Supplementary material

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Article title:
Identification of a novel carotenoid, 2'-isopentenylsaproxanthin, by *Jejuia pallidilutea* strain 11shimoA1 and its increased production under alkaline condition

Author name:
N. Takatani・K. Nishida・T. Sawabe・T. Maoka・K. Miyashita・M. Hosokawa

Affiliation:
Faculty of Fisheries Sciences, Hokkaido University
3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan.
E-mail: hoso@fish.hokudai.ac.jp

Fig. S1. The rooted phylogenetic tree on the basis of the 16S rRNA gene sequences. Scale bar, 0.01 accumulated changes per nucleotide. Figure combines the results of three analyses, NJ, MP and ML. Topology shown was obtained by NJ and the percentage values on the branches are the results of a bootstrap analysis using 500 replications. Only nodes supported by all three analyses are shown with the bootstrap values. GenBank/EMBL/DDBJ accession numbers are listed in parentheses.

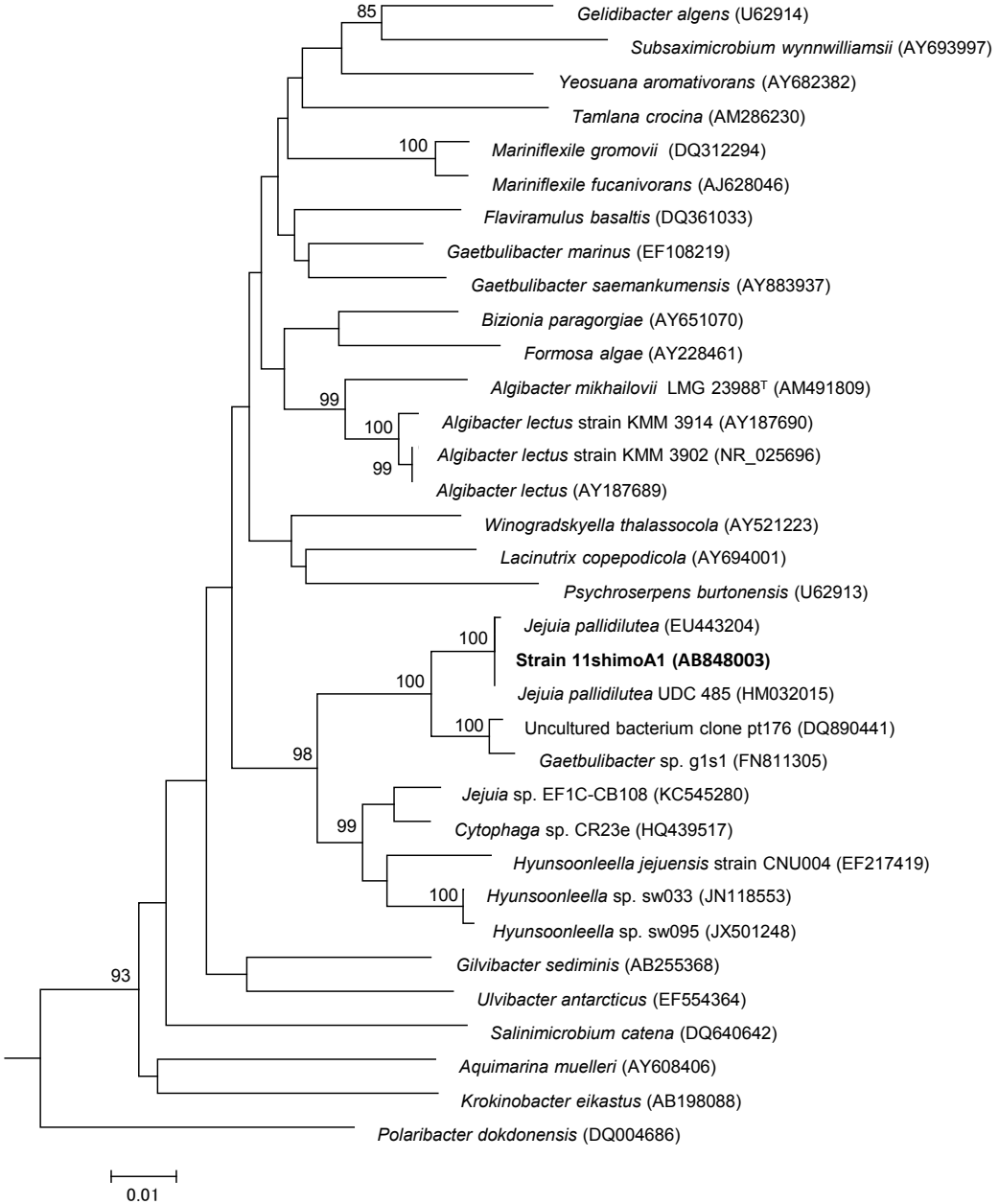
85
Gelidibacter algens (U62914)
Subsaximicrobium wynnwilliamsii (AY693997)
Yeosuana aromativorans (AY682382)
Tamlana crocina (AM286230)
100
Mariniflexile gromovii (DQ312294)
Mariniflexile fucanivorans (AJ628046)
Flaviramulus basaltis (DQ361033)
Gaetbulibacter marinus (EF108219)
Gaetbulibacter saemankumensis (AY883937)
Bizionia paragorgiae (AY651070)
Formosa algae (AY228461)
Algibacter mikhailovii LMG 23988T (AM491809)
99
100
Algibacter lectus strain KMM 3914 (AY187690)
Algibacter lectus strain KMM 3902 (NR_025696)
99
Algibacter lectus (AY187689)
Winogradskyella thalassocola (AY521223)
Lacinutrix copepodicola (AY694001)
Psychroserpens burtonensis (U62913)
100
Jejuia pallidilutea (EU443204)
Strain 11shimoA1 (AB848003)
100
Jejuia pallidilutea UDC 485 (HM032015)
100
Uncultured bacterium clone pt176 (DQ890441)
98
Gaetbulibacter sp. g1s1 (FN811305)
Jejuia sp. EF1C-CB108 (KC545280)
99
Cytophaga sp. CR23e (HQ439517)
Hyunsoonleella jejuensis strain CNU004 (EF217419)
100
Hyunsoonleella sp. sw033 (JN118553)
Hyunsoonleella sp. sw095 (JX501248)
Gilvibacter sediminis (AB255368)
93
Ulvibacter antarcticus (EF554364)
Salinimicrobium catena (DQ640642)
Aquimarina muelleri (AY608406)
Krokinobacter eikastus (AB198088)
Polaribacter dokdonensis (DQ004686)
0.01

