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Study of carotenoids in cyanobacteria by Raman spectroscopy

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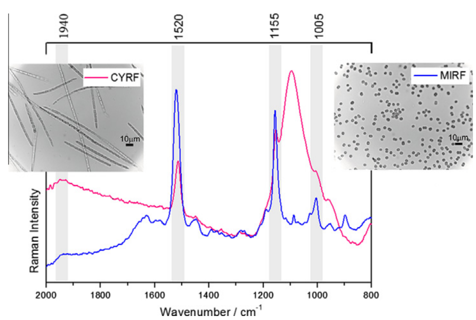
HIGHLIGHTS

- The colours of the cyanobacteria are due to the carotenoids compounds.
- Different protective dyes are synthesized according to the strain necessity.
- Thereby, our proposition is the peridinin pigment as the polyene majority.
- We use the Raman spectroscopy (RS) as a tool for the dye assignment.
- The RS is a preliminary tool for discussion about dyes and the synthetic priority.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacteria have established dominant aquatic populations around the world, generally in aggressive environments and under severe stress conditions, e.g., intense solar radiation. Several marine strains make use of compounds such as the polyenic molecules for their damage protection justifying the range of colours observed for these species. The peridinin/chlorophyll-*a*/protein complex is an excellent example of essential structures used for self-prevention; their systems allow to them surviving under aggressive environments. In our simulations, few protective dyes are required to the initial specimen defense; this is an important data concern the synthetic priority in order to supply adequate damage protection. Raman measurements obtained with 1064 and 514.5 nm excitations for *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* strains shows bands assignable to the carotenoid peridinin. It was characterized by bands at 1940, 1650, 1515, 1449, 1185, 1155 and 1000 cm^{-1} assigned to ν(C=C=C) (allenic vibration), ν(C=C/CO), ν(C=C), δ(C—H, C-18/19), δ(C—H), ν(C—C), and ρ($C—CH_3$), respectively. Recognition by Raman spectroscopy proved to be an important tool for preliminaries detections and characterization of polyene molecules in several algae, besides initiate an interesting discussion about their synthetic priority.

Introduction

Cyanobacteria or blue–green algae are photosynthetic microorganisms commonly found in diverse aquatic environments; these organisms can grow and develop in water columns, attached on

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substrates or water surface [1]. The cyanobacteria uncontrolled growth gives rise to agglomerates known as "bloom-forming" [1,2]. This phenomenon is the most notorious and evident consequence of the eutrophication process and/or ozone depletion with dramatic implications in the aquatic environments [3–6]. Thus, remarkable alterations are observed in the system balance, such as the production of several cyanotoxins, e.g., the hepatotoxins with harmful effects on animal health [7–10]. The uncontrolled liberation of toxic pollutants on the nature promotes a high risk of water contamination and requires intensive studies, control, and continuous monitoring of that region [4,8,11–14]. The ability to form surface blooms is associated with the creation of gas vesicles and is more noticeable after prolonged exposure to high radiation levels [5,15]. The excessive exposure to solar radiation or stressed extremophilic environments results in the production of several photo-protective compounds, for example, mycosporine-like amino acids (MAAs), scytonemin and carotenoids [16–22].

Cylindrospermopsis raciborskii (CYRF) and *Microcystis aeruginosa* (MIRF) strains have established dominant populations in different habitats [2,6,13,23–28]. These strains can be found generally in severe stress environments and under highest radiation levels [29], which contributes to the range of colors observed for these species, from green to orange hues [17,30–35]. In these photosynthetic organisms, an universal pigment is the carotenoids class; carotenoids have important functions for maintenance of the cellular matrix integrity, an important supporting for light harvesting and other, for example in the prevention/repair of the photo-oxidative damage [18,36]. The rather wide range of carotenoids colorations aforementioned has motivated their study and recognition by Raman spectroscopy [37] in particular the *in situ* detection and characterization of polyunsaturated structures [38–43], Fig. 1.

