JOURNAL OF BACTERIOLOGY, Jan. 1982, p. 361–363
0021-9193/82/010361-03$02.00/0

Vol. 149, No. 1

NOTES

Accumulation of Poly-β-Hydroxybutyrate in *Spirulina platensis*

JOHN CAMPBELL III,[1] S. EDWARD STEVENS, JR.,[1]* AND DAVID L. BALKWILL[2]

Program in Microbiology, Department of Microbiology, Cell Biology, Biochemistry and Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802,[1] and Department of Microbiology, University of New Hampshire, Durham, New Hampshire 03824[2]

Received 27 July 1981/Accepted 25 August 1981

Poly-β-hydroxybutyrate has been identified in the cyanobacterium *Spirulina platensis*. The addition of reduced carbon compounds to the growth medium was not required for poly-β-hydroxybutyrate accumulation. Poly-β-hydroxybutyrate accumulated during exponential growth to 6% of the total dry weight and then decreased during the stationary phase.

Cyanobacteria are known to store carbohydrate as glycogen, phosphate as polyphosphate, and nitrogen as multi-L-arginyl-poly (L-aspartic acid) or cyanophycin (11, 14). Little is known about the lipid reserve of cyanobacteria. Poly-β-hydroxybutyrate (PHB) has been demonstrated in a wide range of classical bacteria (6), but has been reported in only one cyanobacterium (1, 4). Carr (1) demonstrated PHB in *Chlorogloea fritschii* (*Chlorogloeopis fritschii*, see reference 9) by chemical analysis, whereas Jensen and Sicko (4) demonstrated PHB granules in this same cyanobacterium by electron microscopy. However, for the production of PHB by *C. fritschii*, it was necessary to supplement the medium with the reduced carbon compound sodium acetate. We report the presence of PHB in the cyanobacterium *Spirulina platensis* and show that PHB accumulated without the addition of reduced carbon compounds.

A unialgal strain of *S. platensis* was the generous gift of H. C. Bold (University of Texas). The culture was repeatedly streaked on agar plates until an axenic culture was obtained. Lack of bacterial contamination of *S. platensis* was demonstrated by lack of bacterial growth when broth suspensions were subcultured on nutrient broth, Luria broth, yeast extract, and Casamino Acids media. Axenic *S. platensis* was grown in the mineral salts medium of Ogawa and Terui (8). Cultures were bubbled with 5% CO_2–95% air. Temperature of growth was 35°C and illumination was by four F72T12 CW lamps providing an incident intensity of 6.17×10^{-9} einsteins $cm^{-2} \cdot s^{-1}$ on the growth tubes. Growth was measured at 550 nm with a Bausch and Lomb Spectronic 20 colorimeter. At the same time growth measurements were made, 10 ml of the culture was removed for PHB determinations which were performed by the method of Law and Slepecky (5). PHB was isolated and purified by the methods of Sutherland and Wilkinson

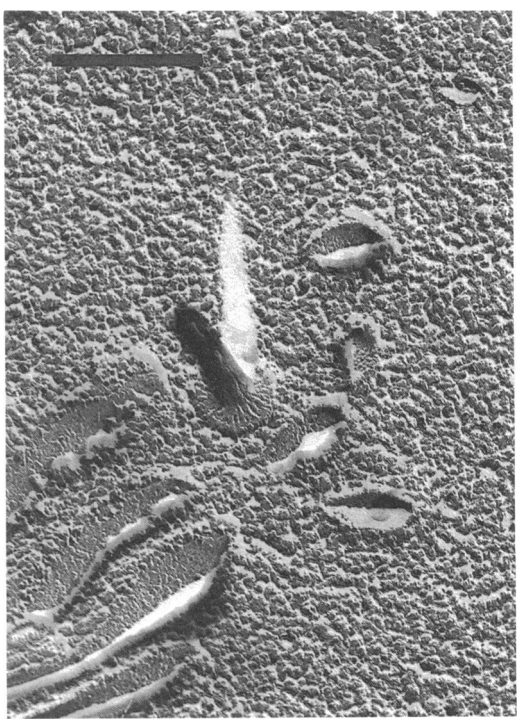

FIG. 1. Poly-β-hydroxybutyrate granule in *Spirulina platensis* stretched out into the unique and characteristic cone structure (2). Bar = 0.3 μm.

