CAROTENOIDS OF *RHODOMICROBIUM VANNIELII*

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The relationship of the anaerobic, photoheterotrophic bacterium *Rhodomicrobium vannielii* to other photosynthetic bacteria is difficult to assess in view of its complex morphological characteristics (Duchow and Douglas, J. Bacteriol. **58**: 409, 1949). A detailed examination of its carotenoid constitution was carried out to establish more clearly its relationship to other photosynthetic bacteria. A partial characterization of its pigment complex has been reported by Volk and Pennington (J. Bacteriol. **59**:169, 1950).

R. vannielii was obtained from H. C. Douglas and grown as described by Duchow and Douglas (J. Bacteriol. **58**:409, 1949), using ethanol or acetate as the primary source of carbon. Pigments were extracted from 6-day-old cells with benzene, and chromatographed on powdered alumina as described by Benedict et al. (Biochim. et Biophys. Acta **43**:525, 1961). The spectrum of pigmented particles, obtained by sonic disruption of whole cells and differential centrifugation, was determined with a Cary model 14 recording spectrophotometer, using opal glass to decrease light scattering.

The absorption spectrum of pigmented particles, which is identical with that obtained with whole cells, is given in Fig. 1. Absorption maxima of the bacteriochlorophyll complex in the intact cells were observed at 590, 800, and 868 mμ.

A typical chromatographic separation of the carotenoids of *R. vannielii* is shown in Table 1. β-Carotene, characterized by its absorption spectrum and cochromatography with authentic samples of purified β-carotene, was present. These results are in agreement with the report of Volk and Pennington. The presence of lycopene, P-481, and lycophyll, in addition to lycoxanthin, demethyllated spirilloxanthin, and spirilloxanthin, was observed. Thus, *R. vannielii* contains, in addition to bacteriochlorophyll, the full complement of carotenoids found in photosynthetic bacteria of the families *Thiorhodaceae* (*Chromatium* strain D; Goodwin and Land, Arch. Mikrobiol. **24**:305, 1956) and *Athiorhodaceae* (*Rhodopseudomonas* sp. and *Rhodospirillum* sp.; Goodwin, Arch. Mikrobiol. **24**:313, 1956). The presence of β-carotene, however, makes *R. vannielii* unique, since it is the only photosynthetic bacterium which contains this cyclic carotenoid. Other cyclic carotenoids (γ-carotene) have been found in green sulfur bacteria (Goodwin and Land, Biochem. J. **62**:553, 1956).

TABLE 1. *A typical chromatographic separation of the carotenoids of Rhodomicrobium vannielii*

Zone	Column color	Eluate color	Absorption maxima (mμ) in 30–60 C petroleum ether	Concn of ether or acetone in petroleum ether for elution	Identification
1	Yellow	Yellow	425, 449, 475	1% Ether	β-Carotene
2	Orange-pink	Yellow	442, 469, 500	4% Ether	Lycopene
3	Red-pink	Pink-yellow	451, 480, 512	15% Ether	P-481
4	Red	Orange-pink	460, 489, 523	20% Ether	Spirilloxanthin
5	Yellow-orange	Yellow	441, 468, 499	35% Ether	Lycoxanthin
6	Pink	Yellow-pink	462, 487, 522	10% Acetone	Demethylated spirilloxanthin
7	Pink	Yellow	441, 468, 501	50% Acetone	Lycophyll

R. vannielii is therefore an exception to the generalization that β-carotene is almost as characteristic of oxygen-evolving photosynthetic bacteria as is chlorophyll (Dougherty and Allen, *Comparative biochemistry of photoreactive systems*, p. 133. Academic Press, Inc., New York, 1960). The presence of a peripheral lamellar system (Vatter et al., J. Bacteriol. **77**:812, 1959) and a cyclic carotenoid indicates a close relationship between *R. vannielii* and the blue-green algae.

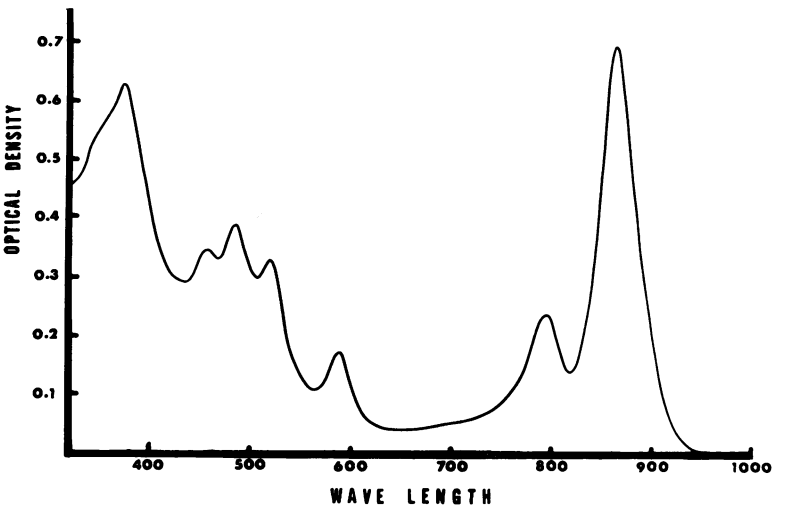

FIG. 1. *Absorption spectrum of particles from Rhodomicrobium vannielii in 0.01 M phosphate buffer (pH 7.0).*

Attempts are now being made to clarify the taxonomic and evolutionary position of this photosynthetic bacterium.

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EFFECT OF FLUOROCARBON TREATMENT ON NEWCASTLE DISEASE VIRUS

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Full hemolytic activity in Newcastle disease virus (NDV) suspensions is not obtained without pretreatment of the virus by techniques such as freezing and thawing or extensive dialysis (Granoff and Henle, J. Immunol. **75:322**, 1954). Girardi (Virology **9:488**, 1959) has reported that fluorocarbon treatment unmasks polyoma virus hemagglutinin. Results are reported here which show that fluorocarbon treatment may also be used to unmask the NDV hemolysin.

Fluorocarbon treatment of virus suspensions was accomplished by mixing equal volumes of allantoic fluid, containing the L. Kansas or NK strains of NDV, and Genetron 113 (trichlorotrifluoroethane). This mixture was stirred at the slowest speed in a Virtis 23 homogenizer for 10 min and then centrifuged to separate the two phases. Hemolysis of chicken red cells (RBC) was measured by mixing 1.0 ml of virus suspension with 3.0 ml citrate saline or 3.0 ml of a 1:120 dilution of NDV antiserum in citrate saline. This mixture was incubated for 1 hr at 37 C, and then 1.0 ml of 4% washed RBC was added and incubation continued for 1 hr. The suspensions were then centrifuged to remove unlysed cells and the optical density of the supernatant fluid was measured at 550 mμ. Hemagglutinin end point titrations were carried out using twofold dilution series in spot plates to which 2 drops of 4% washed RBC were added. Plaque titrations were made by inoculating chick embryo fibroblast tissue cultures, which were incubated with a nutrient agar overlay after infection.

Genetron-treated NK virus suspensions showed greater than ten times as much hemolytic activity as untreated fluids (Table 1). Samples run in parallel series, with and without antiserum, indi-