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Preliminary identification of unicellular algal genus by using combined confocal resonance Raman spectroscopy with PCA and DPLS analysis

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ABSTRACT

The analysis of algae and dominant alga plays important roles in ecological and environmental fields since it can be used to forecast water bloom and control its potential deleterious effects. Herein, we combine in vivo confocal resonance Raman spectroscopy with multivariate analysis methods to preliminary identify the three algal genera in water blooms at unicellular scale. Statistical analysis of characteristic Raman peaks demonstrates that certain shifts and different normalized intensities, resulting from composition of different carotenoids, exist in Raman spectra of three algal cells. Principal component analysis (PCA) scores and corresponding loading weights show some differences from Raman spectral characteristics which are caused by vibrations of carotenoids in unicellular algae. Then, discriminant partial least squares (DPLS) classification method is used to verify the effectiveness of algal identification with confocal resonance Raman spectroscopy. Our results show that confocal resonance Raman spectroscopy combined with PCA and DPLS could handle the preliminary identification of dominant alga for forecasting and controlling of water blooms.

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1. Introduction

Nowadays, under influences of human activity, sewage and industrial wastewater, rainfall and surface runoff which contain large amounts of nitrogen, phosphorus, other inorganic salt and plant nutrients are input into water bodies such as reservoirs, lakes, estuaries and gulfs. Then, increasing nutrients of water bodies has prompted growth and rapidly reproduction of autotrophic organisms and planktonic algae. The uncontrolled growth and reproduction of algae has led to water blooms frequently. It has been reported that water blooms have serious consequences that deplete local oxygen or destruct habitat of submerged vegetation. And, some toxins which were released from algal cells in water blooms have particularly harmful to organisms (including human beings) with its neurotoxic or hepatotoxic [1,2]. Moreover, the identification of dominant alga can help us to forecast and control the potentially deleterious effects of water blooms. Therefore, the algal identification plays important roles in ecological and environmental fields. However, it is challenging to find a sensitive, non-destructive, time saving, automated method for the identification of dominant alga so as to prevent water blooms worldwide.

Traditionally, algae are identified by microscopy based on their morphology by experienced scientists [3]. Recently, a variety of molecular biology techniques have also been reported for the identification of algae, such as enzyme-linked immunosorbent assay [4], and high performance liquid chromatography [5]. These techniques have been used specifically to analyze the composition or distribution of intracellular proteins, lipids, and pigments in planktonic algal cells. However, the destructive, time-consuming, and high-specialized characteristics have limited their application for algal identification rapidly.

Raman spectroscopy is a versatile powerful technique due to its non-destructive and minimal sample preparation. It has been explored for chemical analysis of organic compounds, including biomolecules and microbial pigments, especially for composition or distribution of intracellular proteins, lipids, and pigments in planktonic algal cells with resonant and mapping technologies [6,7]. Based on the fundamental vibrations of pigments, various marine algal species are identified

Abbreviations: PCA, principal component analysis; DPLS, discriminant partial least squares; FACHB, Freshwater Algae Culture Collection at the Institute of Hydrobiology; AsLS, asymmetric least squares; RMSE, root mean squared error; Lw, loading weights; PC, principal components.

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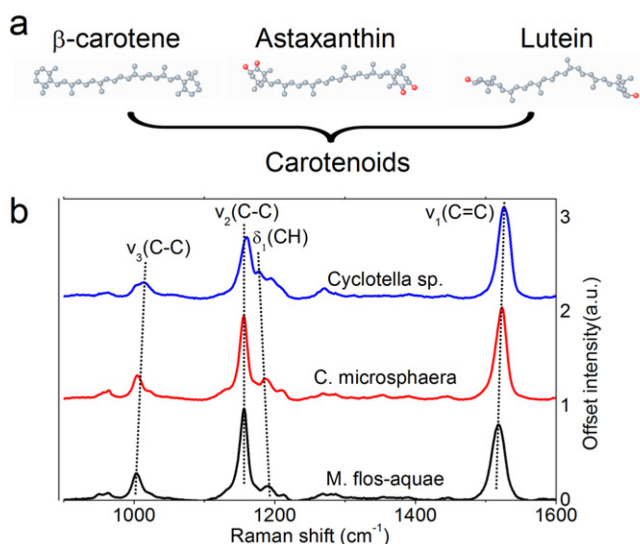

Fig. 1. 3D structures of three carotenoids (a), average resonance Raman spectra of three algal cells and its characteristic vibration information (b).

from aqueous suspensions which contain different algae with resonance Raman spectroscopy [8]. Non-toxic and toxic algal strains are also discriminated with weaker resonance Raman features from composition of different carotenoids [9]. Others report that portable Raman spectrometer combining with acoustic levitation device can obtain some different characteristic features of chromophores in algal cells [10–12]. Moreover, coherent anti-stokes Raman scattering microscopy with nonlinear light-matter interaction could suppress the two-photon-excitation fluorescence in microalgae and be used as a label-free technique for non-invasion monitoring of accumulation and movement of chemical compounds at the subcellular level either [13].

