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NOTES

Photosynthetic Sulfide Oxidation by *Chloroflexus aurantiacus*, a Filamentous, Photosynthetic, Gliding Bacterium

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Chloroflexus, a newly described genus of filamentous, photosynthetic, gliding bacteria, oxidizes sulfide anaerobically under photoautotrophic or photoheterotrophic growth conditions and deposits elemental sulfur outside the cell. The formation of sulfur granules outside the cell supports the idea that this organism is related to the green sulfur bacteria (*Chlorobiaceae*).

The genus *Chloroflexus* has recently been established to describe a unique new group of gliding photosynthetic bacteria (9). There are numerous similarities between *Chloroflexus* and the established members of the green sulfur bacteria (*Chlorobiaceae*). The production of bacteriochlorophyll-rich "chlorobium vesicles" (9), and the synthesis of lipids containing a monogalactosyldiglyceride- and digalactosyldiglyceride-like glycolipid (6), along with a deoxyribonucleic acid base ratio of 53 to 55% (as mol% guanine plus cytosine) (9), are three important characteristics *Chloroflexus* shares in common with the *Chlorobiaceae*. In contrast to the strictly anaerobic, photoautotrophic nature of the green bacteria however, *Chloroflexus* is capable of growing as an aerobic heterotroph, as well as an anaerobic photoheterotroph or photoautotroph (7, 9).

In the original work characterizing *Chloroflexus* (9), the organism had only been grown under photoheterotrophic conditions. We have succeeded in culturing this organism under photoautotrophic conditions anaerobically with bicarbonate, conditions under which growth is dependent on sulfide or another suitable reductant; thus making it possible to study the sulfide oxidation process.

As received, *Chloroflexus aurantiacus* strain OK-70-fl (9) had been cultured in mineral salts medium D (4) containing yeast extract. Photoautotrophic growth was achieved by first adapting this strain to a defined medium containing the dipeptide glycyl-glycine (added originally as a pH buffer) as the sole source of organic carbon (7). After successive transfers in this medium the organism was transferred to the autotrophic medium described by Madigan et al. (7) which contains medium D supplemented with vitamins, sulfide, and bicarbonate following the procedure of Pfennig (8). For photoheterotrophic growth medium D (pH 8.2) supplemented with 0.025% yeast extract and 0.05% $Na_2S \cdot 9H_2O$ (Mallinckrodt Chemical Works, St. Louis, Mo.) was used. Screw-capped tubes (18.5 ml) filled completely with autotrophic- or yeast extract-supplemented medium were inoculated with 0.2 ml of homogenized cell suspensions and incubated at 50 C in a light cabinet (Controlled Environments, Pembina, N.D.) at a light intensity of approximately 50 foot candles (548 lx). Darkened conditions, when required, were created by wrapping the tubes in two layers of aluminum foil. Sulfide was assayed colorimetrically using the method of Pachmayr (3). After removing a sample of the growth medium for the sulfide determination, the remainder of the sample was acidified and bubbled with O_2-free N_2 for 15 min to remove any remaining H_2S. Half of this suspension was filtered through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) and the filter containing elemental sulfur was dried overnight at 55 C. (Experiments by J. L. Mosser in this laboratory had shown that elemental sulfur was removed quantitatively by the filtration process.) The filter was extracted in 5 ml of carbon disulfide (Matheson Coleman and Bell Co., Los Angeles, Calif.) for 30 min and then the extract was placed in a large test tube and the CS_2 was allowed to evaporate. The resultant elemental sulfur was dissolved in 10 ml of petroleum ether (Skelly "C", boiling range 77 to 110 C), and assayed by the colorimetric method of Bartlett

and Skoog (2). Sulfate was determined on the filtrate from the above filtration as the $BaSO_4$ precipitate (1). Bacteriochlorophyll "c" was determined on methanol extracts by measuring the absorbance at 668 nm with a Beckman DB-G spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.), using the extinction coefficient of Stanier and Smith (10). Crystalline elemental sulfur was converted into a colloidal, amorphous suspension to test for utilization during photoautotrophic growth by the procedure of Brierley (J. Brierley, Ph.D. thesis, Montana State Univ., 1966). The results of one sulfide oxidation experiment are shown in Fig. 1.

As can be seen in Fig. 1, autotrophically grown cells oxidized sulfide over the growth period and produced elemental sulfur, but significant concentrations of sulfate were not produced. In other experiments under photoheterotrophic conditions, similar results were obtained, and only insignificant amounts of sulfate were produced. Thus, elemental sulfur is a major product of sulfide oxidation whether the organism is growing photoautotrophically or photoheterotrophically. However, at most, only 70% of the sulfide oxidized was accounted for as elemental sulfur, so that other reduced sulfur species such as thiosulfate and tetrathionate may also be formed.

Sulfur produced during the sulfide oxidation process is deposited in the medium as amorphous, elemental sulfur (Fig. 2). This allotrop of sulfur is also known to be produced by other photosynthetic sulfur bacteria (5). Microscopic observations frequently revealed filaments that contained elemental sulfur deposited about the long axis of the filament (Fig. 3). These elongated deposits are apparently quite loosely attached to the filament since they are frequently seen free in the medium; they probably become directly deposited on the outside of the filaments during the sulfide oxidation process.

