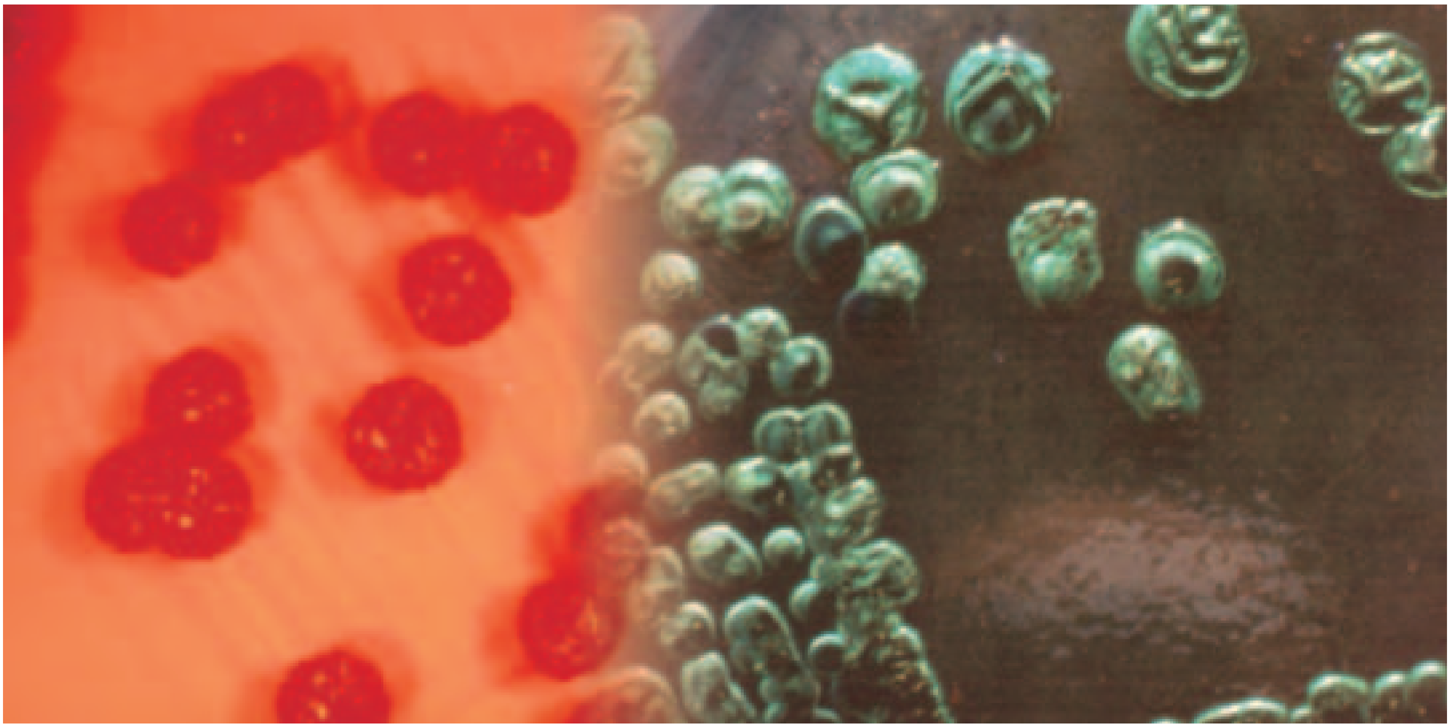


Bacterial Pigments

Maurice Moss explores the colourful world of microorganisms

IF one leaves a plate of nutrient agar exposed to the air for about 30 min, or makes a spread plate of an appropriate dilution of river water, and incubates at 25ºC for a few days, a number of coloured colonies of bacteria will usually appear. The yellow and pink colonies from the air exposure plate will usually be Gram-positive micrococci whereas the much wider range of colours from the river water will often be Gram-negative rods such as *Flavobacterium*, *Cytophaga*, *chromobacteria*, *Serratia* and pseudomonads.

The genus *Micrococcus* includes a full range of colours associated with carotenoid pigments from the yellow of *M. luteus* and *M. varians*, through the orange of *M. nishinomiyaensis*, pink of *M. roseus* to red of *M. agilis*. The carotenoids are widespread amongst bacteria and undoubtedly play an important role in protecting them from the damaging effects of light and, in an aerobic environment, the oxidative damage from activated forms of oxygen. There are several hundred different carotenoids known but they all have an extended system of conjugated double bonds such as that of β-carotene (Fig. 1). One or both of the cyclohexene rings at the ends of the chain may be replaced by aromatic rings, or aliphatic chains, and it is the extent of the conjugation, and the presence or absence of oxygen functions, which determines the depth of colour of these molecules.