Fig. S1.

Fig. S2. Regulation of carotenoid synthesis in strain 11shimoA1 by nicotine.

The strain 11shimoA1 was cultured on Marine Agar 2216 (Difco) containing 2 mM nicotine for 48 h. After cultivation, the strain 11shimoA1 was scrapped off, and total lipid was extracted by the method described by Folch et al. (1957). The extracted lipid was applied to high performance liquid chromatography (HPLC) with Develosil ODS column (4.6 x 250 mm, Nomura Chemical, Japan), eluting with acetonitrile: dichloromethane (75:25, v/v) at a flow rate of 1.0. Carotenoids were detected with UV-VIS detector (Hitach L-2400) at 450 nm and retention time and UV/Vis spectra of a major carotenoid (i) was compared to that of lycopene standard (ii).

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509

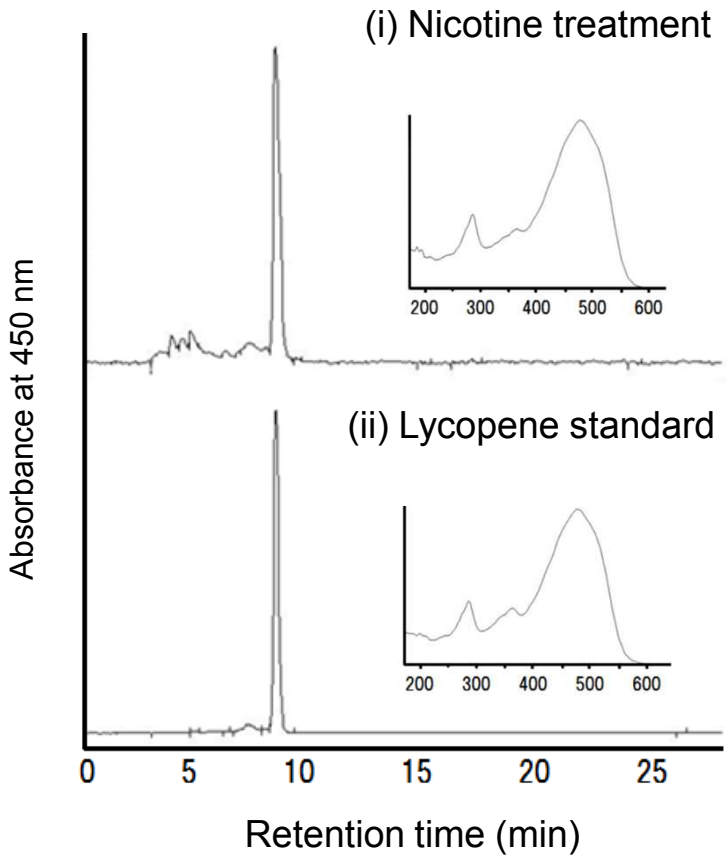
(i) Nicotine treatment
200 300 400 500 600
(ii) Lycopene standard
200 300 400 500 600
Absorbance at 450 nm
0 5 10 15 20 25
Retention time (min)

Fig. S2.

