. nov., co-isolated with a
444 colonial choanoflagellate. *International Journal of Systematic and Evolutionary*
445 *Microbiology*, *63*, 163–168. https://doi.org/10.1099/ijs.0.038646-0

446 Andrewes, A. G., Foss, P., Borch, G., & Liaaen-Jensen, S. (1984). Bacterial carotenoids.
447 50. On the structures of (3*S*)-flexixanthin and (3*S*,2′*S*)-2′-hydroxyflexixanthin. *Acta*
448 *Chemica Scandinavica*, *B38*, 337–339.
449 https://doi.org/10.3891/acta.chem.scand.38b-0337

450 Bowman, J. P., Nichols, C. M., & Gibson, J. A. E. (2003). *Algoriphagus ratkowskyi* gen.
451 nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen.
452 nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria
453 isolated from various polar habitats. *International Journal of Systematic and*
454 *Evolutionary Microbiology*, *53*, 1343–1355. https://doi.org/10.1099/ijs.0.02553-0

455 Buchin, Y., Sakemi, Y., Hamashima, R., Morioka, Y., Yamanaka, D., Hakuno, F.,
456 Takahashi, S., & Shindo, K. (2019). Structures and biological activities of new
457 carnosic acid- and carnosol-related compounds generated by heat treatment of
458 rosemary. *Phytochemistry Letters*, *30*, 43–48.
459 https://doi.org/10.1016/j.phytol.2019.01.005

460 Chen, L., Teng, H., Xie, Z., Cao, H., Cheang, W. S., Skalicka-Woniak, K., Georgiev, M.
461 I., & Xiao, J. (2018). Modifications of dietary flavonoids towards improved
462 bioactivity: An update on structure-activity relationship. *Critical Reviews in Food*
463 *Science and Nutrition*, *58*(4), 513–527.
464 https://doi.org/10.1080/10408398.2016.1196334

465 Čikoš, Š., Bukovská, A. & Koppel, J. (2007). Relative quantification of mRNA:
466 comparison of methods currently used for real-time PCR data analysis. *BMC*
467 *Molecular Biology*, *8*, Article 113. https://doi.org/10.1186/1471-2199-8-113

468 Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation
469 and purification of total lipides from animal tissues. *Journal of Biological Chemistry*,
470 *226*, 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

471 Fujiwara, N., & Kobayashi, K. (2005). Macrophages in inflammation. *Current drug*

targets. Inflammation and allergy, *4*(3), 281–286. https://doi.org/10.2174/1568010054022024

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gianazza, E., Brioschi, M., Martinez Fernandez, A., Casalnuovo, F., Altomare, A., Aldini, G., & Banfi, C. (2021). Lipid peroxidation in atherosclerotic cardiovascular diseases. *Antioxidants & Redox Signaling*, *34*(1), 49–98. https://doi.org/10.1089/ars.2019.7955

Kim, S., Kim, M., Lee, B., & Lee, P. (2016). Generation of structurally novel short carotenoids and study of their biological activity. *Scientific Reports*, *6*, Article 21987. https://doi.org/10.1038/srep21987

Klassen, J. L., McKay, R., & Foght, J. M. (2009). 2′-Methyl and 1′-xylosyl derivatives of 2′-hydroxyflexixanthin are major carotenoids of *Hymenobacter* species. *Tetrahedron Letters*, *50*(22), 2656–2660. https://doi.org/10.1016/j.tetlet.2009.03.117

Konishi, I., Hosokawa, M., Sashima, T., Maoka, T., & Miyashita, K. (2008). Suppressive effects of alloxanthin and diatoxanthin from *Halocynthia roretzi* on LPS-induced expression of pro-inflammatory genes in RAW264.7 cells. *Journal of Oleo Science*, *57*(3), 181–189. https://doi.org/10.5650/jos.57.181

Kuzucu, M. (2023). Extremophilic solutions: The role of deinoxanthin in counteracting UV-induced skin harm. *Current Issues in Molecular Biology*, *45*(10), 8372–8394. https://doi.org/10.3390/cimb45100528