Generally, for algae and cyanobacteria, the main pigments identified in their matrix are peridinin [32,44,45], β-carotene, nostoxanthin, xanthophyll, caloxanthin, echinenone, myxoxanthophyll, echinenone, canthaxanthin, oscillaxanthin, zeaxanthin and scytonemin [17,22,30–32,46–48]. Carotenoids are non-water soluble dyes and, jointly with chlorophylls compounds, are present in lipophilic environments inside of sturdy membranes [49]. These dyes are often associated with proteins which renders their characterization and measurements *in situ* extremely problematic for the conventional analytical techniques. The preferential cellular localization of β-carotene is inside of the thylakoid membranes, while the cytoplasmic membranes provide preferential location for xanthophyll compounds [15]. Carotenoids are a very special family consisting over 400 conjugated polyenes with C40 substituted and unsubstituted/unsaturated carbon–carbon chains and, jointly with chlorophyll compounds, are responsible for radiation absorptions, mainly in the visible region of the electromagnetic spectrum [33,50–53]. The Raman spectra profile of these type of polyunsaturated chain present three characteristic bands located at specific wavenumbers, in the region between 1600 and 1000 cm^{-1} which are definitive data for those pigments characterization by Raman spectroscopy [38,39,43,54,55]. These bands are specifically located at ~1500 (ν_1), 1160 (ν_2) and 1000 cm^{-1} (ρ_3), assigned respectively to (C=C) and (C—C) stretching and ($C—CH_3$) deformation modes. Those three bands are assigned to vibrational modes of the polyenic chain, which may also contain substituents in the terminal positions, such as methyl groups and aliphatic or aromatic rings.

Several studies have been undertaken about the composition and the functional properties of carotenoids in living systems; however, this work is generally quite laborious

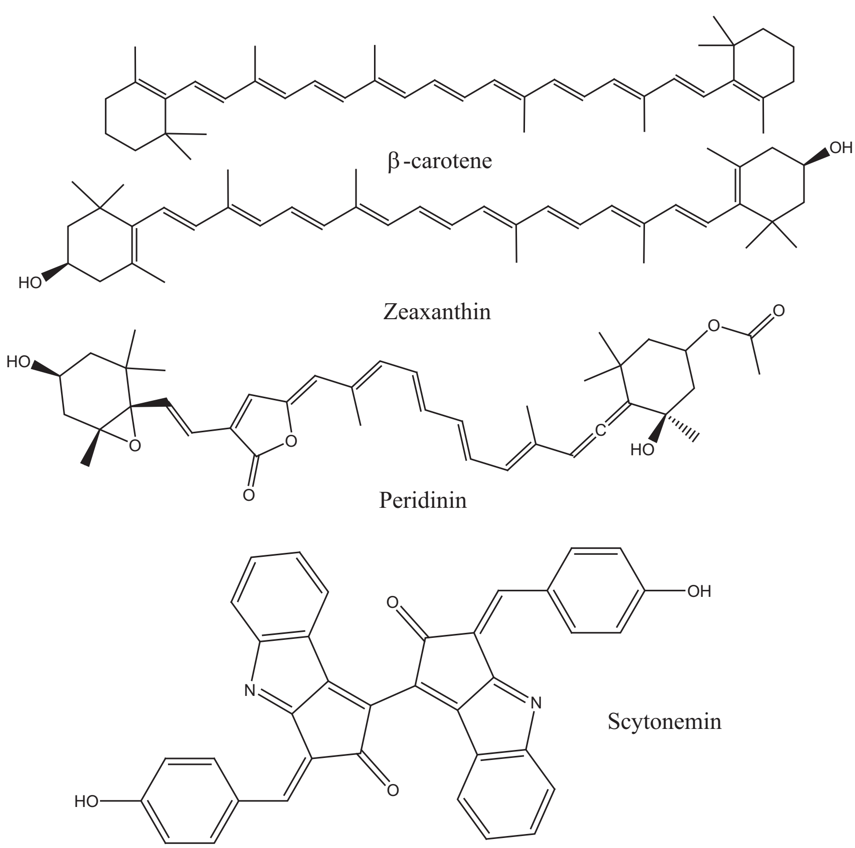

Fig. 1. Molecular structures of the carotenoids zeaxanthin, β-carotene, peridinin and scytonemin.