Fig. S3. Analysis of carotenoid produced by the mutant 11shimoA1R.

Strain 11shimoA1 was treated with ethyl methane sulphonate (EMS) in PBS at 30 ^{o}C for 45 min. After centrifugation at 3,500 g, surpernatunt was moved and the pellet was suspended in Marine Broth 2216 (Difco). Aliquot of suspension was plated on Marine Agar 2216 (Difco) and incubated at 25^{o}C for 48 h. A red-colony mutant (11shimoA1R) was isolated and cultivated in Marine Broth 2216 at 25^{o}C for 48 h again. The mutant 11shimoA1R was collected by centrifugation, and the total lipid was extracted from the pellet by Folch method (J Biol Chem 226:497-509,1957). The extracted lipid was then applied onto a preparative thin-layer chromatography (TLC) plate with silica gel 60 (Merck, Germany) and developed with n-hexane:ethyl acetate (4:6, v/v)(Fig. S3(i).). A pigmented fraction, which produced only by the mutant 11shimoA1R, but not the wild type, was scraped and a carotenoid was eluted from silica gel with acetone. The isolated carotenoid was applied to HPLC with Develosil ODS column (4.6 x 250 mm, Nomura Chemical, Japan), eluting with acetonitrile: dichloromethane (75:25, v/v) at a flow rate of 1.0 ml/min and detected with UV-VIS detector (Hitach L-2400) at 450 nm. The retention time and UV/Vis spectra of the isolated carotenoid was compared to that of lycopene (Fig. S3(ii).).

(i) TLC analysis of carotenoids in mutant 11shimoA1R and wild type 11shimoA1

(ii) HPLC analysis of a carotenoid isolated from mutant 11shimoA1R

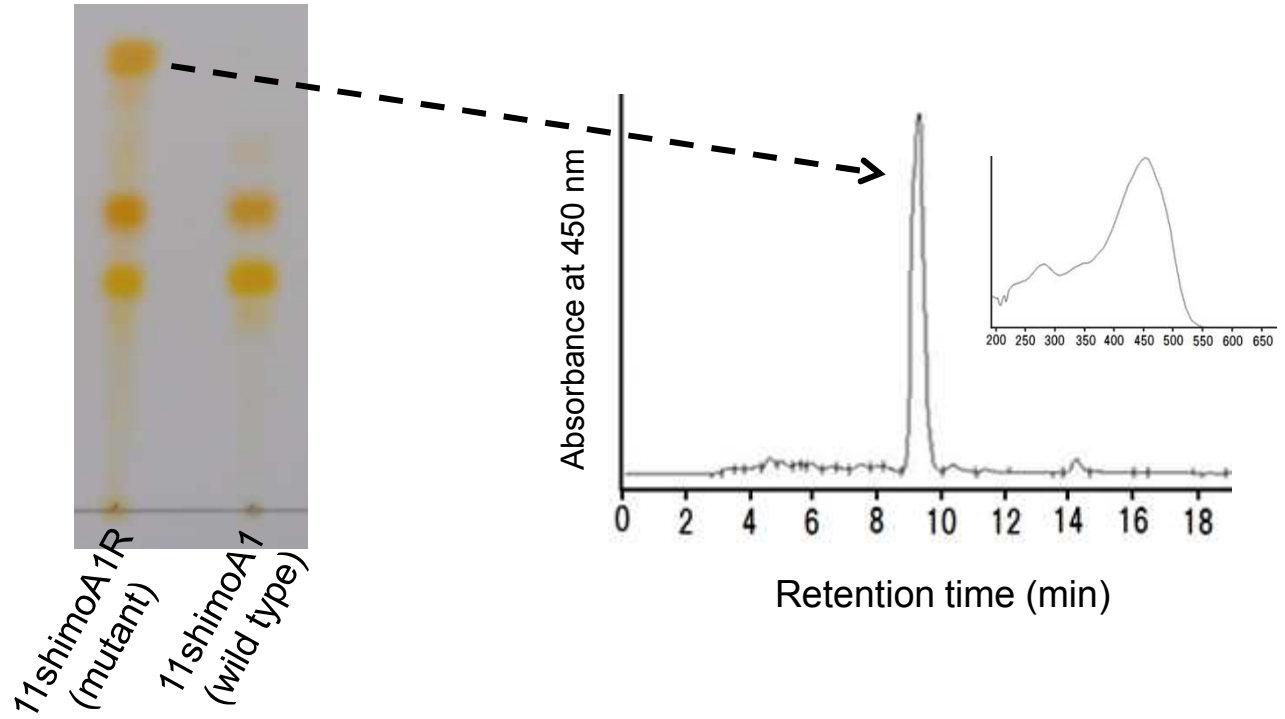

Fig. S3.