(13). The physical and chemical properties of purified *S. platensis* PHB were determined according to the procedures of Herbert et al. (3). A Perkin-Elmer (model 237) infrared spectrophotometer was used to obtain infrared spectra of PHB. For the electron microscopic studies, cells were concentrated by centrifugation to prepare them for freeze-etch transmission electron microscopy. Droplets of concentrated cell suspension were frozen in liquid Freon 22 and transferred quickly to liquid nitrogen. Frozen samples were fractured, etched for 3 min, and replicated in a Balzers BA 360 M freeze-etching device at −100°C by the method of Moor and Mühlethaler (7). The platinum-carbon replicas were viewed with a Philips EM-300 transmission electron microscope operating at an accelerating potential of 60 kV.

When the ultraviolet absorption spectrum of crotonic acid derived from PHB purified from *S. platensis* was compared with the spectrum of commercial crotonic acid, they were found to be identical (data not shown). The infrared spectrum of *S. platensis* PHB was identical to that for PHB purified from *Bacillus megaterium* (data not shown) and other bacteria (10). The dry polymer of PHB had a melting point of 165°C which corresponds to what has been reported by Lundgren et al. (6).

Freeze-etching followed by transmission electron microscopy indicated that PHB granules in *S. platensis* (Fig. 1) possessed the same ultrastructural features as did those of *Bacillus cereus* described by Dunlop and Robards (2). After freeze-etching, PHB granules stretched out into the cone-shaped structures which are unique and characteristic of PHB.

PHB accumulation increased during exponential growth of *S. platensis* (Fig. 2), reached a maximum as the culture entered the stationary phase, and then decreased. Up to 6% of the total dry weight of *S. platensis* was recoverable as PHB when cells were grown in medium without sodium acetate. The presence of sodium acetate

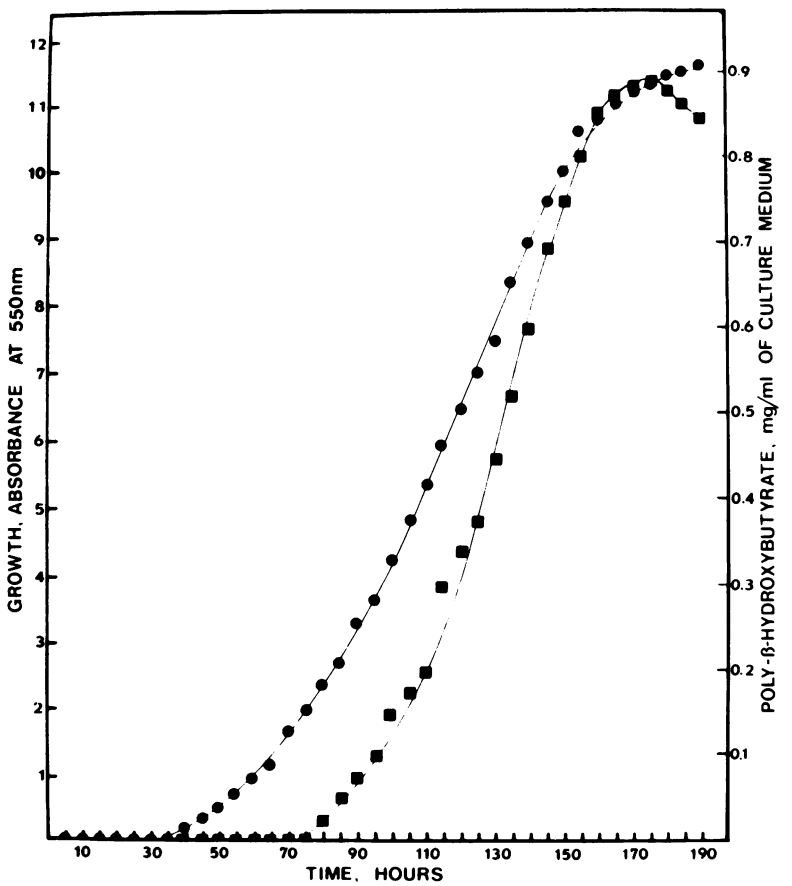

FIG. 2. Growth of *Spirulina platensis* and accumulation of poly-β-hydroxybutyrate. Growth is plotted as absorbance (●). PHB (■) was assayed by converting it to crotonic acid (5, 13).