Lee, D. H., Kahng, H. Y., & Lee, S. B. (2012). *Algoriphagus jejuensis* sp. nov., isolated from seawater. *International Journal of Systematic and Evolutionary Microbiology*, *62*, 409–413. https://doi.org/10.1099/ijs.0.030106-0

Maejima, Y., Iino, T., Muraguchi, Y., Ohkuma, M., Kimbara, K., & Shintani, M. (2019). *Algoriphagus sanaruensis* sp. nov., a member of the family *Cyclobacteriaceae*, isolated from a brackish lake in Hamamatsu, Japan. *International Journal of*

Systematic and Evolutionary Microbiology, *69*(7), 2108–2113. https://doi.org/10.1099/ijsem.0.003447

Manabe, Y., Tomonaga, N., Maoka, T., & Sugawara, T. (2021). Multivariate analysis reveals that unsubstituted β-ring and C8-keto structures are important factors for anti-inflammatory activity of carotenoids. *Nutrients*, *13*(11), Article 3699. https://doi.org/10.3390/nu13113699

Miller, A. P., Hornero-Méndez, D., Bandara, S., Parra-Rivero, O., Limón, M. C., von Lintig, J., Avalos, J., & Amengual, J. (2023). Bioavailability and provitamin A activity of neurosporaxanthin in mice. *Communications Biology*, *6*(1), Article 1068. https://doi.org/10.1038/s42003-023-05446-1

Mount, D. W. (2007). Using the Basic Local Alignment Search Tool (BLAST). *Cold Spring Harbor Protocols*, *2007*, pdb. top17. https://doi.org/10.1101/pdb.top17

Nakanishi, M., Meirelles, P., Suzuki, R., Takatani, N., Mino, S., Suda, W., Oshima, K., Hattori, M., Ohkuma, M., Hosokawa, M., Miyashita, K., Thompson, F. L., Niwa, A., Sawabe, T., & Sawabe, T. (2014). Draft genome sequences of marine Flavobacterium *Nonlabens* strains NR17, NR24, NR27, NR32, NR33, and Ara13. *Genome Announcements*, *2*(6), Article e01165-14. https://doi.org/10.1128/genomea.01165-14

Nedashkovskaya, O. I., Vancanneyt, M., Van Trappen, S., Vandemeulebroecke, K., Lysenko, A. M., Rohde, M., Falsen, E., Frolova, G. M., Mikhailov, V. V., & Swings, J. (2004). Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from sea water and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman *et al*. 2003 and *Hongiella* Yi and Chun 2004. *International Journal of Systematic and Evolutionary Microbiology*, *54*, 1757–1764. https://doi.org/10.1099/ijs.0.02915-0

Niki, E., Yoshida, Y., Saito, Y., & Noguchi, N. (2005). Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications*, *338*(1), 668–676. https://doi.org/10.1016/j.bbrc.2005.08.072

Nishida, Y., Adachi, K., Kasai, H., Shizuri, Y., Shindo, K., Sawabe, A., Komemushi, S.,

Miki, W., & Misawa, N. (2005). Elucidation of a carotenoid biosynthesis gene cluster encoding a novel enzyme, 2,2'-beta-hydroxylase, from *Brevundimonas* sp. strain SD212 and combinatorial biosynthesis of new or rare xanthophylls. *Applied and Environmental Microbiology*, *71*(8), 4286–4296. https://doi.org/10.1128/aem.71.8.4286-4296.2005

Oishi, Y., & Manabe, I. (2018). Macrophages in inflammation, repair and regeneration. *International Immunology*, *30*(11), 511–528. https://doi.org/10.1093/intimm/dxy054

Peng, Y., Cho, D. H., Humaira, Z., Park, Y. L., Kim, K. H., Kim, C. Y., & Lee, J. (2024). Improving plant salt tolerance through *Algoriphagus halophytocola* sp. nov., isolated from the halophyte *Salicornia europaea*. *Frontiers in Microbiology*, *15*, Article 1466733. https://doi.org/10.3389/fmicb.2024.1466733

