RAMAN MICROSPECTROSCOPY OF BACTERIAL MICROCOLONIES

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Abstract: Spectra taken directly from an imprints of colonies showed spectral differences between various parts of the colony from which molecular structural differences and correlation to phases of the growth cycle can be inferred. In particular, a pigmented species showed a ring structure in carotene distribution not previously reported.

A recent FT-IR ATR study of the growth phase of several bacteria [1], including two halophiles, one thermophile and an acid tolerant species, showed that significant spectral changes occurred in all species. The bacterial cells were harvested at various times during growth, separated by centrifugation and washed thoroughly to remove all media components. However, studying the regions of bacterial microcolonies could save much time and labour in growth phase studies compared to studies utilising broth media, because of the elimination of the harvesting and washing steps. It has been demonstrated by infrared microspectroscopy [2,3] that yeast and bacterial microcolonies are not homogeneous. An investigation by Naumann et al. [2] differentiated regions within the microbial microcolony. Three regions were identified, edge (or periphery), intermediate and centre. As suggested by Orsini et al.[4] the periphery might represent the exponential growth phase of the organism, while the centre represents the stationary phase. Preliminary results of the investigation of the halophile *H. salinarium* are described below.

Microcolonies of the halophile *Halobacterium salinarium* grown on solid growth medium (7 days @ 45 ^{o}C) were stamped from the agar plate to a BaF_2 window following the method devised by Naumann's group [4]. Spatially accurate replicas of the microcolonies were consequently transferred to the optical substrate, which are air dried and ready for spectral acquisition.

Raman spectra were taken along a line traversing the diameter (line map) of a microcolony. The excitation wavelength was 633 nm. The exposure time for each spectrum was 13 minutes 20 seconds (80 accumulations at 10 sec each). The line map consisted of 46 spectra at 2 µm intervals for the total diameter of the microcolony (~92 µm), as shown in figure 1 below.

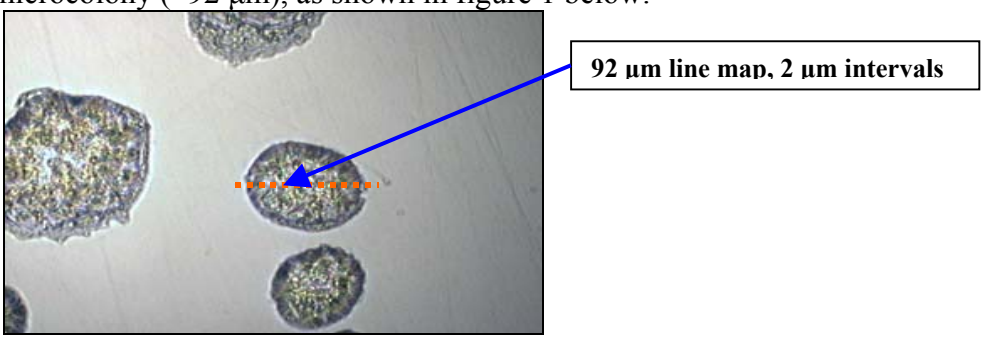

Fig. 1. Micrograph of microcolonies of *H. salinarium*. Position of Raman line map is indicated by the orange line.

Each of the 46 Raman spectra was baseline corrected and normalised to amide 1 (~1657 cm^{-1}). The spectra are dominated by β-carotene bands at 1495 cm^{-1}, 1142 cm^{-1}, and 998 cm^{-1}, due to

resonance enhancement. The most intense β-carotene band (1142 cm^{-1}) was integrated for each spectrum, thus yielding a measure of concentration. Figure 2 below shows selected overlaid spectra, indicative of the varying amount of β-carotene present in the microcolony as a function of distance from the periphery.

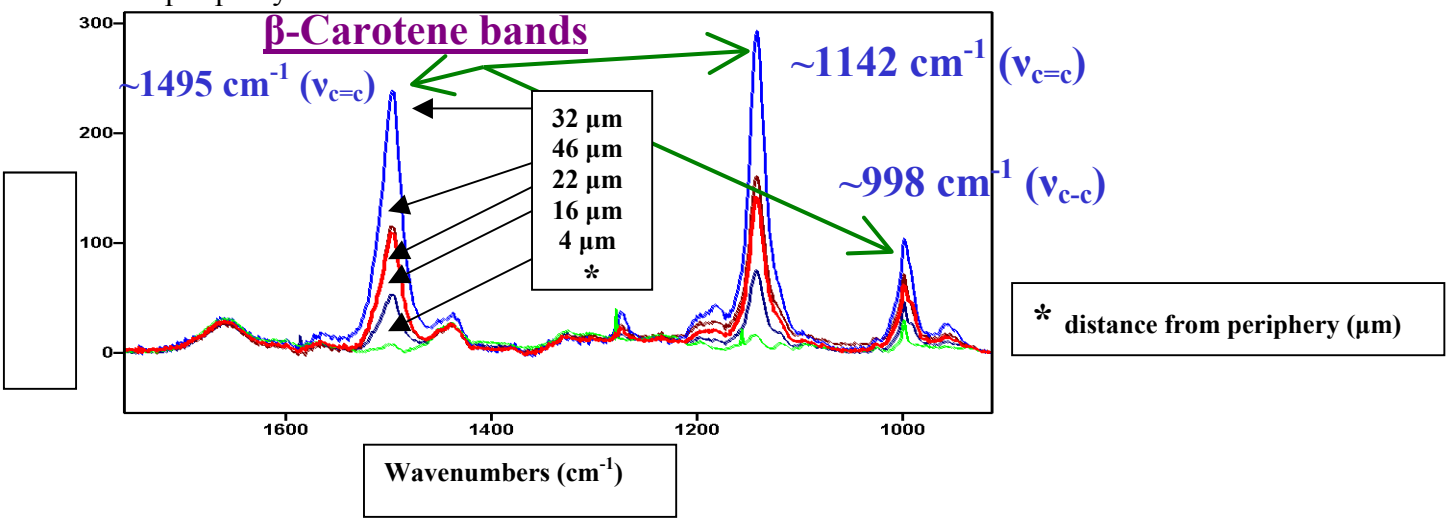

Figure 2. Selected Raman spectra from the line map at various distances from the periphery of a microcolony of *H. salinarium*. Spectra normalised to Amide 1 band near 1657 cm^{-1}.

A graph of the area of the most intense β-carotene band (1142 cm^{-1}) versus distance from the periphery (µm) for each of the spectra shows an interesting near symmetric trend, which relates to the concentric growth pattern of the microcolony, as shown in Figure 3 below.

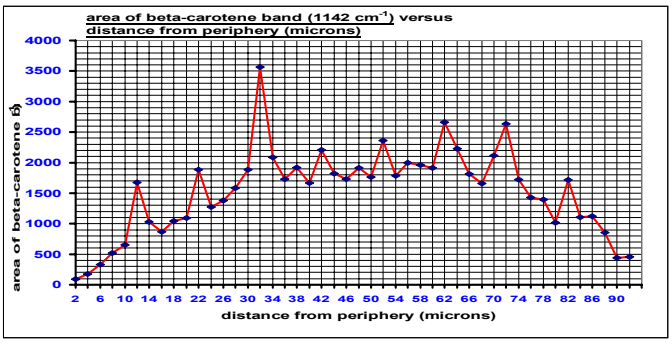

Figure 3. Graph of *area of β-carotene band* versus *distance from periphery (µm)* for the line map (92 µm) of the microcolony, *H. salinarium*.

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