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Deoxyribonucleic Acid Homologies of Some So-Called "*Hydrogenomonas*" Species

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Evidence based on deoxyribonucleic acid homology supports the abandonment of the genus *Hydrogenomonas*. *Pseudomonas facilis* (formerly *Hydrogenomonas facilis*) is closely related to the nonautotrophic species *P. delafieldii*. *P. facilis* and *Alcaligenes eutrophus* (often called *H. eutropha*) are not related to each other or to other hydrogen bacteria and pseudomonads studied.

A systematic study of a number of gram-negative "hydrogen bacteria" led to the proposal that the genus *Hydrogenomonas*, to which most of them had been previously assigned, should be abandoned (1). Two of the arguments used were that the genus contained a heterogeneous collection of morphologically and physiologically distinct types and that some of the hydrogen bacteria appeared to be phenotypically much more closely related to nonautotrophic members of other genera than to one another. The various species were, therefore, tentatively reassigned to the genera *Pseudomonas*, *Alcaligenes*, and *Paracoccus*. We report here some in vitro deoxyribonucleic acid (DNA) hybridization (DNA-DNA) studies with a few selected hydrogen bacteria and nonautotrophic *Pseudomonas* species that support our previous conclusions. DNA homologies were determined by the competition and direct-binding methods used by Palleroni and Doudoroff (4). The strains are designated as in our previous studies (2, 4-6) except for *Pseudomonas mallei* and *P. pseudomallei*, which are designated by the method of M. Rogul, of the Walter Reed Army Institute of Research, Washington, D.C. M. Rogul kindly supplied us with the extracted DNA of his strains.

Competition experiments were conducted at 25 degrees (C) below T_m with the DNA of the obligately heterotrophic species *P. delafieldii* strain 134 (2) as reference. One-hundred per cent DNA homology was found with three other strains of the same species (strains 133, 135, and 137) and with one strain of the hydrogen bacterium *P. facilis* 332 (formerly *Hydrogenomonas facilis*). The homology with *P. facilis* 458 was 42%. It should be noted that the guanine plus cytosine (G + C) content of the DNA of *P. delafieldii* has been reported to be 65 to 66 moles per cent, whereas that of *P. facilis* 332 and 458 was found to be 64 and 62%, respectively. These two strains are, however, practically indistinguishable from one another in phenotype. In the same experiment, no DNA homology whatever was found between *P. delafieldii* and the hydrogen bacteria *P. saccharophila*, *P. palleronii* 362 (2), or any of the following nonautotrophic *Pseudomonas* strains: *P. aeruginosa* 131, *P. pseudoalcaligenes* 63, *P. mallei* 3873, *P. pseudomallei* 165, *P. caryophylli* 720, and *P. acidovorans* 14. Results with *P. cepacia* 382 and *P. solanacearum* 769 were somewhat erratic. As a check of the DNA competition experiments, direct-binding experiments were conducted with immobilized DNA of *P. delafieldii* and *P. solanacearum* and selected samples of sheared ^{14}C-labeled DNA of several strains. By this method, a homology of 83% was found between *P. delafieldii* 134 and *P. facilis* 332. *P. solanacearum* showed virtually no homology with either *P. delafieldii* or *P. facilis* 332 (4 to 11%), and *P. cepacia* showed essentially no homology with *P. delafieldii* (6%). A melting profile of the *P. delafieldii*-*P. facilis* DNA hybrid obtained in the above experiment gave a T_m of 4 degrees (C) lower than that of the *P. delafieldii* homologous hybrid, suggesting a mismatch of ca. 6% of the hybridized portion (3). In our experience, relatively few strains of different *Pseudomonas* species or even strains assigned to a single species or biotype are as closely related as are the strains of *P. delafieldii* and *P. facilis*.

Competition experiments were conducted with reference DNA from the hydrogen bacterium, *Alcaligenes eutrophus* strain 337 (1, 2). (This peritrichously flagellated bacterium is commonly and incorrectly called *H. eutropha* in the literature and in culture collections.) A

homology value of 93% was obtained with the holotype strain 335 (*H. eutropha* ATCC 17697). No homology whatever was detected with the DNA of the other hydrogen bacteria, *P. facilis* 332 and 458, *P. saccharophila*, and *P. palleronii* 362, or with that of the heterotrophic species *P. acidovorans* 105, *P. testosteroni* 78, *P. delafieldii* 134, *P. stutzeri* 221, *P. mendocina* CH50, and *P. aeruginosa* 132. It should be noted that the peritrichously flagellated *A. eutrophus* (67% G + C) shares many phenotypic characters with both *P. acidovorans* (67% G + C) and *P. testosteroni* (61% G + C). In spite of the large difference in DNA composition, *P. acidovorans* and *P. testosteroni* show some DNA homology with each other (33% competition at 72 C) but very little if any with other *Pseudomonas* species.

The above observations give support to the view that the genus *Hydrogenomonas* is untenable. *P. facilis* and *P. delafieldii* clearly belong to a distinct subgroup of the genus *Pseudomonas*. Interestingly enough, *P. saccharophila*, which shows many phenotypic similarities to this subgroup, appears to be unrelated with respect to DNA homology. Limited studies with this species have shown that it is also unrelated to all other *Pseudomonas* species that have been examined. The peritrichously flagellated species, *A. eutrophus*, is unrelated to any of the polarly flagellated hydrogen bacteria or to other *Pseudomonas* species that have been tested. The DNA homologies between this species and other peritrichously flagellated aerobic bacteria of the genera *Alcaligenes*, *Achromobacter*, *Rhizobium*, and *Agrobacterium* remain to be studied.

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LITERATURE CITED

1. Davis, D. H., M. Doudoroff, R. Y. Stanier, and M. Mandel. 1969. Proposal to reject the genus *Hydrogenomonas:* taxonomic implications. Int. J. Syst. Bacteriol. **19**:375–390.
2. Davis, D. H., R. Y. Stanier, M. Doudoroff, and M. Mandel. 1970. Taxonomic studies on some gram negative polarly flagellated "hydrogen bacteria" and related species. Arch. Mikrobiol. **70**:1–13.
3. Laird, C. D., B. L. McConaughy, and B. J. McCarthy. 1969. Rate of fixation of nucleotide substitutions in evolution. Nature (London) **223**:149–154.
4. Palleroni, N. J., and M. Doudoroff. 1971. Phenotypic characterization and deoxyribonucleic acid homologies of *Pseudomonas solanacearum*. J. Bacteriol. **107**:690–696.
5. Palleroni, N. J., M. Doudoroff, R. Y. Stanier, R. E. Solanes, and M. Mandel. 1970. Taxonomy of the aerobic pseudomonads: the properties of the *Pseudomonas stutzeri* group. J. Gen. Microbiol. **60**:215–231.
6. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. **43**:159–271.