Riyanti, Zumkeller, C. M., Spohn, M., Mihajlovic, S., Schwengers, O., Goesmann, A., Choironi, N. A., Schäberle, T. F., & Harwoko, H. (2023). Draft genome sequences of *Algoriphagus* sp. strain PAP.12 and *Roseivirga* sp. strain PAP.19, isolated from marine samples from Papua, Indonesia. *Microbiology Resource Announcements*, *12*(4), Article e0126422. https://doi.org/10.1128/mra.01264-22

Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S. A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J. T., & Sahebkar, A. (2018). Macrophage plasticity, polarization, and function in health and disease. *Journal of Cellular Physiology*, *233*(9), 6425–6440. https://doi.org/10.1002/jcp.26429

Shindo, K., Kikuta, K., Suzuki, A., Katsuta, A., Kasai, H., Yasumoto-Hirose, M., Matsuo, Y., Misawa, N., & Takaichi, S. (2007). Rare carotenoids, (3*R*)-saproxanthin and (3*R*,2′*S*)-myxol, isolated from novel marine bacteria (*Flavobacteriaceae*) and their antioxidative activities. *Applied Microbiology and Biotechnology*, *74*, 1350–1357. https://doi.org/10.1007/s00253-006-0774-y

Takaichi, S., Maoka, T., & Masamoto, K. (2001). Myxoxanthophyll in Synechocystis sp. PCC 6803 is myxol 2'-dimethyl-fucoside, (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-alpha-L-fucoside), not rhamnoside. *Plant and Cell Physiology*, *42*(7), 756–762. https://doi.org/10.1093/pcp/pce098

Takatani, N., Beppu, F., Yamano, Y., Maoka, T., Miyashita, K., & Hosokawa, M. (2022).

Preparation of apoastaxanthinals and evaluation of their anti-inflammatory action against lipopolysaccharide-stimulated macrophages and adipocytes. *ACS Omega*, *7*(26), 22341–22350. https://doi.org/10.1021/acsomega.2c01164

Takatani, N., Maoka, T., Sawabe, T., Beppu, F., & Hosokawa, M. (2024a). Identification of a novel monocyclic carotenoid and prediction of its biosynthetic genes in *Algoriphagus* sp. oki45. *Applied Microbiology and Biotechnology*, *108*(1), Article 102. https://doi.org/10.1007/s00253-023-12995-2

Takatani, N., Miyafusa, H., Yamano, Y., Beppu, F., & Hosokawa, M. (2024b). Apo-12'-capsorubinal exhibits anti-inflammatory effects and activates nuclear factor erythroid 2-related factor 2 in RAW264.7 macrophages. *Archives of Biochemistry and Biophysics*, 760, Article 110125. https://doi.org/10.1016/j.abb.2024.110125

Takatani, N., Nishida, K., Sawabe, T. Maoka, T., Miyashita, K., & Hosokawa, M. (2014). Identification of a novel carotenoid, 2′-isopentenylsaproxanthin, by *Jejuia pallidilutea* strain 11shimoA1 and its increased production under alkaline condition. *Applied Microbiology and Biotechnology*, *98*, 6633–6640. https://doi.org/10.1007/s00253-014-5702-y

Takatani, N., Sawabe, T., Maoka, T., Miyashita, K., & Hosokawa, M. (2015). Structure of a novel monocyclic carotenoid, 3″-hydroxy-2′-isopentenylsaproxanthin ((3*R*,2′*S*)-2′-(3-hydroxy-3-methylbutyl)-3′,4′-didehydro-1′,2′-dihydro-β, ψ-carotene-3,1′-diol), from a flavobacterium *Gillisia limnaea* strain DSM 15749. *Biocatalysis and Agricultural Biotechnology*, *4*, 174–179. https://doi.org/10.1016/j.bcab.2015.02.005

