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Short Communications

Photosynthetic Bacteria and Carotenoids from a Sea Sponge *Halichondrium panicea*

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Aryl carotenoids were first isolated by Yamaguchi [1-6] from a sea sponge, *Reniera japonica*, and have since exclusively been found in various green,[7] brown,[8] and purple [9] photosynthetic sulphur bacteria. Considering the wide phylogenetic gap between the taxonomic groups to which these organisms belong, it has been suggested [10] that the aryl carotenoids extracted from *R. japonica* could possibly orginate from microorganisms either collected by this specialized sea-animal or inhabiting the same. Both alternatives might easily be conceived by consideration of the feeding habits and the construction of the sponge.

As an attempt to test the possible general validity of the hypothesis that the carotenoids of sea sponges might be of direct microbial origin the carotenoid pigments of the yellow-brown *Halichondrium panicea* were examined. The total carotenoid content was 0.016 % of the dry extracted residue and comprised β-carotene (72 %) and three other pigments tentatively identified as rubixanthin-like (19 %), canthaxanthin-like (5 %) and lycoxanthin-like (3 %). Aryl carotenoids or other carotenoids characteristic of photosynthetic bacteria were absent.

Aliquots of the same sponge material, treated aseptically after collection, were simultaneously examined for microorganisms with the normal aseptic precautions. Tiny drops (0.02 ml) of the yellow-brown very turbid "juice" obtained by squeezing bits of the sponge were streaked out directly on sea water agar media containing 0.1 % yeast extract (Difco) or 0.5 % yeast extract + 0.5 % glycerol for isolation of aerobic bacteria and the plates placed at 15 and 28°C, in dark as well as in illuminated incubators. Amazingly few colonies (20–50 on each plate) of bacteria appeared, indicating a very low number of live aerobic bacteria inside the sponge cells. None of the colonies were pigmented.

Small cuts of the sponge were used as inoculation material for enrichment cultures of photosynthetic sulphur bacteria (in Pfennig's inorganic sulphide medium [11] with 3 % NaCl, pH 6.8 and 7.2) and non-sulphur bacteria (in 0.2 % Na_2-succinate – 0.1 % yeast extract or 1 % peptone – 0.05 % Na-ascorbate medium, both in sea water at pH 6.9) incubated in light from tungsten lamps as well as in filtered light of wavelengths above 890 mμ.[12] In the organic media only anaerobic proteolytic bacteria developed, none with pigments, and the sulphide media incubated in filtered light gave rise to no growth in one month. In the same sulphide media illuminated by full-spectrum light, however, enrichment cultures developed within 10 days; at pH 7.2 green bacteria, at pH 6.8 a mixed culture of two morphological types of purple bacteria prevailed. Pure cultures of the three species (Isolates I–III) of photosynthetic bacteria were isolated by standard procedures using the corresponding enrichment medium with agar.

Isolate I consisted of immotile, short, rodshaped cells, unable to utilize thiosulphate as electron donor for growth. Analysis showed the cells to contain bacteriochlorophylls *c* [13] and chlorobactene[7] as the major carotenoid. These properties identified Isolate I as a strain of *Chlorobium limicola*. The cells of Isolate II were coccoid, immotile, and contained predominantly spirilloxanthin consistent with the properties of the genera in subgroup A of the spirilloxanthin producing purple sulphur bacteria.[14]

The deep violet-purple cultures of Isolate III contained spherical (diameter ~2 μ), very motile cells typical of *Thiocystis* spp. The tentative identification was further supported by the carotenoid analysis which revealed carotenoids (total 0.22 % of d.w.) of the rhodopinal (warmingone) series,[15] *i.e.* lycopene (4 %), a lycopenal (3 %), rhodopin (31 %), rhodopinal (57 %), and rhodopinol (5 %), as previously encountered in many genera of the Thiorhodaceae, among them species of *Thiocystis*.

All three isolates were marine strains in the sense that they failed to grow in media with no NaCl added.

No attempt was made to isolate microscopic algae from this material.

This investigation has shown that a sea sponge do contain bacteria synthesizing aryl carotenoids but the comparative carotenoid and microbiological analysis of *H. panicea* failed to support the general hypothesis that the carotenoids extractable from sponges might wholly or in part be derived from bacteria associated with the higher animal. If not genuine to the sponge, the nature of the carotenoids of *H. panicea* points to a possible plant origin. However, no mass accumulation of pigmented, morphologically intact algae was discovered in the interior of the sponge cells.

The isolated marine *Thiocystis* sp. was easy to grow in large cultures of 5 to 20 l, and thus proved to be an excellent source of the rhodopinal (warmingone), rhodopinol (warmingol) and a lycopenal (anhydrowarmingone) of the rhodopinal (warmingone) series. A total of 830 g w.w. of cells were harvested from autotrophically grown cultures in Pfennig's medium (average 2.7 g w.w./l) and used for the isolation and the structural elucidation of these carotenoids described in a separate paper.[15]

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Correction to "Properties and Structure of the Decanolic Solutions in the Sodium Caprylate-Decanol-Water System. II. Density and Viscosity of the Solutions"*

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Eqn. (4) on p. 1628 should read

$$\log \eta_{rel} = A_3 C/(1-Q'C)$$

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