Carotenoids may also play a role as light-gathering pigments in photosynthetic bacteria but a major group of light-gathering pigments, in the cyanobacteria, is the phycobiliproteins which are red or blue and have a very different structure from the carotenoids. The chromophore coupled to a protein is a chain of four nitrogen containing pyrrole rings, a typical example being phycocyanin (Fig. 2). A detailed and beautifully illustrated introduction to the pigments associated with photosynthetic bacteria can be found in Madigan, Martinko & Parker (2000). There is almost certainly a biosynthetic relationship between these open chain tetrapyrroles and the elegant closed rings of the chlorophylls but pyrrole rings are also present in the red and purple pigments of non-photosynthetic bacteria.

Prodigiosin (Fig. 3) is a tripyrrole first characterised from *Serratia marcescens* which forms beautiful pillar-box red colonies. A wide variety of bacterial taxa, including Gram negative rods such as *S. rubidaea*, *Vibrio gazogenes*, *Alteromonas rubra*, *Rugamonas rubra*, and Gram positive actinomycetes, such as *Streptoverticillium rubrireticuli* and *Streptomyces longisporus* ruber form prodigiosin and/or derivatives of this molecule (see Austin and Moss, 1986 for references). On some media *Rugamonas rubra* produces so much prodigiosin that, as the pH drops, it precipitates out within the cells and colonies change from pillar box red to deep maroon, often with a green metallic sheen under reflected light (Fig. 4). At this stage most organisms in the colony are no longer viable.

Although, in the early days of bacteriology, the name *Chromobacterium* was used for many organisms producing bright colours whether they were yellow, red or purple, it was soon restricted, not only to those organisms producing purple colonies, but to those in which the purple pigment is violacein (Fig. 5). It is now appreciated that violacein producing bacteria can be assigned to at least three genera - *Chromobacterium*, *Janthinobacterium* and *Iodobacter* (see Moss and Ryall, 1981 and Logan, 1994 for discussions of the early and subsequent characterisation of these genera). Many of these organisms are isolated from soil and water and one possible role of violacein is to make the bacteria ▷

