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Short communication

Rhodospirillum centenum, sp. nov., a thermotolerant cyst-forming anoxygenic photosynthetic bacterium

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Abstract. A novel non-sulfur purple photosynthetic bacterium, designated *Rhodospirillum centenum*, was isolated from an enrichment culture designed to favor growth of anoxygenic photosynthetic N_2-fixing bacteria. *R. centenum* grows optimally at 40–42° C and has the capacity to produce cytoplasmic 'R bodies', refractile structures not observed hitherto in photosynthetic prokaryotes. The bacterium is also unusual among photosynthetic bacteria in that it forms desiccation-resistant cysts when grown aerobically in darkness with butyrate as the sole carbon source.

The first pure culture of an anoxygenic photosynthetic bacterium, *Spirillum rubrum*, was isolated from the dry residue of a dead mouse by Erwin Esmarch (1887). Although he was unaware of the fact that the red-colored organism, later renamed *Rhodospirillum rubrum*, had the capacity to grow phototrophically, he was the first investigator to observe the inhibitory effect of molecular oxygen on photopigment synthesis by purple bacteria. In 1987, we isolated a previously unknown species of *Rhodospirillum* and have named it *Rhodospirillum centenum* in recognition of the centennial of Esmarch's publication.

R. centenum was isolated from a water sample (temperature, ca. 55° C) collected at the edge of the source pool at Thermopolis Hot Springs, Wyoming. The bacterium was enriched, at 40° C, using a procedure that is selective for anoxygenic photosynthetic bacteria that fix N_2 readily (Gest et al. 1985). Isolation of *R. centenum* in pure culture and characterization of its morphological, physiological and other properties were accomplished using techniques detailed by Pellerin & Gest (1983) and Gest et al. (1985). Electron micrographs of thin sections of the bacterium were made essentially as described by Gest & Favinger (1983).

R. centenum cells are typically short (1×2 to $3\ \mu$m) and, depending on the composition of the growth medium, are vibrioid to spiral in shape. When

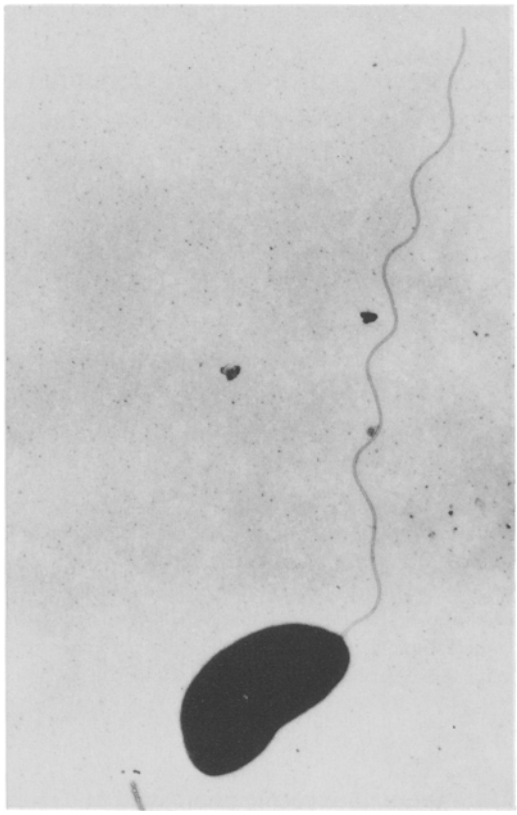

Fig. 1. Electron micrograph of a vibrioid cell of *R. centenum*, with polar flagellum. Negative stain using 2% uranyl acetate; magnification: × 14,570.

grown phototrophically, the cells are actively motile by means of a single, long polar flagellum (Fig. 1). Lamellar photosynthetic membranes are localized in the cell periphery, and the in vivo absorption spectrum of the photopigments is virtually identical to that of *R. rubrum* (peaks at ca. 800 and 875 nm; Biebl & Drews 1969). The bacterium requires biotin and vitamin B_{12} for growth, whereas biotin is the only growth factor necessary for *R. rubrum*. *R. centenum* grows photoheterotrophically at temperatures up to 45° C, and optimally between 40° and 42° C, whereas most isolates of non-sulfur purple bacteria are unable to grow at temperatures above 39° C. The G + C content of *R. centenum* DNA is 68.3 Mol%; values reported for *R. rubrum* DNA range from 62.4 to 65 (Silver et al. 1971).

