Raman and fluorescence lifetime imaging of cellular carotenoids distribution in algae

A.N.Semenov[1], E.A.Protasova[1], Eu.Yu.Parshina[1], K.A.Chekanov[1], T.A.Fedorenko[1], D.N.Ahaev[1], E.S.Lobakova[1], E.G.Maksimov[1]

[1]Department of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia

Abstract—The results of the complex multimodal optical study utilizing Raman and fluorescence lifetime imaging of the cells of microalga *Bracteacoccus aggregatus* BM5/15 are presented.

Keywords— carotenoids, Raman, FLIM, algae.

I. Introduction

Antioxidants, including carotenoids, provide promising opportunities to significantly increase quality of life of the patients suffering from various pathologies associated with increased oxidative stress such as cardiovascular deceases. However, due to the poor bioavailability and fast photodegradation of carotenoids in the native forms, their targeted delivery to the cells is very limited [1]. Recently, a new strain of the microalga *Bracteacoccus aggregatus* BM5/15 was reported to be capable of industrial production of carotenoids, namely, β-carotene and astaxanthin [2]. In the present report we demonstrate the results of multimodal optical research of different states of *Bracteacoccus aggregatus* using Raman and fluorescence lifetime imaging. This study was aimed at the assessment of optical characteristics and spatial distribution of the cellular pigments in order to determine the possibilities of optical imaging techniques to investigate the structural features and types of producible carotenoids. Results are important to control the efficiency of carotenoids extraction from the production substance and subsequent targeted delivery.

II. Materials and Methods

Three states of *Bracteacoccus aggregatus* – green, orange and red, which are characterized with different content of carotenoids – were studied. Raman imaging and spectroscopy were performed using a confocal microscope system Ntegra Spectra (NT-MDT, Russia) under excitation at 532 nm. Raman data analysis was performed in Spectragryph software. Fluorescence Lifetime Imaging (FLIM) was performed in TCSPC regime on the confocal system by Becker&Hickl (Germany) on Eclipse Ti2 (Nikon, Japan) microscope with two-photon excitation at 750 nm utilizing parametric laser source TOPOL (Avesta, Russia). Autofluorescence was detected in 350-650 nm range, lifetime kinetics were analyzed in the SPCImage software (Becker&Hickl, Germany).

III. Results

Mean Raman spectra, measured on a single-cell level for different states of algae, are presented in Fig. 1. First of all, we observed an increase in Raman intensity during cultivation of cells under exposure to strong white light indicating an increase of astaxanthin concentration. Secondly, we observed evolution of line shape of Raman spectra, namely at 1160 cm^{-1} and 1520 cm^{-1}, and heterogeneity of signals on the single-cell level. Autofluorescence data analysis (Fig. 2) shows that mean lifetime significantly decreases from green (510 ps) to orange (320 ps) and red (180 ps) forms. We suppose that such short lifetimes correspond to autofluorescence originated from carotenoids in monoester form.

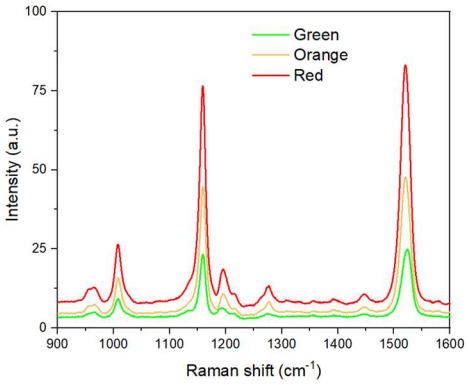

Fig. 1. Raman spectra (excitation at 532 nm) of green, orange and red states of microalga *Bracteacoccus aggregatus* BM5/15.

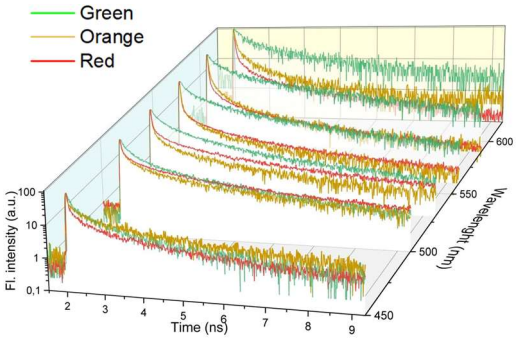

Fig. 2 Fluorescence lifetime kinetics of green, orange and red states of microalga *Bracteacoccus aggregatus* BM5/15 (two-photon excitation at 750 nm), detected in various spectral channels.

Acknowledgment

The study was supported by Russian Science Foundation grant № 22-25-00183.

References

[1] E. Maksimov, et al. “Soluble cyanobacterial carotenoprotein as a robust antioxidant nanocarrier and delivery module,” Antioxidants, vol. 9, 869, pp. 1-23, 2020.

[2] K. Chekanov, et al.”Combined production of astaxantin and β-carotene in a new strain of the microalga *Bracteacoccus aggregatus* BM5/15 (IPPAS C-2045) cultivated in photobioreactor,” Biology, vol. 10, 643, pp. 1-17, 2021.

2022 International Conference Laser Optics (ICLO) | 978-1-6654-6664-6/22/$31.00 ©2022 IEEE | DOI: 10.1109/ICLO54117.2022.9839720