Takatani, N., Taya, D., Katsuki, A., Beppu, F., Yamano, Y., Wada, A., Miyashita, K., & Hosokawa, M. (2021). Identification of paracentrone in fucoxanthin-fed mice and anti-inflammatory effect against lipopolysaccharide-stimulated macrophages and adipocytes. *Molecular Nutrition & Food Research*, *65*(2), Article e2000405. https://doi.org/10.1002/mnfr.202000405

Tao, L., Yao, H., Kasai, H., Misawa, N., & Cheng, Q. (2006). A carotenoid synthesis gene cluster from *Algoriphagus* sp. KK10202C with a novel fusion-type lycopene beta-cyclase gene. *Molecular Genetics and Genomics*, *276*, 79–86. https://doi.org/10.1007/s00438-006-0121-0

Tian, B., Xu, Z., Sun, Z., Lin, J., & Hua, Y. (2007). Evaluation of the antioxidant effects

of carotenoids from *Deinococcus radiodurans* through targeted mutagenesis, chemiluminescence, and DNA damage analyses. *Biochimica et Biophysica Acta*, *1770*(6), 902–911. https://doi.org/10.1016/j.bbagen.2007.01.016

Woznica, A., Cantley, A. M., Beemelmanns, C., Freinkman, E., Clardy, J., & King, N. (2016). Bacterial lipids activate, synergize, and inhibit a developmental switch in choanoflagellates. *Proceedings of the National Academy of Sciences of the U S A*, *113*(28), 7894–7899. https://doi.org/10.1073/pnas.1605015113

Xu, J., Ericson, C. F., Lien, Y. W., Rutaganira. F. U. N, Eisenstein, F., Feldmüller, M., King, N., & Pilhofer, M. (2022). Identification and structure of an extracellular contractile injection system from the marine bacterium *Algoriphagus machipongonensis*. *Nature Microbiology*, *7*(3), 397–410. https://doi.org/10.1038/s41564-022-01059-2

Yokoyama, A., & Miki, W. (1995). Isolation of myxol from a marine bacterium Flavobacterium sp. associated with a marine sponge. *Fisheries Science*, *61*, 684–686. https://doi.org/10.2331/fishsci.61.684

Yoon, J. H., Kang, S. J., Jung, S. Y., Lee, C. H., & Oh, T. K. (2005). *Algoriphagus yeomjeoni* sp. nov., isolated from a marine solar saltern in the Yellow Sea, Korea. *International Journal of Systematic and Evolutionary Microbiology*, *55*, 865–870. https://doi.org/10.1099/ijs.0.63479-0

Yu, S., Kim, S., Kim, J., Kim, J. W., Kim, S. Y., Yeom, B., Kim, H., Choi, W. II., & Sung, D. (2023). Highly water-dispersed and stable deinoxanthin nanocapsule for effective antioxidant and anti-inflammatory activity. *International Journal of Nanomedicine*, *18*, 4555–4565. https://doi.org/10.2147/ijn.s401808

Figure captions

Fig. 1 High-performance liquid chromatography profiles of the total lipid extracted from the strain Fs4. (A) Chromatogram at 470 nm. (B), (C), and (D) are the absorption spectra of peaks I, II, and III, respectively, as depicted in (A).

Fig. 2 The molecular structures of monocyclic carotenoids identified in this study.

