A SURVEY OF THE PIGMENTS OF A NUMBER OF CHROMOGENIC MARINE BACTERIA, WITH SPECIAL REFERENCE TO THE CAROTENOIDs

DORIS P. COURINGTON AND T. W. GOODWIN

Department of Marine Microbiology, Scripps Institution of Oceanography, La Jolla, California, and Department of Biochemistry, The University of Liverpool, England

Received for publication April 15, 1955

More than half the bacteria occurring in the sea are chromogenic. In a general survey of several thousand colonies isolated from marine sources ZoBell and Feltham (1934) found that 31.3 per cent were yellow, 15.2 per cent orange, 9.9 per cent brown and 5.4 per cent red or pink. Later, ZoBell and Upham (1944) described 60 new marine species of which were yellow, 5 brown, 5 pink or salmon colored, 4 orange and one red. Many species of bacteria which spoil fish are also chromogenic and the general information on this subject has been summarized by ZoBell (1946). In spite of the easy availability of this material, only one investigation (Hodgkiss et al., 1954) has so far been published on the nature of the pigments of marine bacteria. Two primary isolates from the skin slime of Gadus morrhuae, A1032 and A1062, which are coryneform, both synthesize the carotenoids, neo-xanthin, sarmcaxanthin and corynxanthin but in different relative amounts. A1062, but not A1032, also produces coproporphyrin under certain cultural conditions. The present paper reports a general survey on the nature of the pigments in a number of species isolated by Dr. ZoBell and his collaborators.

EXPERIMENTAL METHODS

Cultural methods. The medium used throughout consisted of peptone, 5.0 g; yeast extract (Difco), 1.0 g; FePO₄, 0.1 g; glucose 1.0 g; and 75 per cent sea water, 1000 ml; the pH of the medium was adjusted to 7.6; sterilization was obtained by autoclaving. Cultures were grown in mass (300 to 350 ml of medium) in either Roux bottles or Fernbach flasks at 25°C for as long as was necessary for heavy growth and pigmentation to occur. The cells were harvested by centrifuging in a Servall G/1 angle centrifuge and then decanting the supernatant. For pressure studies cultures were grown in specially constructed leucite tubes holding approximately 200 ml of medium. After inoculation with a heavy suspension in order to obtain maximal growth, the cells were incubated in pressure cylinders (ZoBell and Oppenheimer, 1950) for 8 days. The pressures used were the highest which the species under examination could tolerate (Oppenheimer and ZoBell, 1952).

The species examined included those previously described by ZoBell and Upham (1944) together with a number of as yet unclassified isolates contributed by C. H. Oppenheimer, D. E. Contois and H. L. Scotten.

Extraction and identification of the pigments. The pigments were extracted by grinding the wet centrifuged cells with ethanol and, if necessary, warming. An extraction procedure often used for micro-organisms involves first grinding the cells to a fine dry powder with anhydrous Na₂SO₄, prior to treatment with ethyl ether (Garton, Goodwin and Lijinsky, 1951); this method could not be used with most of the bacteria examined here. As observed by Hodgkiss et al. (1954), with their marine bacteria, the pigments cannot be extracted from the dry mass, either because they are so tightly adsorbed to the Na₂SO₄ or because the pigment-protein complex, the state in which the pigments exist naturally, is not broken down by this treatment. The latter explanation is probably correct, for in the present experiments it was obvious that the various species differed considerably in the ease with which they yielded their pigments to ethanol. The critical factor always appeared to be the ease with which the cell proteins coagulated; immediately the proteins were denatured, the pigment was liberated into the ethanol.

