INVESTIGATION OF PHOTOSYNTHETIC BACTERIA AND ALGAE BY RESONANCE RAMAN SPECTROSCOPY

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Cyanobacteria and algae comprise a large and diverse group of organisms that are widely distributed in freshwater, marine, terrestrial, and extreme environments. Cyanobacterial and algal identification and classification rely on morphological characterization and 16S rRNA gene sequence analysis. Morphology however, can be misleading in certain circumstances, while 16S rRNA gene sequencing can be time consuming. Analysis of pigments that have taxonomic value may be an alternative approach to the identification, classification, and discrimination of cyanobacteria and algae in environmental samples.

It is well-known that the chloroplasts of photosynthetic organisms contain a wide variety of pigments which in turn are known to be taxonomic markers (Fawley, 1989; Andersen et al., 1996). The primary biological function of these pigments is the absorption of light. The light energy is then either transferred to photosynthetic reaction centres to drive photosynthesis or re-emitted at longer wavelengths, to avoid photodamage to cells. These pigments fall into three main categories: chlorophylls and carotenoids, both soluble in organic solvents; and phycobiliprotein which is soluble in water. There are several types of chlorophyll, including chlorophyll *a*, *b*, *c1*, *c2*, *c3*, and *d*. All appear green. In contrast there are more than 60 carotenoid pigments found in cyanobacteria and algae which range in colour from orange to red. Some carotenoids, such as β-carotene and zeaxanthin, are widely distributed throughout algal groups, whereas others are highly specific to individual groups. Phycobiliproteins are unusually found in cyanobacteria, red algae, and a small group of flagellates (Trainor, 1978).

If a biological molecule absorbs light in the visible portion of the electromagnetic spectrum, then new possibilities are opened up for Raman spectroscopy of these compounds of interest, via the resonance Raman (RR) effect. Judicious selection of tuning the excitation wavelength to the electronic absorption spectrum can produce selective enhancement of certain Raman bands. These Raman bands correspond to vibrational modes which involve motions of the atoms in the chromophore, being that portion of the molecule where the electronic transition is localised. Therefore, RR spectroscopy provides a means whereby vibrations of biological chromophores can be distinguished from many of the vibrational modes associated with the complex biological matrix. Significantly, the resonance enhancement factor can be quite large, in the order of 10^3 to 10^6 orders of magnitude, thereby allowing the analysis of chromophore concentrations as low as 10^{-4} to 10^{-6} M.

A total of nine photosynthetic bacteria and micro-algae were studied by RR spectroscopy ($\lambda_{excitation}$ 488 nm). The cyanobacteria investigated include *Nostoc sp*, *Anabaena sp*, *Microcystis sp*, Antarctic isolated cyanobacteria, and Yellowstone National Park isolated cyanobacteria. The micro-algae investigated include two Chlorophyta green micro-alga, namely *Botryococcus braunii* Race A and B and two Dinophyta, namely *Lingulodinium machaerophorum* and *Tuberculodinium vancampoae.* All spectra contain major features at approximately 1005, 1155, and 1525 cm^{-1}, which are strongly resonance enhanced due to carotenoids. These bands at 1500-1500 cm^{-1} and 1150-1170 cm^{-1} are due to in-phase C=C (ν_1) and C-C stretching (ν_2) vibrations of the polyene chain. Additionally, in-plane rocking modes of CH_3 groups attached to the polyene chain coupled with C-C bonds occur in the 1000-1020 cm^{-1} region. The most significant spectral change observed was the position of the C=C (ν_1) band. The position of the ν_1 band is strongly dependent on the length of the carotenoid chain (Withnall et al., 2003; Veronelli et al., 1995).

The position of the ν_1 band acquired from the RR spectra of the cyanobacteria and algae follow the trend: Peridinin carotenoid (*Lingulodinium machaerophorum* and *Tuberculodinium vancampoae*) > Botryoxanthin carotenoid (*Botryococcus braunii* Race A and B) > β-Carotene (*Nostoc sp*, *Anabaena sp*, *Microcystis sp*, Antarctic isolated cyanobacteria, and Yellowstone National Park isolated cyanobacteria). RR spectroscopy has shown to be a useful rapid and non-destructive tool in the discrimination of photosynthetic bacteria and eukaryote micro-algae based on carotenoid analysis.

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