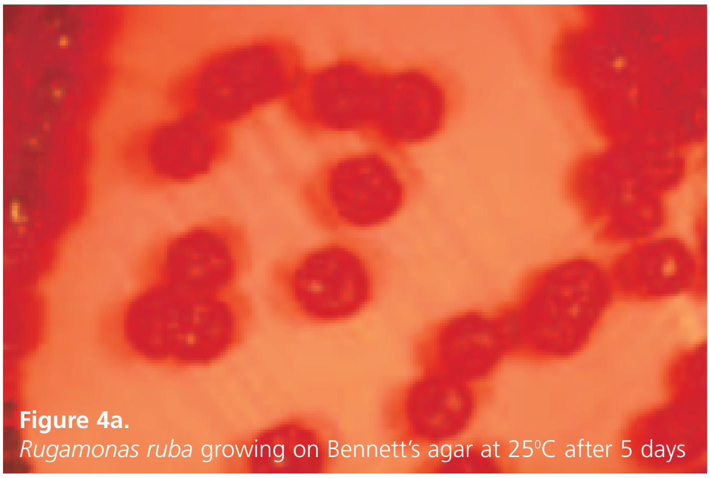


Figure 4a.
Rugamonas ruba growing on Bennett's agar at 25°C after 5 days

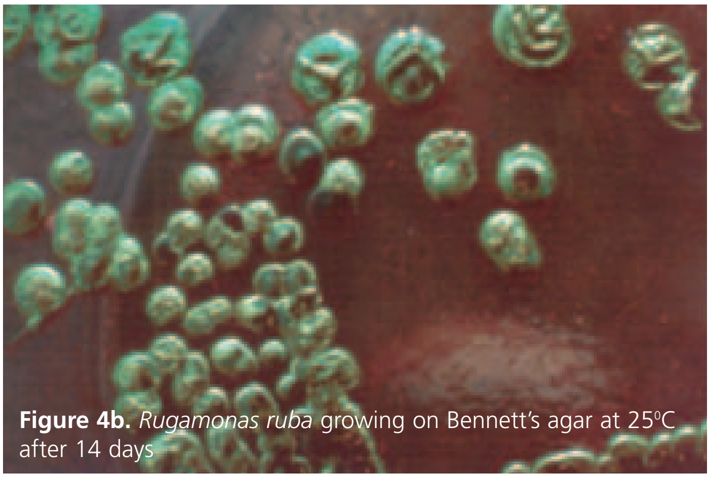


Figure 4b. *Rugamonas ruba* growing on Bennett's agar at 25°C after 14 days

Figure 1. The structure of β-carotene

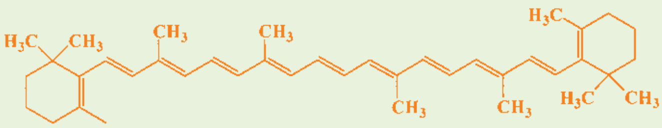
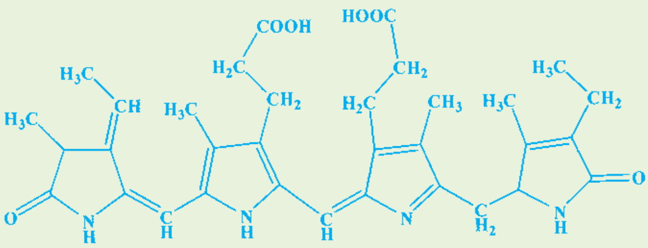


Figure 2. The structure of phycocyanin



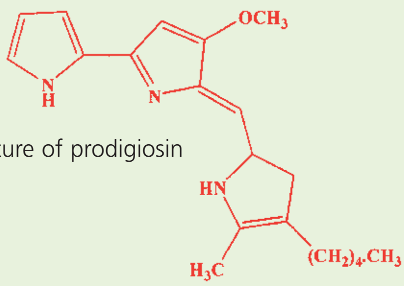
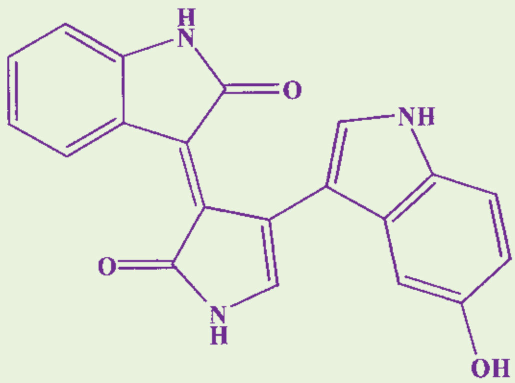


Figure 3. The structure of prodigiosin

Figure 5. The structure of violacein



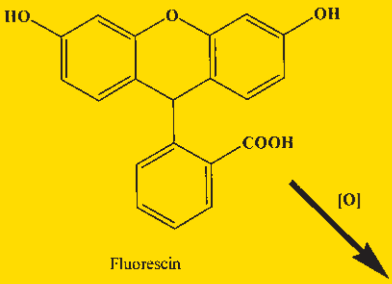
unpalatable to bacteriophagous species of protozoa and nematodes.

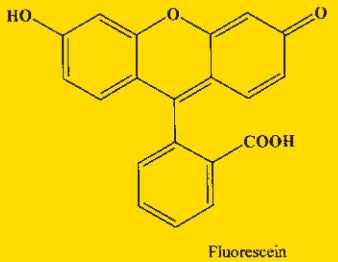
Perhaps the most familiar examples of coloured colonies seen in the routine soil, water and medical laboratory are those of pseudomonads such as the blue-green colonies of *Pseudomonas aeruginosa* or the yellow fluorescent colonies of *Ps. fluorescens* and related species. An example of a water soluble, non-fluorescent blue-green pigment produced by *Ps. aeruginosa* is pyocyanin (Fig. 6) which crystallises as beautiful blue needles and may have a role in respiration. The yellow water soluble fluorescent pigments produced by a number of Pseudomonas species, especially under conditions of iron limitation, are variously known as pyoverdin, pyofluorescein or simply fluorescein.

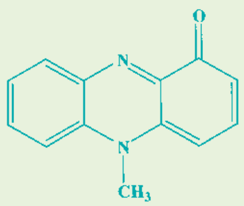
There is some confusion in my own mind over the naming of these compounds but it may be that one of the compounds actually secreted by pseudomonads is fluorescin which is subsequently oxidised to the yellow fluorescent fluorescein (Fig. 7).

The pyoverdines are a large family of complex siderophores in which a dihydroxyquinoline derivative is linked to a peptide which itself may be linked to a cyclic depsipeptide. They are able to bind metal ions, especially iron, and for those who would like to follow up the structure of these beautifully complex molecules I can commend starting with Poppe et al. (1987). But there are other pigments too, pyorubins, pyomelanins and the pseudomonads have provided a considerable challenge to students of natural products.

Figure 7. The oxidation of fluorescin to fluorescein







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