Confocal Raman microscopy technology has brought algal analysis up to in vivo unicellular scale. It has been shown that distribution of carotenoids and spectral signatures of highly similar chromophores in living algal cells can be resolved with confocal Raman microscopy [14,15]. Some investigations have also shown that Raman spectroscopy is highly sensitive and selective for pigment-astaxanthin distribution, concentration and molecular structures in the cyst of unicellular microalgae [16,17]. Moreover, light-induced conformation changes, synthetic of protective dyes under aggressive conditions and photopigment reorganization in algal cells are visualized with confocal resonance Raman spectroscopy at unicellular scale in a non-destructive manner respectively [18–20]. However, the identification of algal genus which leads to water blooms has not been in vivo explored at unicellular scale by using confocal resonance Raman spectroscopy technology.

Herein, we propose an algal identification technique that uses confocal resonance Raman spectroscopy combining with multivariate analysis to discriminate the three primary algal genera from water blooms in *Three Gorges reservoir* at unicellular scale. The different characteristic information from average Raman spectra of each algal genus cells is firstly obtained with statistical analysis among three algal genera. Then, an unsupervised exploratory analysis principal component analysis (PCA) [21,22] is also employed to preliminarily mine Raman characteristic differences among three algae from water blooms at living unicellular scale. Thereafter, discriminant partial least squares (DPLS) classification method [23,24] will be used to identify algal genus with Raman spectra of cultivated and real cells. These heuristic approaches could provide a possibility non-destructive dominant algal identification method for forecasting and controlling of water blooms.

2. Materials and Methods

2.1. Organisms and Growth Conditions

All microalgae including cyanophyta *microcystis flos-aquae* (FACHB-1341, *M. flos-aquae*), *microcystis sp.* (FACHB-1314), bacillariophyta *cyclotella sp.* (FACHB-1631) and chlorophyta *chlamydomonas microsphaera* (FACHB-52, *C. microsphaera*) were obtained from Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-collection), Chinese Academy of Sciences. All of them were cultivated under 25 ± 2 °C, with a 12:12 h light/dark cycle at a photon flux of 2000 Lux in 50 ml Erlenmeyer flasks with BG11, SE and CSI medium (FACHB) respectively. In addition, real algal suspension was obtained from *Pengxi River* of *Three Gorges reservoir* in May 2017.

2.2. Raman Spectroscopy and Measurements

A Renishaw (Wotton-under-Edge, Gloucestershire, UK) inVia micro-Raman spectroscopy system, equipped with a Peltier cooled CCD detector (−70 °C) and a 532 nm DPSS laser (the maximum laser power: 50 mW), was used to collect back-scattered Raman signals of algal cells upon 2400 lines mm^{-1} grating. A Leica microscope with 50× objective was used to focus incident laser light on the samples. So, the diameter of laser spot on sample was approximately 1 μm. The spectral resolution was 1 cm^{-1}. Prior to Raman measurement of algal cells, the spectrometer was calibrated with Raman shift of silicon at 520 cm^{-1}. Thereafter, the experimental data were all obtained under the same conditions except for special instructions.

For each alga, 1 μl algal suspensions were harvested at the exponential growth phase. The algal cells were adhered to the surface of glass slides overcoated with poly-L-lysine (Sigma) and maintained about 10 min before Raman measurements. Then, Raman spectra of 15 single algal cells were acquired with the aid of the microscope. The algal harvest and measurements were repeated one time every day for five consecutive days. The spectral range was from 596 cm^{-1} to 1760 cm^{-1}, the number of acquisition was 1 time, the acquisition time of each spectrum was 1 s. The excitation power on the sample was 0.5% of laser power (approximately 0.1 mW). It was low enough to prevent photo damage of algal cells during Raman measurements, as verified by visual inspection (shown in Fig. S1). For the identification of algal genus, Raman spectra of *M. flos-aquae* were used in classification modeling processes, whereas Raman spectra of *microcystis sp.* were applied to externally verify the effectiveness of proposed classification models.

2.3. Spectral Preprocessing

The characteristic Raman spectral peaks of algal cells were fitted with Lorenz function (WiRE3.4) which was provided by Renishaw

Table 1
The characteristic Raman shifts (cm^{-1}) for three algae.

Bands	*M. flos-aquae*	*Cyclotella sp.*	*C. microsphaera*	Band assignments [8,10,12]
v_3	1003.97	1011.95	1005.39	Carotenoids: symmetry CH_3, in-plane stretch CC.
v_2	1155.85	1159.47	1155.75	Carotenoids: in-plane stretch CC, deformation CH.
δ_1	1191.47	1178.96	1188.34	Carotenoids: deformation CH.
δ_2	1212.36	1195.74	1210.46	Carotenoids: in-plane stretch CC, deformation CH.
v_1	1517.85	1526.29	1522.13	Carotenoids: in-plane stretch (C=C).

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