Fig. 3 Carotenoid analysis of 24 species of *Algoriphagus* bacteria. (a) *A. winogradskyi* JCM 13505^T, (b) *A. yeomjeoni* JCM 12598^T, (c) *A. locisalis* JCM 12597^T, (d) *A. aquimarinus* DSM 23399^T, (e) *A. faecimaris* JCM 16561^T, (f) *A. hitonicola* DSM 19315^T, (g) *A. terrigena* DSM 22685^T, (h) *A. trabzonensis* DSM 24576^T, (i) *A. boritolerans* NBRC 101277^T, (j) *A. aquatilis* NBRC 104237^T, (k) *A. confluentis* NBRC 111222^T, (l) *A. taiwanensis* JCM 19755^T, (m) *Algoriphagus* sp. oki45 JCM 19877, (n) *A. chordae* DSM 19830^T, (o) *A. aestuarii* NBRC 110552^T, (p) *A. machipongonensis* DSM 24695^T, (q) *A. halophilus* DSM 15292^T, (r) *A. marincola* JCM 12319^T, (s) *A. ornithinivorans* DSM 15282^T, (t) *A. vanfongensis* DSM 17529^T, (u) *A. zhangzhouensis* DSM 25035^T, (v) *A. jejuensis* JCM 16112^T, (w) *A. taeanensis* NBRC 