The ethanol extract was freed from cellular debris by centrifuging, and an equal volume of ethyl ether or light petroleum (b.p. 30 to 60°C) was added, followed by water dropwise until two layers formed. The upper layer was removed and taken to dryness at 40°C in a stream of illuminating gas. The residue was treated in one of two ways: (a) dissolved in a small volume (5 ml) of
light petroleum (or, if insoluble, in a few drops of ethyl ether followed by dilution to 5 ml with light petroleum) ready for chromatography; (b) saponified by heating to boiling for 2 min with 5 ml of ethanolic KOH (20 per cent, w/v); the unsaponifiable fraction, containing the pigments, was extracted and prepared for chromatography as described by Goodwin and Morton (1946).

Chromatography of the extracts was carried out either on columns of alumina (Merck-Brockmann) deactivated according to the method of Goodwin and Srisukh (1949) or on CaCO₃ (analytical grade).

The pigments were identified by the position and shape of their absorption spectra and by their relative positions on a chromatogram.

Absorption spectra were measured using either a Beckman DU or Unicam SP600 instrument.

RESULTS

Micrococcus species. Micrococcus aquisinus, M. maripunicus and M. infimus yield a pigment mixture to ethanol only with difficulty. On saponifying the extract one of the components (λ max 404 μ) is destroyed leaving a carotenoid (figure 1) in the unsaponifiable fraction. This pigment is completely hypophasic when partitioned between light petroleum and 90 per cent aqueous methanol. It is unstable, disappearing from a CaCO₃ column during development of the chromatogram, while it is so tightly absorbed that it cannot be eluted from alumina even with glacial acetic acid. It is almost certainly a polyhydroxy carotenoid which, because of the large difference in the position of its absorption maximum in ethanol and light petroleum solutions, probably also contains a carbonyl group. It cannot be identified with any previously described pigment. It differs from the pigments in the only other Micrococcus spp. recently examined, M. luteus and M. flavus (Sobin and Stahly, 1942).

The nature of the material with a band at 404 μ is unknown, but it appeared in many of the bacteria examined during this investigation. The amount produced also varies in different cultures of the same bacterium. M. sedentarius produced no detectable amounts of carotenoids under our conditions.

Flavobacterium species. Flavobacterium marinotypicum yielded a bright yellow ethanol extract which when chromatographed separated into two fractions with the same absorption spectrum (λmax 418, 439, 468) as neoxanthin, sarcina-

xanthin and corynexanthin. Both fractions are epiphasic in the partition test and one (A) is absorbed about as strongly as lycopene and the other (B) as a monohydroxy carotenoid. From these properties A is almost certainly sarcinicene first observed in Sarcina lutea by Char- gaff & Dieryck (1932) and B sarcincaxanthin first isolated from the same source. Sarcinicene has previously been observed in F. sulphureum by Sobin & Stahly (1942).

P. okeanокоites and F. marinovirosum give easily extractable yellow extracts with absorption bands having maxima around 410 μ. This pigment is destroyed by saponification and is therefore not a carotenoid.

Pseudomonas species. Pseudomonas xanthochrus and P. aestumaria produce two carotenoids A and B which, from their absorptive behavior, are a mono- and a dihydroxy carotenoid respectively. Their absorption spectra are identical with those of cryptoxanthin and zeaxanthin. They are almost certain that A and B are these pigments. Figure 2 shows the absorption spectrum of A compared with that of authentic cryptoxanthin (Goodwin, 1954) (the spectra of cryptoxanthin and zeaxanthin are virtually indistinguishable).

Vibrio species. Vibrio adaptatus synthesizes the same pigments as P. xanthochrus, i.e., cryptoxanthin and zeaxanthin. Other Vibrio species examined, V. hyphalus, V. phytoplanktis and V. marinofulvus, produced no carotenoids. V. algensis is unique among the marine bacteria examined in producing two carotenoids containing different chromophoric systems. Figure 2 shows the ab-
Figure 2. The absorption spectra in light petroleum (b.p. 30-60°C) of: Pigment A from *Pseudomonas zanthochrous*, ———. Authentic cryptoxanthin, - - - - - . Pigment A from *Vibrio algoeus*, ——— .