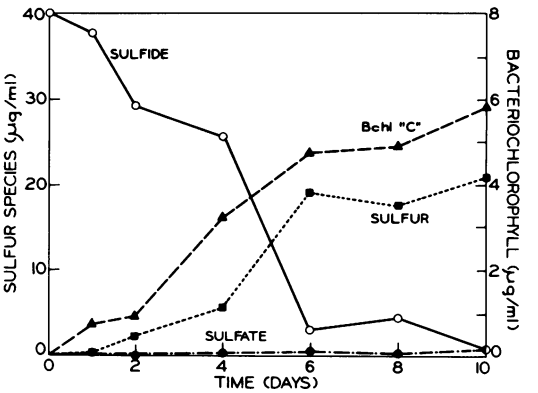

FIG. 1. *Oxidation of sulfide with the production of elemental sulfur during photoautotrophic growth of Chloroflexus. Sulfate expressed in terms of its sulfur content.*

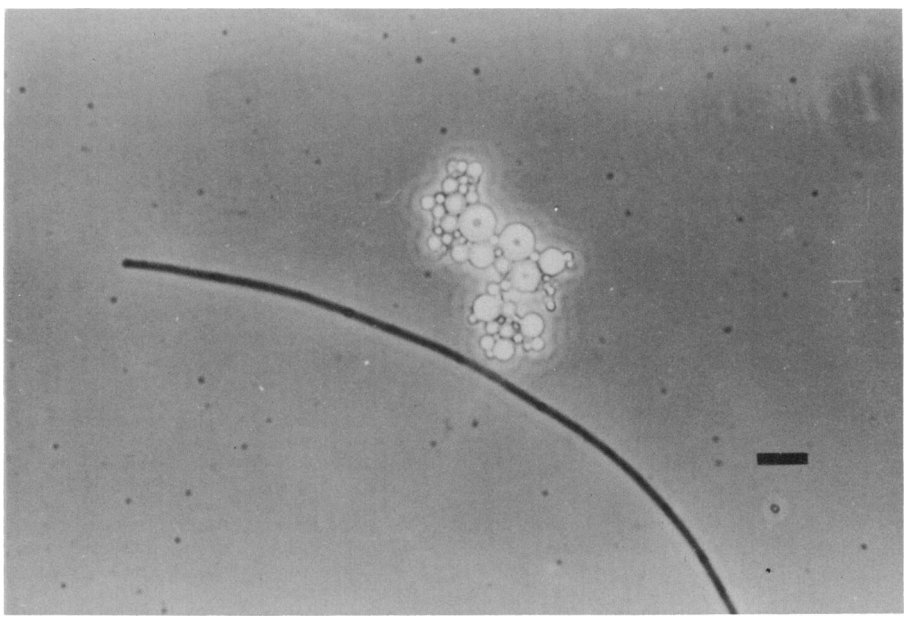

FIG. 2. *Phase contrast photomicrograph of elemental sulfur deposited in the medium during growth of Chloroflexus, strain OK-70-fl. Magnification ×625. Marker bar represents 10 μm.*

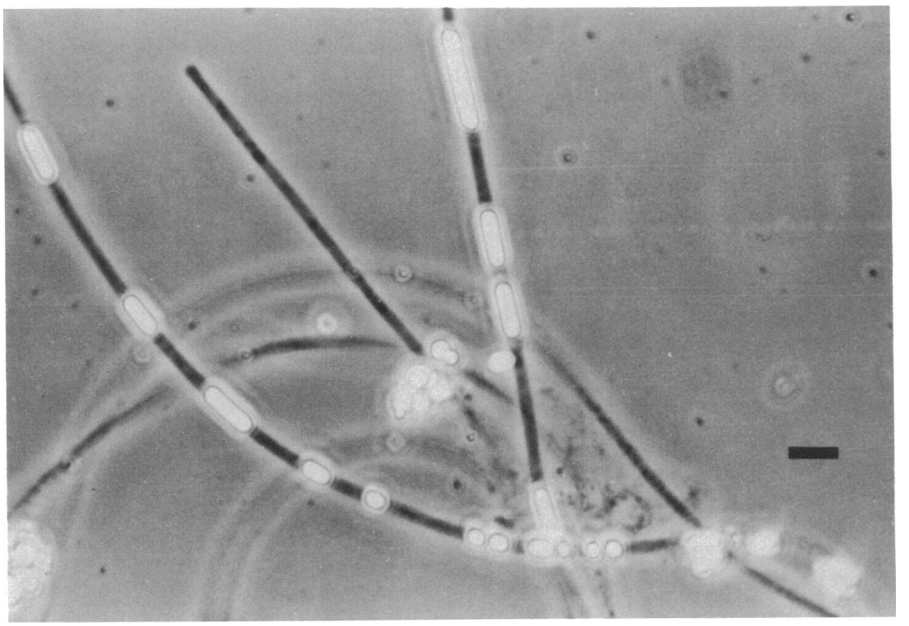

FIG. 3. *Phase contrast photomicrograph of filaments of Chloroflexus, strain OK-70-fl with deposited sulfur. Magnification ×1,560. Marker bar represents 4 μm.*

This same phenomenon has also been observed in strains J-10-fl, and 244-3 (7).

To determine whether other reductants could support the growth of *Chloroflexus* strain OK-70-fl, several sulfur-containing compounds were tested for their ability to support growth in the absence of sulfide. Of the compounds tested other than sulfide (thiosulfate, tetrathionate, sulfite, thioglycolate, methionine, cysteine, and elemental sulfur), only elemental sulfur and cysteine supported growth. Crystalline elemental sulfur did not support growth, but a colloidal suspension of sulfur did support slow growth. Since the utilization of numerous organic compounds under anaerobic conditions in the light has been shown (7), growth with cysteine as reductant possibly reflects the photoheterotrophic influence of this amino acid, rather than its suitability strictly as an electron donor.

Thus, despite its filamentous, gliding nature and its ability to grow aerobically in the dark, *Chloroflexus* resembles a typical green sulfur bacterium in its ability to use sulfide as a reductant with the deposition of elemental sulfur outside the cell. Our results further substantiate the suggestion (9) that this organism should be classified as a member of the *Chlorobiaceae*.

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