105728^T, and (x) *A. mannitolivorans* DSM 15301^T. The blue, green, light blue, and red dashed lines represented lycopene, flexixanthin, 2-hydroxyflexixanthin, and 2'-hydroxyflexixanthin, respectively.

Fig. 4 Inhibitory effect of monocyclic carotenoids on inflammatory cytokines and mediator gene expression in lipopolysaccharide (LPS)-activated RAW264.7 macrophages. Cells are pretreated with 10 μM of carotenoids for 24 h and then stimulated with LPS (10 ng/mL) for 6 h in the presence of carotenoids. (A) Cell viability. (B), (C), and (D) are mRNA levels of inflammatory cytokines and mediator. Data are represented as the mean ± SEM (n = 5 (A) or 3 (B–D)), with different letters indicating statistical significance ($p < 0.05$).

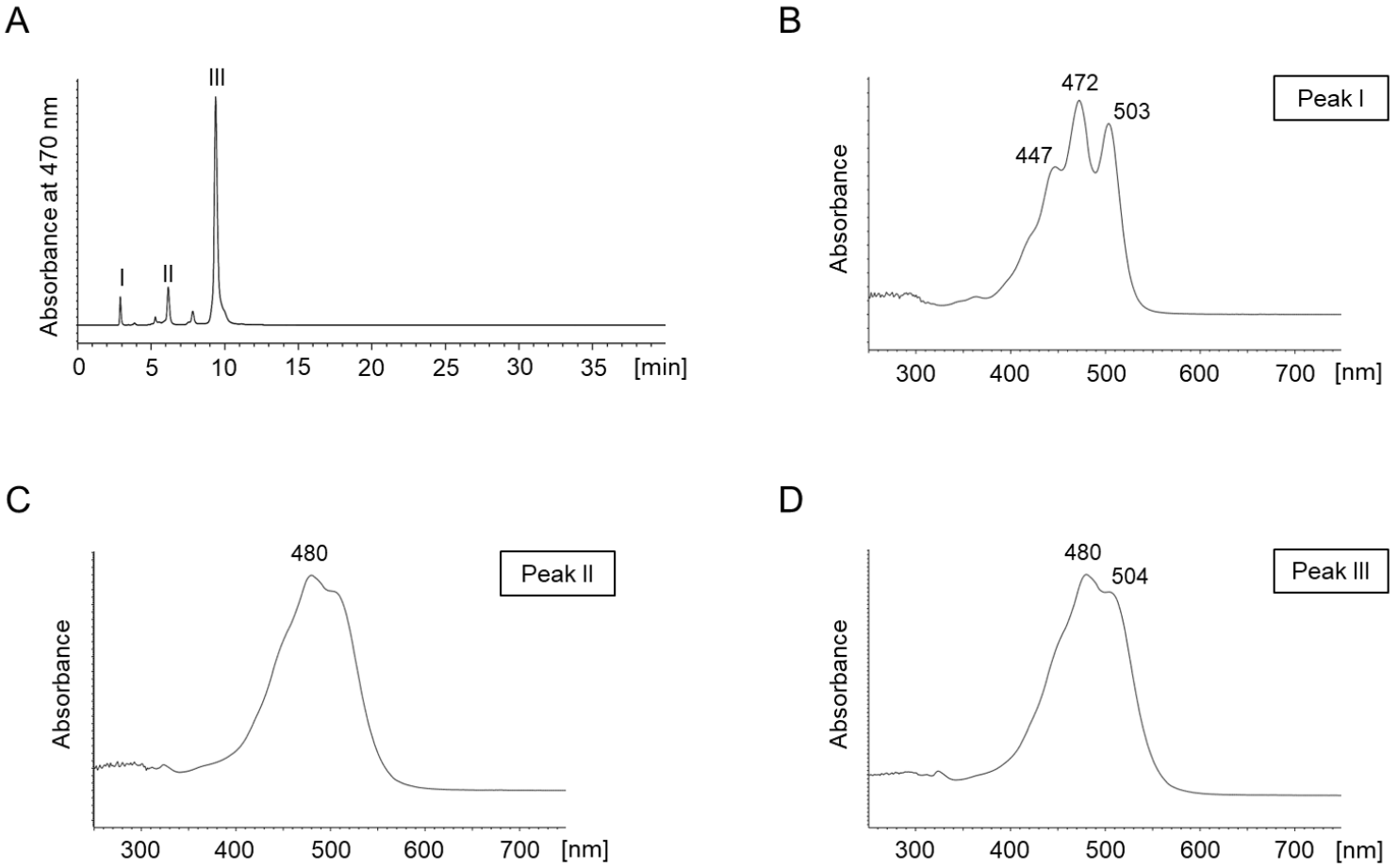
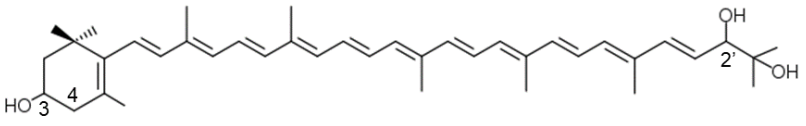
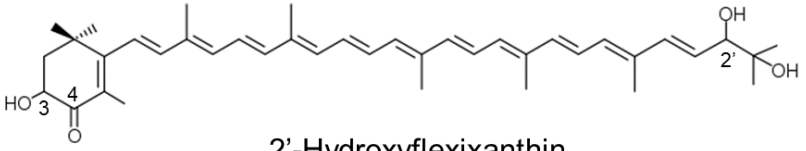

