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Fucoxanthin production by heterokont microalgae

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ABSTRACT

Fucoxanthin is one of the most abundant carotenoids in nature. It is a major carotenoid in heterokont and haptophyte groups of algae that includes >20 thousand species. Fucoxanthin also has many beneficial health effects including anticancer, antihypertensive, anti-inflammatory, and antiobesity effects. Microalgae are a promising source of fucoxanthin for commercial production. To find the most efficient fucoxanthin producing strains of microalgae, we isolated seven new strains of microalgae from multiple locations in Russia and Vietnam and studied their growth parameters and fucoxanthin content. Based on these results we isolated a novel strain of *Mallomonas* sp. (Synurophyceae) with the highest known content of fucoxanthin in biomass (26.6 mg g^{-1} DW). The analysis of available data on heterokont and haptophyte algae shows that three groups of algae are the most promising for commercial production of fucoxanthin: diatoms (up to 21.67 mg g^{-1} DW), Synurophyceae (up to 26.6 mg g^{-1} DW) and Prymnesiophyceae (up to 18.23 mg g^{-1} DW).

1. Introduction

Fucoxanthin is one of the most abundant carotenoids in nature, accounting for about 10% of the estimated total natural production of carotenoids [1]. It is a major carotenoid in heterokont and haptophyte groups of algae that includes >20 thousand species [2,3]. Some of them are dominant phytoplankton groups in the global ocean (diatoms, *Phaeocystis* and coccolithophores) and brown seaweed species are dominant in the intertidal zone [4].

In algae, fucoxanthin acts as a primary light-harvesting carotenoid that transfers energy to chlorophyll-protein complex. Fucoxanthin molecule exhibits high energy transfer efficiency (>80%) that is thought to be related to the unique structure of this carotenoid [5]. Fucoxanthin also participates in photoprotection from excess light and has strong antioxidant activity [6,7]. Recently, it was shown that fucoxanthin and its derivatives have many beneficial health effects including anticancer, antihypertensive, anti-inflammatory, and antiobesity effects [8,9,10].

Commercial-scale production of fucoxanthin faces several challenges [11]. Chemical synthesis of fucoxanthin is not efficient for commercial-scale production [12]. Currently fucoxanthin is produced mainly from the waste parts of brown macroalgae such as *Laminaria japonica*, *Eisenia bicyclis*, *Undaria pinnatifida*, and *Hijikia fusiformis*. However, these macroalgae are mostly harvested for food in Asia and they contain very low concentrations of fucoxanthin [13].

Microalgae can be considered as promising source of fucoxanthin for commercial production. The concentration of fucoxanthin in microalgae is much higher than in macroalgae and the techniques of industrial production of microalgae are constantly developing. Despite the abundance and diversity of fucoxanthin producing microalgae only a few species were studied for commercial production of fucoxanthin [6,11].

To find the most efficient fucoxanthin producing strains of microalgae, we isolated seven new strains of microalgae from multiple locations in Russia and Vietnam and studied their growth parameters and fucoxanthin content. Based on these results we isolated a novel strain of *Mallomonas* sp. (Synurophyceae) with the highest known content of fucoxanthin in biomass.

2. Materials and methods

2.1. Culture isolation and cultivation

Cultures were isolated in 2012 and 2014 from Baikal Lake (*Cyclotella meneghiniana* SBV23 (N 52°33.855′, E 107° 08.125); *Cyclotella* cf. *cryptica* SBV12 (N 52° 28.997′, E 106° 57.601′), *Nitzschia* sp. SBV25 (N 53° 51.018′, E 108° 39.489′) and *Nitzschia* sp. SBV26 (N55° 22,429′,

