Confused History of *Chloropseudomonas ethylica* 2K

JOHN M. OLSON

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Chloropseudomonas ethylica 2K cultured at Brookhaven National Laboratory since 1962 has always been a mixed culture of *Prosthecochloris aestuarii* and *Desulfuromonas acetoxidans*.

In 1960 Shaposhnikov et al. (10) proposed the name *Chloropseudomonas ethylica* for a new species of green sulfur bacteria isolated from mud samples from Kulal'nik estuary near Odessa and Lake Saksii in the Crimea. This new species was characterized by "motile, short, rod-shaped cells measuring about $1.0\text{--}1.5 \times 0.8\text{--}0.9 \mu\text{m}$." (Inconsistencies between this description and an earlier one [11] have been pointed out previously [2, 7].)

In August 1961, E. N. Kondrat'eva of Moscow State University gave me a culture of *Cps. ethylica* 2K, which I brought to Brookhaven National Laboratory. From this culture, single-colony isolates were made and then grown up in liquid culture. In June 1962, Carol Romano examined several cultures in my laboratory and noted the presence, in each, of two distinct cell types, nonmotile "raspberries" and motile rods (unpublished data), but we did not grasp the significance of this observation at that time. Over the next 10 years, subcultures were sent to several other laboratories including the one where Gray et al. (3, 4) showed in 1972 that *Cps. ethylica* 2K was really a mixed culture of a nonmotile, green photosynthetic bacterium and one or more motile, colorless, non-photosynthetic bacteria. Very recently Gray (2) proposed that the names *Chloropseudomonas* and *Chloropseudomonas ethylica* be declared nomina rejicienda, since the original descriptions of these "organisms" were almost certainly based on mixed cultures.

A difference of opinion exists over the correct name for the green bacterium in the mixed culture. Gray (2-4) named the green bacterium *Chlorobium limicola*, but Norbert Pfennig (personal communication) in October 1976 identified the organisms in the Brookhaven culture as *Prosthecochloris aestuarii* and *Desulfuromonas acetoxidans* (9, 12). He stated unequivocally that there was no *Chlorobium* in the culture. (However, the *Cps. ethylica* N2 culture does contain *C. limicola* [9].)

Biochemical evidence supports Pfennig's classification based on morphology. Soluble cytochromes c_{555} have been extracted from the green

bacterium in *Cps. ethylica* 2K and from strains PM (NCIB 8346) and L (NCIB 8327) of *C. limicola* f. sp. *thiosulfatophilum*. The sequence of amino acid residues (86) for strains PM and L are identical, but the sequence of residues (99) for the green bacterium in the *Cps. ethylica* 2K culture can be matched with only 47 residues at best in cytochrome c_{555} from *C. limicola* f. sp. *thiosulfatophilum* (13). This degree of matching indicates that the green bacterium in the *Cps. ethylica* 2K culture belongs to a different species than *C. limicola* f. sp. *thiosulfatophilum*.

I am confident that the Brookhaven culture of *Cps. ethylica* 2K has always contained *P. aestuarii* and *D. acetoxidans*. For the sake of continuity, I shall designate my strain of *P. aestuarii* as strain 2K. This bacterium is and always has been the sole source of bacteriochlorophyll *a*—protein from *Cps. ethylica* strain 2K (5, 6, 8) or *C. limicola* 2K (1).

The work at Brookhaven National Laboratory was supported by the U.S. Atomic Energy Commission and by the U.S. Energy Research and Development Administration.

LITERATURE CITED

1. Fenna, R. E., and B. W. Matthews. 1975. Chlorophyll arrangement in a bacteriochlorophyll-protein from *Chlorobium limicola*. *Nature* 258:573-577.
2. Gray, B. H. 1977. Rejection of *Chloropseudomonas ethylica* as a Nomina Rejicienda. *Int. J. Syst. Bacteriol.* 27:168.
3. Gray, B. H., C. F. Fowler, N. A. Nugent, and R. C. Fuller. 1972. A reevaluation of the presence of low midpoint potential cytochrome 551.5 in the green photosynthetic bacterium *Chloropseudomonas ethylica*. *Biochem. Biophys. Res. Commun.* 47:322-327.
4. Gray, B. H., C. F. Fowler, N. A. Nugent, N. Rigopoulos, and R. C. Fuller. 1973. Reevaluation of *Chloropseudomonas ethylica* strain 2-K. *Int. J. Syst. Bacteriol.* 23:256-264.
5. Olson, J. M. 1966. Chlorophyll-protein complexes derived from green photosynthetic bacteria, p. 413-425. *In* L. P. Vernon and G. R. Seely (ed.), *The chlorophylls*. Academic Press, New York.
6. Olson, J. M. 1971. Bacteriochlorophyll-protein of green photosynthetic bacteria, p. 636-639. *In* A. San Pietro (ed.), *Methods in enzymology*. Academic Press, New York.
7. Olson, J. M. 1973. Historical note on *Chloropseudomonas ethylica* strain 2-K. *Int. J. Syst. Bacteriol.* 23:265-266.
8. Olson, J. M., D. Filmer, R. Radloff, C. A. Romano,

- and C. Sybesma. 1963. The protein-chlorophyll-770 complex from green bacteria, p. 423-431. In H. Gest, A. San Pietro, and L. P. Vernon (ed.), Bacterial photosynthesis. Antioch Press, Yellow Springs, Ohio.
9. Pfennig, N., and H. Biebl. 1976. *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch. Microbiol. 110:3-12.
10. Shaposhnikov, V. V., E. N. Kondratieva, and V. D. Federov. 1960. A new species of green sulphur bacteria. Nature (London) 187:167-168.
11. Shaposhnikov, V. N., E. N. Kondrat'eva, E. N. Krasil'nikova, and A. A. Ramenskaya. 1959. Green bacteria utilizing organic compounds. Dokl. Akad. Nauk SSR 129:1424-1426.
12. Shioi, Y., K. Takamiya, and M. Nishimura. 1976. Isolation and properties of NAD⁺ reductase of the green photosynthetic bacterium *Prosthecochloris aestuarii*. J. Biochem. (Tokyo) 79:361-371.
13. Van Beeumen, J., R. P. Ambler, R. E. Meyer, M. D. Kamen, J. M. Olson, and E. K. Shaw. 1976. Amino acid sequences of cytochromes c-555 from two green bacteria of the genus *Chlorobium*. Biochem. J. 159:757-769.