in the culture medium had no observable effect on the extent of PHB accumulation in *S. platensis*. PHB accounted for 10% of the total dry weight of *C. fritschii* when grown in sodium acetate-supplemented medium (1). However, Carr (1) was not able to detect any PHB when *C. fritschii* was grown in the absence of acetate. Thus, although *S. platensis* accumulates PHB as does *C. fritschii*, it is different in that reduced carbon compounds need not be supplied for PHB accumulation.

S. platensis and *C. fritschii* were assigned generically to sections III and V, respectively, by Rippka et al. (9) and are considered to be of quite distant relatedness. However, both cyanobacteria accumulate PHB. We suggest that PHB may occur much more generally in this group of microorganisms. We observed previously (12) that accumulation of cyanophycin in *Agmenellum quadruplicatum* required a condition of unbalanced nutrition resulting from phosphate starvation. We offer the suggestion that PHB might accumulate in other cyanobacteria if they are grown under conditions of high CO_2 concentration.

This study was supported by grant 7900238 from the U.S. Department of Agriculture.

We thank A. T. Phillips for helpful comments.

LITERATURE CITED

1. **Carr, N. G.** 1966. The occurrence of poly-β-hydroxybutyrate in the blue-green alga, *Chlorogloea fritschii*. Biochim. Biophys. Acta **120**:308–310.
2. **Dunlop, W. F., and A. W. Robards.** 1973. Ultrasructural study of poly-β-hydroxybutyrate granules from *Bacillus cereus*. J. Bacteriol. **114**:1271–1280.
3. **Herbert, D., P. J. Phipps, and R. E. Strange.** 1971. Chemical analysis of microbial cells, p. 304–307. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press, Inc., New York.
4. **Jensen, T. E., and L. M. Sicko.** 1971. Fine structure of poly-β-hydroxybutyric acid granules in a blue-green alga, *Chlorogloea fritschii*. J. Bacteriol. **106**:683–686.
5. **Law, J. H., and R. A. Slepecky.** 1961. Assay of poly-β-hydroxybutyric acid. J. Bacteriol. **82**:33–36.
6. **Lundgren, D. G., R. Alper, C. Schnaitman, and R. H. Marchessault.** 1965. Characterization of poly-β-hydroxybutyrate extracted from different bacteria. J. Bacteriol. **89**:245–251.
7. **Moor, H., and K. Mühlethaler.** 1963. Fine structure in frozen-etched yeast cells. J. Cell Biol. **17**:609–628.
8. **Ogawa, T., and G. Terui.** 1970. Studies on the growth of *Spirulina platensis* (I) on the pure culture of *Spirulina platensis*. J. Ferment. Technol. **48**:361–367.
9. **Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier.** 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. **111**:1–61.
10. **Rouf, M. A. and J. L. Stokes.** 1962. Isolation and identification of the sudanophilic granules of *Sphaerotilus natans*. J. Bacteriol. **83**:343–347.
11. **Stanier, R. Y., and G. Cohen-Bazire.** 1977. Phototrophic prokaryotes: the cyanobacteria. Annu. Rev. Microbiol. **31**:225–274.
12. **Stevens, S. E., Jr., D. A. M. Paone, and D. L. Balkwill.** 1981. Accumulation of cyanophycin granules as a result of phosphate limitation in *Agmenellum quadruplicatum*. Plant Physiol. **67**:716–719.
13. **Sutherland, I. W., and J. F. Wilkinson.** 1971. Chemical extraction methods of microbiol cells, p. 345–359. *In* J. R. Norris, and D. W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press, Inc., New York.
14. **Wolk, C. P.** 1973. Physiology and cytologial chemistry of blue-green algae. Bacteriol. Rev. **37**:32–101.