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E109° 12,652′)) and freshwater pond in Vietnam (*Mallomonas* sp. SBV13, (N 12°15.016′, 109°09.083′ E), *Cyclotella meneghiniana* SBV11 (N 12°15.016′, 109°09.083′ E)) using WC medium, one culture was isolated from South-China Sea, Vietnam (*Paralia longispina* SBV19, N 12° 19.896′, E 109° 12.582′) using f/2 medium [14,15]. We also used *Phaeodactylum tricornutum* UTEX L642 as a positive control in experiments. Monoclonal cultures were established by micropipetting single cells under an inverted microscope. Microalgae were cultured in laboratory incubator shaker Multitron (Infors HT) at temperature 24 °C, constant shaking at 150 rpm and 5% CO_2 in air supply. Light intensity was 140 μmol m^{-2} s^{-1} with 16:8 h light/dark photoperiod. 20% inoculate was used and the culture was grown for 14 days. All cultures were deposited in the BOROK WDCM602 collection of the Papanin's Institute for Biology of Inland Waters of the Russian Academy of Sciences. For each replicate seven flasks with 50 mL of medium were used. The optical density was measured every two days using UV–Vis spectrophotometer DR 6000 (HACH-Lange). At the end of experiment, the remaining flask was used for dry mass and fucoxanthin content measurements. The experiments for optimization of growth conditions were performed in laboratory incubator shaker Multitron at constant shaking at 150 rpm and 5% CO_2 in air supply. The light intensity range of growth was tested from 10 to 226 μmol photons m^{-2} s^{-1}. The pH range of growth was tested with MES and Tris buffers at concentrations from 4.1 to 30 mM. The pH was measured at the beginning and at the end of cultivation. The final pH values were used for analysis. The temperature range was tested from 16 to 30 °C. The influence of NaCl, phosphate and nitrate concentrations on a growth of culture was tested using different concentrations of reagents added to a medium before autoclaving.

2.2. Dry weight and fucoxanthin content determination

To obtain dry weight data, the biomass was harvested by centrifugation (2900 ×g, 15 min) and lyophilized. Dry weight was recorded and biomass was stored in fridge at −70 °C until extraction. Different conditions were studied for extraction of fucoxanthin from the lyophilized biomass: chloroform-methanol 3:1, 96% ethanol, and acetonitrile. All reagents were HPLC grade. In all cases extraction was performed as follows: to 10.0 mg of lyophilized biomass 1.0 mL of extraction solvent was added and the suspension was shaken at room temperature for 20 min at 1800 rpm. Then the mixture was centrifuged for 3 min at 15000 g. The supernatant (0.8 mL) was collected for further analysis. Analysis was performed on HPLC system Nexera X2 (Shimadzu) with SPD M20A diode array detector and HPLC column Discovery C18 150 × 4,6 mm, 5 μm (Supelco). Isocratic elution with water-acetonitrile mixture, 1:3, during 12 min was carried out at 1 mL/min, monitoring absorbance at 450 nm. Solutions of Sigma-Aldrich fucoxanthin standard PN F6932 (3.2 mg in 25.0 mL of acetonitrile) at various dilutions (up to 50× in case of minimal concentration) were used for calibration (Fig. 1). Analysis of chromatograms showed that two successive extractions with pure acetonitrile proved most efficient in terms of fucoxanthin yield and stability in extract. Particularly, fucoxanthin content did not change in acetonitrile extract for 24 h at +4 °C.

2.3. Molecular analysis

DNA was isolated using InstaGene Matrix (BioRad). Fragments of 18S rDNA (375 bp, including V4 domain) were amplified using primers from Zimmerman and coauthors [16]. The amplifications of 18S rDNA were carried out in reaction volumes of 25 μL, with 10 ng of gDNA, 0.4 mM of forward primer, 0.4 mM of reverse primer, 3 mM $MgCl_2$, 0.5 μL BSA (10 g/L), 125 μM dNTP, 10 mM Tris-HCl, 50 mM KCl, 2.5 nL Tween 20, and 1.75 units of PeqLab Hot Taq DNA Polymerase. The conditions of amplification were: an initial denaturation of 2 min at 94 °C, 5 initial cycles at 94 °C for denaturation (45 s), annealing at 52 °C (45 s), and extension at 72 °C (1 min), followed by 35 cycles at 94 °C for denaturation (45 s), 50 °C for annealing (45 s) and 72 °C for extension (1 min), and a final extension of 10 min at 72 °C [16]. The obtained sequences were edited manually and assembled using BioEdit v7.1.3 [17]. Phylogenies of these sequences were constructed based on the GTR + G + I model using Bayesian inference (BI) and Maximum likelihood (ML) analysis. BI analysis was conducted with MrBayes-3.1.2 [18] and was run with 5 Markov chains (four heated chains, one cold) for 2 × 10 6 generations and 2 independent runs in each analysis. Trees were sampled every 100th generation, the first 25% samples were discarded as "burn-in". The average standard deviation of split frequencies, convergence diagnostics for the posterior probabilities of bipartitions were used to check for convergence. ML analysis was conducted using the MEGA6 with 1000 bootstrap replicates [19]. Sequences were deposited to Genbank under accession numbers *Nitzschia* cf. *carinospeciosa* SBV25 (KX905098), *Nitzschia* cf. *carinospeciosa* SBV26 (KX905099), *Cyclotella meneghiniana* SBV11 (KX905100), *Cyclotella meneghiniana* SBV23 (KX905101), *Cyclotella* cf. *cryptica* SBV12 (KX905102), *Paralia longispina* SBV19 (KX905103), *Mallomonas* sp. SBV13 (KX905104).

