Occurrence of Bacteriochlorophyll *a* in a Strain of an Aerobic Heterotrophic Bacterium

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A strain of a Gram-negative heterotrophic bacterium was isolated from a surface of a thallus of a green seaweed, *Enteromorpha lynza*, collected from Aburatsubo Inlet, Kanagawa Prefecture, in April, 1975. This bacterium, tentatively called OCh 101, grew only under aerobic conditions and produced water-insoluble orange pigments. These and following characteristics, details of which will be reported elsewhere, indicate the resemblance of OCh 101 to some species of genus *Flavobacterium*: non-motile rod, $0.7 \times 1.5 \sim 2.5 \mu\text{m}$; no acid formation from glucose and galactose; no spreading growth on agar plate media; halophilic; oxidase positive; catalase positive. Thin-layer chromatography of the water-insoluble pigments revealed the presence of a blue pigment showing a maximum absorption in the near infrared region. This report concerns the identification of this pigment.

OCh 101 was grown on the liquid PPES-II medium¹⁾ (1.5 liter in a 5 liter Erlenmeyer flask) under rotatory shaking (160 rpm) for 3 days at 27°C. About 15 g of packed cells harvested by centrifugation at $9000 \times g$ (10 min) was extracted successively twice with each 45 ml of aqueous 90% acetone and twice with each 45 ml of acetone, so that the bulk of carotenoids was washed out. The blue pigment and polar carotenoids remaining unextracted were extracted finally from the residue with 45 ml of methanol. Shaking of the methanolic solution with 45 ml of diethyl ether

FIG. 1. Visible Absorption Spectra of the Crude Preparation of the Blue Pigment in Diethyl Ether.

A: The absorption spectrum of the ethereal solution obtained as described in the text. $\lambda_{\text{max}} = 358, 391, 575$ and $770 \sim 1 \text{ nm}$.

B: The absorption spectrum of the ethereal solution after the addition of 1 drop of concentrated hydrochloric acid and 0.1 ml of methanol/2 ml. $\lambda_{\text{max}} = 357, 384, 490, 525$ and $747 \sim 8 \text{ nm}$.

Light absorbancies were recorded with a Hitachi's spectrophotometer, Model 124.

and 53 ml of distilled water resulted in transference of the blue pigment to the ethereal layer. Thorough washing of the ethereal layer with aqueous saturated solution of sodium chloride followed by dilution to 136 ml gave a solution showing the visible absorption spectrum illustrated in Fig. 1 (A) and similar to that of bacteriochlorophyll *a*. The ratio of bacteriochlorophyll *a* to pheophytin was calculated to be higher than 99 by the method of van der Rest and Gingras.²⁾ Treatment of this solution with hydrochloric acid afforded a solution showing an absorption spectrum of bacteriopheophytin *a* (Fig. 1, B). Purification of the crude preparation according to Sato and Murata³⁾ gave a pheophytin-free preparation giving a positive phase test. Tests by thin-layer chromatography,^{3,4)} as illustrated in Fig. 2, substantiated the identity of the blue pigment with the authentic bacteriochlorophyll *a* isolated from cells of *Chromatium vinosum*.

Now it is evident that the blue pigment of OCh 101 is bacteriochlorophyll *a* which has been found only in photosynthetic bacteria. Therefore careful experiments should be undertaken concerning a possible utilization of light

FIG. 2. Thin-layer Chromatograms of the Blue Pigment.

A: Hyflosuper Cel-triolein/methanol-acetone-water-triolein (80:16:9: a few drops).⁴⁾

B: Hyflosuper Cel-Wakogel B-0-CaCO₃-Ca(OH)₂-ascorbate (12:3:3:0.02:0.02) petroleum ether-isopropanol (95:5).⁸⁾

1: The blue pigment from OCh 101, 2: Bacteriochlorophyll *a* from *Chromatium vinosum*.

Beside the main blue spots, pink spots of bacterio-pheophytin *a* and yellow-green spots of oxidized products were observed.

energy by OCh 101. However, since this bacterium did not grow under anaerobic conditions so far examined even under illumination of white light, obviously it does not belong to photosynthetic bacteria in the ordinary meaning.⁵⁾ While the amount of bacteriochlorophyll *a** contained in 5 mg of wet packed cells (corresponding to about 1 mg of dry weight) of an early stationary phase growing at 25°C in the dark was 0.8~0.9 nmol,

* Amounts of bacteriochlorophyll *a* were calculated from light absorbancies at 770 nm on the bases of the values of millimolar extinction coefficients: 65.3 in acetone-methanol (7:2)²⁾ and 42.0 in methanol.⁹⁾

that in the cells growing in the light (300~600 lux) was 0.6 nmol. These values are considerably higher than those reported for *Rhodospseudomonas sherooides* grown in the dark aerobically.⁶⁾

Meanwhile Sato and Shimizu^{7,8)} found, independently from the authors, the synthesis of a bacteriochlorophyll-like pigment by a methanol-utilizing bacterium, *Protaminobacter ruber*. The amount of bacteriochlorophyll *a* found in the dark grown cells of OCh 101 is about 5 times as much as that of "bacteriochlorophyll" found by Sato⁷⁾ in the cells of *P. ruber* grown on 1, 2 propanediol under intermittent illumination. Other examples of the occurrence of bacteriochlorophylls in larger amounts in chemoorganotrophic bacteria may remain to be found in the near future.

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REFERENCES

- 1) N. Taga, *Bull. Misaki Mar. Biol. Inst. Kyoto Univ.*, **122**, 65 (1968).
- 2) M. van der Rest and G. Gingras, *J. Biol. Chem.*, **249**, 6446 (1974).
- 3) N. Sato and N. Murata, *Biochim. Biophys. Acta*, **501**, 103 (1978).
- 4) K. Egger, *Planta*, **58**, 664 (1962).
- 5) Pfennig, *Ann. Rev. Microbiol.*, **31**, 275 (1977).
- 6) J. Lascelles, *Biochem. J.*, **92**, 508 (1959).
- 7) K. Sato, *FEBS Lett.*, **85**, 207 (1978).
- 8) K. Sato and S. Shimizu, Abstracts of Papers, Annual Meeting of The Agricultural Chemical Society of Japan, April, 1978, p. 51.
- 9) J. H. Smith and A. Benitez, "Moderne Methode der Pflanzen-Analyse," IV ed. by K. Paech and M. V. Tracey, Springer-Verlag, Berlin, 1955, p. 179.