R. rubrum and most other non-sulfur purple bacteria readily use malate as a sole carbon source, but *R. centenum* is unable to grow on malate or related C_4 dicarboxylic acids. With NH_4 as nitrogen source, carbon sources utilizable by

R. centenum include pyruvate, lactate, and acetate; when supplemented with bicarbonate (9 mM), butyrate, caproate, and caprylate also support phototropic growth. For routine purposes (for either anaerobic phototrophic or dark aerobic chemoheterotrophic growth), we employ pyruvate as the carbon source in a medium designated as CENMED, which contains per liter of deionized water: 2.2 g sodium pyruvate, 0.9 g K_2HPO_4, 0.6 g KH_2PO_4, 1 g NH_4Cl, 5 mg disodium EDTA, 200 mg $MgSO_4 \cdot 7H_2O$, 1 ml trace element solution (see Fry et al. 1984), 75 mg $CaCl_2 \cdot 2H_2O$, 2 ml chelated iron solution (prepare by dissolving 1 g $FeCl_2 \cdot 4H_2O$ and 2 g disodium EDTA in 1 liter deionized water, and adding 3 ml concentrated HCl), 20μg vitamin B_{12}, 15μg biotin, 0.5 g $Na_2S_2O_3 \cdot 5H_2O$; pH adjusted to 6.8 with NaOH.

With pyruvate as the carbon source, NH_4^+, N_2, glutamate, glutamine, and alanine serve as sole nitrogen sources for rapid phototrophic growth. The nitrogenase activity of *R. centenum* cells grown on N_2 (estimated by the acetylene reduction assay) is comparable to that observed with *R. rubrum*, namely, of the order of 500–1000 nmoles acetylene reduced/hour/mg dry weight of cells (see Madigan et al. 1984 for nitrogenase activities of species in the family *Rhodospirillaceae)*.

R. centenum is a typical non-sulfur purple bacterium in respect to ability to grow aerobically as a chemoheterotroph in darkness on various organic carbon sources. An unusual feature was observed in cultures growing in this way with butyrate as the sole carbon source, namely, the conversion of the spiral-shaped cells to cysts (Figs 2 and 3). These forms were not seen in cultures grown photosynthetically on butyrate + bicarbonate. The relative dessication resistance of cysts and growing cells was determined as follows. The two kinds of cell forms were filtered onto separate membrane filters, which were incubated in a drying oven at 38° C. After drying for prolonged periods under these conditions, the filter discs were placed on agar growth medium in Petri dishes, and the latter incubated in illuminated Gas Pak jars. Within 48 hours, discs of cysts that had been dried for 8 days showed extensive red growth over the entire filter whereas the controls (growing cells) dried for 4 days showed virtually no growth. Increased resistance of cysts to desiccation has been observed in several other bacterial genera (Brock and Madigan 1988). Cyst formation by *Azotobacter* cells has been correlated with the accumulation of poly-β-hydroxybutyrate (Stevenson and Socolofsky 1973) and this may well be the case in *R. centenum* also; *R. centenum* cells grown under cyst-forming conditions have a high content of the polymer (ca. 30% of the dry weight). The formation of cysts and the relatively high temperature optimum for growth presumably would favor long-term survival of *R. centenum* under conditions extant in niches adjacent to hot springs.

Electron micrographs of thin sections of *R. centenum* revealed still another remarkable feature of this organism. The cytoplasm of many growing cells

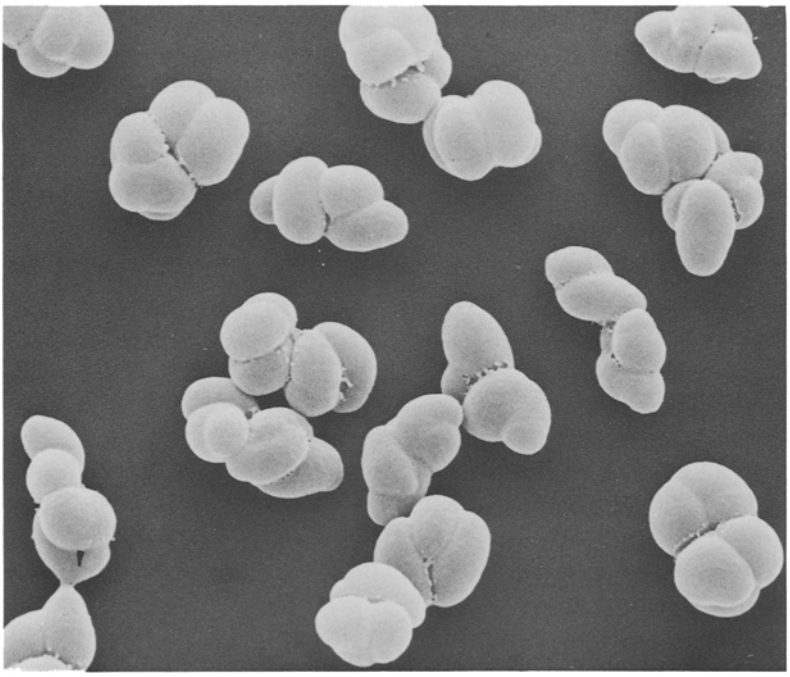

Fig. 2. Scanning electron micrograph showing *R. centenum* in various stages of cyst formation. The preparation was critical point-dried; magnification: × 8,250.