Absorption spectra of pigment A, which appears to be a carotenoid with about the same absorbability as lycopene. Pigment B is probably a dihydroxy carotenoid with its main absorption spectral maxima at 450 μm. Its full spectrum has not been recorded because of the difficulty of obtaining it in a pure state.

*Serratia marinorubra*. The purple pigment in this organism was easily extracted from the bacteria mass with ethanol. In acidified ethyl ether it is purplish-pink, and in alkaline ether yellowish-brown; the absorption spectra of these two forms were identical with those of prodigiosin (Hubbard and Rimington, 1950) (λ_max 470 μm in alkali and 536 μm in acid). Furthermore, the isosbestic point for the two forms of the pigment was the same as for prodigiosin (495 μm). These facts combined with the partition behavior of the pigment, which is also identical with that of prodigiosin, make it certain that it is prodigiosin.

Undescribed species. The following bacteria, which have not yet been identified, were examined for carotenoids (the numbers are reference numbers in the Scripps collection):

623: Produces small amounts of a pigment very similar to that produced by *M. aquivius*.

7 C: A single strongly absorbed carotenoid is produced with a symmetrical absorption spectrum with a maximum at 468 μm in ethanol (figure 2). The lack of fine structure suggests that it is a ketocarotenoid. It is not acidic.

102-6-3: Produces a strongly absorbed yellow pigment (λ_max 418, 441, 472 μm in ethanol) with a spectrum identical with that of the sarcinene-sarcinoxanthin, corynosanthin group. Its absorptive behavior suggests that the pigment is corynosanthin.

9 B and 102-8-FB: Produce the two pigments found in *P. zanthochrous* and *P. aequitorum*—probably cryptoxanthin and zeaxanthin.

102-8-F: Produces a pigment very similar to that of *M. aquivius*.

The following bacteria, not discussed above, were slightly yellow, but produced no significant amounts of carotenoids under our experimental conditions: *Actinomyces halotrichis*, *P. coenobio*, *P. obscura*, *P. oceanica*, *P. perfectomarinus*, and *Sarcina pelagia*.

Effect of pressure. The following marine bacteria were cultured under the pressure indicated. *F. marinotypicum*, 200 atm; *M. aquivius*, 400 atm; and *P. zanthochrous*, 100 atm. These bacteria were chosen because they produced different carotenoids. In every case the pigments produced under pressure were the same as those produced under normal conditions.

**DISCUSSION**

The carotenoids produced by chromogenic marine bacteria do not appear to differ greatly from those produced by other bacteria. The main general characteristics in each case are (a) comparative lack of hydrocarbon carotenoids, (b) synthesis of polyhydroxy compounds, and (c) failure to synthesize lutein, the characteristic xanthophyll of the higher plants.

It is not possible to attach any far-reaching taxonomic importance from the present limited survey or even to suggest that a wider study would demonstrate this importance, but the following should be noted: (a) the pigments of *M. aquivius*, *M. marinus* and *M. infimus* have not been observed in other genera; (b) the cryptoxanthin and zeaxanthinlike pigments occur in members of the genera *Vibrio* and *Pseudomonas*; and (c) the pigments in *F. marinotypicum* are those normally also found in some chromogenic *Corynebacterium* and *Sarcina* sp.

**ACKNOWLEDGMENTS**

We wish to thank Drs. C. E. ZoBell and D. L. Fox for their help and encouragement during this
investigation. One of us (T. W. G.) is grateful to The University of California and The Rockefeller Foundation for financial assistance.

SUMMARY

A survey of a number of chromogenic marine bacteria indicates that the pigments present are generally carotenoids. Polyhydroxyxanthophylls predominate and carotenes are rarely observed. This picture is also characteristic of non-marine bacteria.

The pigment in *Serratia marinorubra* is prodigiosin.

High pressure has no effect on the synthesis of carotenoids by marine bacteria.

REFERENCES