A
Absorbance at 470 nm
I
II
III
0
5
10
15
20
25
30
35
[min]
B
Peak I
472
503
447
Absorbance
300
400
500
600
700
[nm]
C
Peak II
480
Absorbance
300
400
500
600
700
[nm]
D
Peak III
480
504
Absorbance
300
400
500
600
700
[nm]

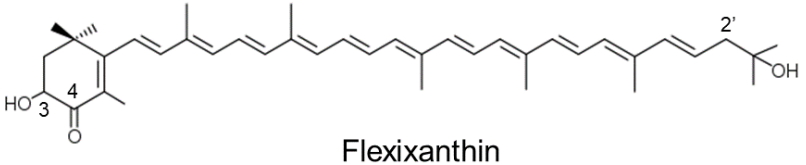
Fig. 1



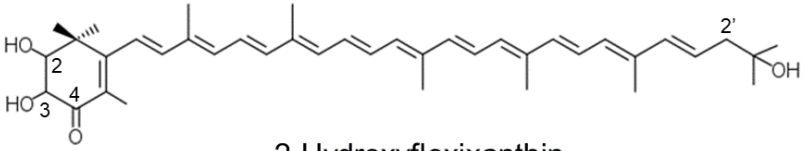
Myxol



2'-Hydroxyflexixanthin



Flexixanthin



2-Hydroxyflexixanthin

Fig. 2

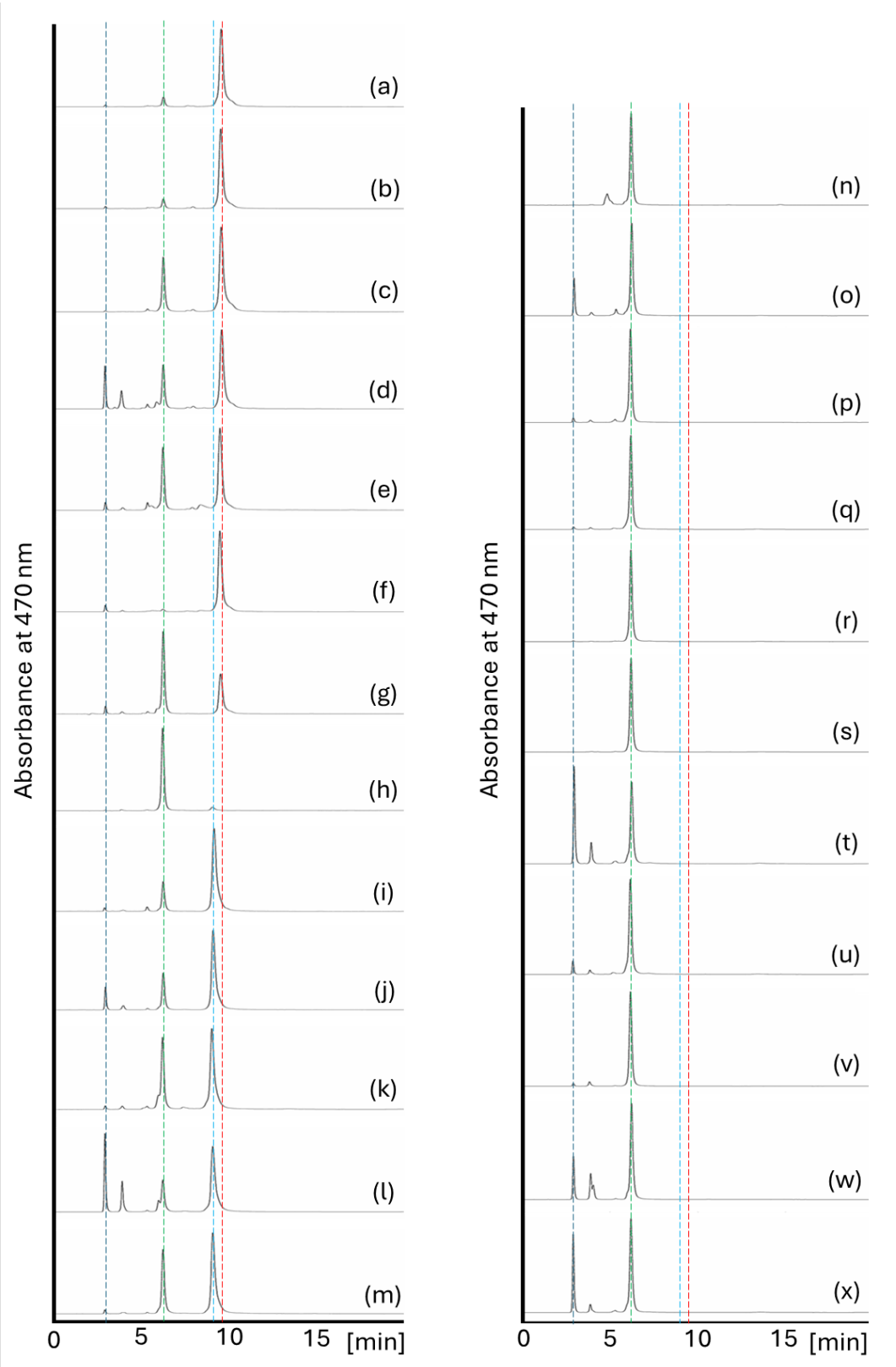

Absorbance at 470 nm
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
0
5
10
15
[min]
Absorbance at 470 nm
(n)
(o)
(p)
(q)
(r)
(s)
(t)
(u)
(v)
(w)
(x)
0
5
10
15
[min]