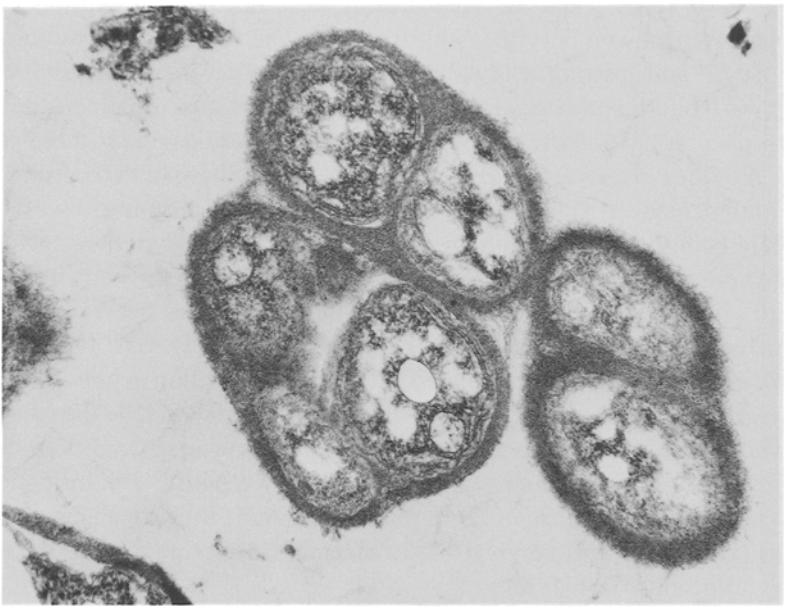

Fig. 3. Electron micrograph of a thin section of *R. centenum* cysts. Magnification: × 37,180.

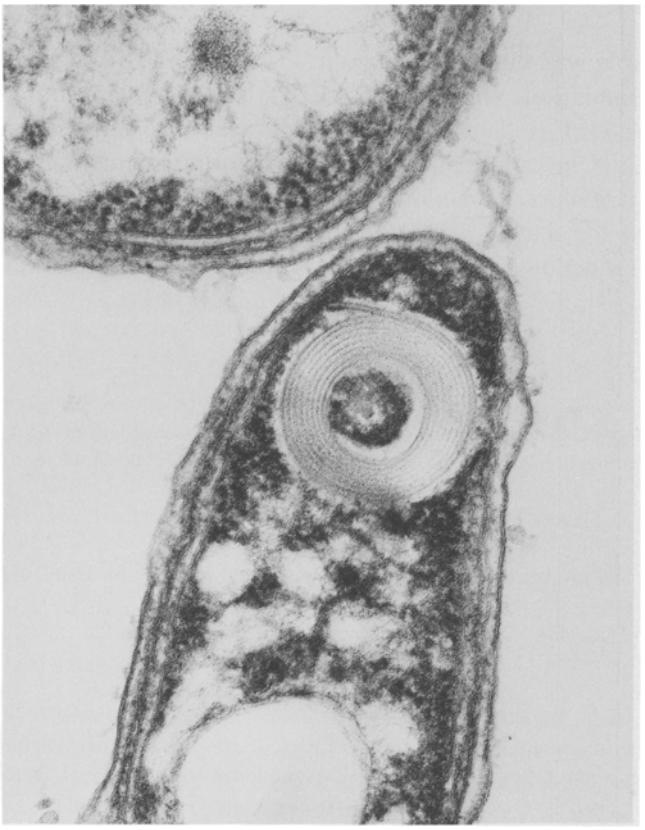

Fig. 4. Electron micrograph of a thin section of an *R. centenum* cell containing an R body, which resembles a coiled rope. Note the large poly-β-hydroxybutyrate granule also present in the cell. Magnification: × 85,800.

contains refractile bodies that have the unique structure shown in Fig. 4. The refractile inclusions were kindly identified by Dr J.R. Preer, Jr. as 'R bodies', which have been observed thus far in a very limited number of prokaryotes, notably in endosymbionts of paramecia (Preer & Preer 1984). In such endosymbionts, the refractile R body consists of a coiled proteinaceous 'ribbon', and in some instances is known to be a plasmid-determined trait (Quackenbush & Burbach 1983). This is the first instance in which R bodies have been observed in photosynthetic bacteria, and their significance for *R. centenum* is now under investigation. *R. centenum,* strain Favinger/Gest, has been deposited in the American Type Culture Collection (no. 43720).

Acknowledgements

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