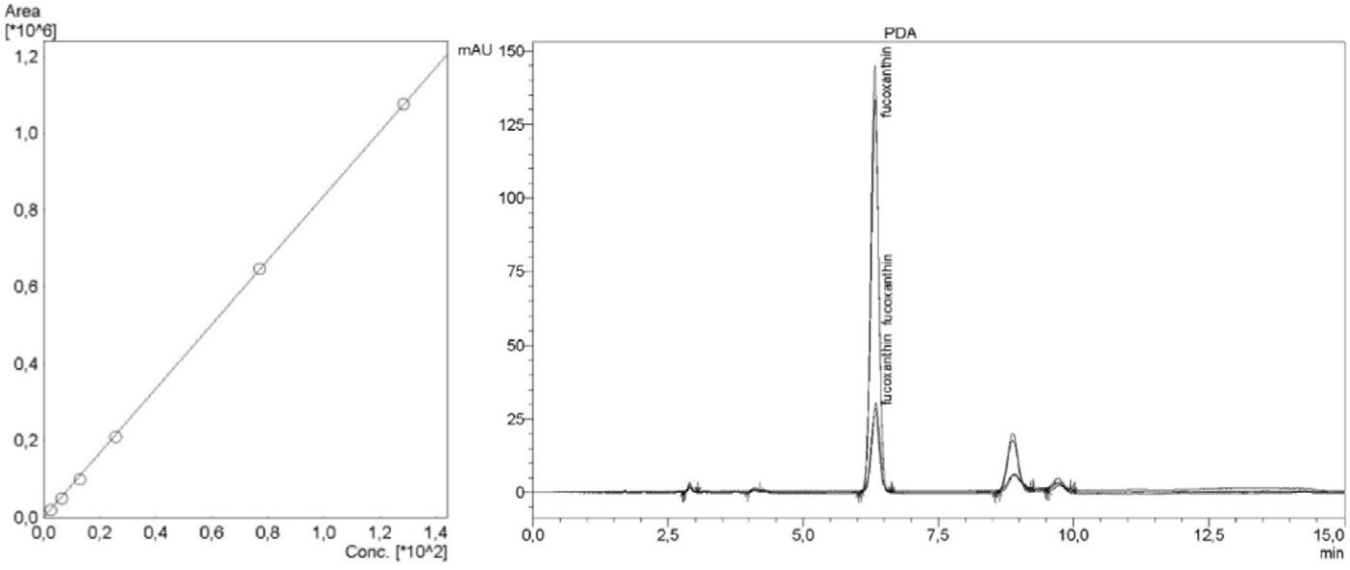

Fig. 1. Fucoxanthin determination in acetonitrile by HPLC: (a) calibration curve (fucoxanthin standard solutions 3.2 mg in 25.0 mL of acetonitrile at 1.0×, 0.60×, 0.25×, 0.10×, 0.050×, and 0.020× dilutions); (b) chromatograms of acetonitrile extracts from lyophilized *Mallomonas* sp. SBV13 biomass, absorbance profiles at 450 nm are shown.