Fig. 3

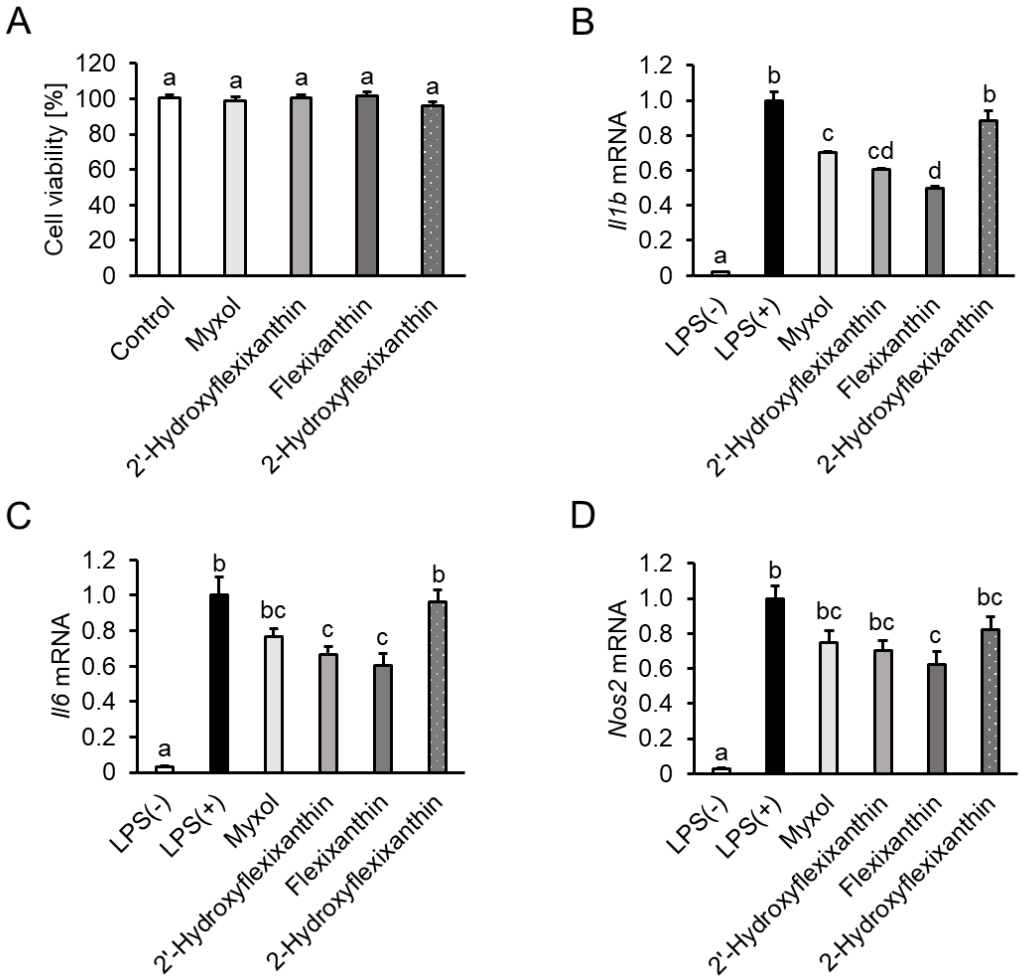

A
Cell viability [%]
120
100
80
60
40
20
0
a
a
a
a
a
Control
Myxol
2'-Hydroxyflexixanthin
Flexixanthin
2-Hydroxyflexixanthin
B
Il1b mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
a
b
c
cd
d
b
LPS(-)
LPS(+)
Myxol
2'-Hydroxyflexixanthin
Flexixanthin
2-Hydroxyflexixanthin
C
Il6 mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
a
b
bc
c
c
b
LPS(-)
LPS(+)
Myxol
2'-Hydroxyflexixanthin
Flexixanthin
2-Hydroxyflexixanthin
D
Nos2 mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
a
b
bc
bc
c
bc
LPS(-)
LPS(+)
Myxol
2'-Hydroxyflexixanthin
Flexixanthin
2-Hydroxyflexixanthin

Fig. 4