NOTES

Recommendation that the Names *Ferrobacillus ferrooxidans* Leathen and Braley and *Ferrobacillus sulfooxidans* Kinsel Be Recognized as Synonyms of *Thiobacillus ferrooxidans* Temple and Colmer

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The only characters currently used to distinguish *Ferrobacillus ferrooxidans* and *F. sulfooxidans* from *Thiobacillus ferrooxidans* are the inability of the first species to oxidize sulfur or thiosulfate and of the second to use thiosulfate. These differences are demonstrated to be invalid inasmuch as all three species can grow on either sulfur or thiosulfate. The names *F. ferrooxidans* and *F. sulfooxidans* are therefore regarded as later, subjective synonyms of *Thiobacillus ferrooxidans*.

At present, three species of bacteria capable of autotrophic development using ferrous iron oxidation as a sole source of energy are described in *Bergey's Manual* (3). These are *Thiobacillus ferrooxidans* Temple and Colmer (5, 22), *Ferrobacillus ferrooxidans* Leathen and Braley (11; Leathen and Braley, *Bacteriol. Proc.*, 1954, p. 44) and *F. sulfooxidans* Kinsel (9). Morphologically these species are indistinguishable. They were separated solely by alleged differences in ability to use inorganic sulfur compounds as oxidizable substrates (Table 1). *T. ferrooxidans*, the earliest named of these species, was described as capable of oxidizing and growing on thiosulfate as well as on iron as a source of energy (4, 5, 22). The only feature distinguishing *F. ferrooxidans* from *T. ferrooxidans* was its reported inability to grow on sulfur or thiosulfate (11; Leathen and Braley, *Bacteriol. Proc.*, 1954, p. 44). Similarly, *F. sulfooxidans* was reported to grow on sulfur but not to use thiosulfate, thus distinguishing it from the other two species (9). No additional biochemical differences apparently exist between the species as so far studied.

Since the initial description of the *Ferrobacillus* species, some taxonomic studies have been made on them (6, 7, 8), and their sulfur metabolism has been further investigated (10, 14, 17-21, 25). These observations, and our

TABLE 1. Features of inorganic sulfur metabolism used to characterize and distinguish the original isolates of *Thiobacillus ferrooxidans*, *Ferrobacillus ferrooxidans*, and *F. sulfooxidans*

Organism	Energy substrates used for growth			Reference
	Ferrous iron	Sulfur	Thio-sulfate	
<i>T. ferrooxidans</i>	+	±	+	3, 5, 22
<i>F. ferrooxidans</i>	+	—	—	3, 11; Leathen and Braley ^a
<i>F. sulfooxidans</i>	+	+	—	9

^a *Bacteriol. Proc.*, 1954, p. 44.

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own using culture-collection strains of *T. ferrooxidans*, *F. Ferrooxidans*, and newly isolated strains of these organisms, all indicate that the characters used to distinguish ferro-

TABLE 2. Utilization of sulfur compounds as oxidizable substrates by strains of *T. ferrooxidans*, *F. ferrooxidans*, and *F. sulfooxidans*

Organism	Sulfur compound	Observation ^a	Reference
<i>T. ferrooxidans</i>	Sulfur	A	6, 15, 23
	Thiosulfate	A,B,C	4, 6, 23-25 ^b
NCIB 9490	Sulfur	A,D	1, 2, 6, 10, 13
	Thiosulfate	A	6, 10
ATCC 13598 13728 NCIB 8451	Sulfur or thiosulfate	A	6, 24
<i>F. ferrooxidans</i>	Sulfur	A,D	6, 12, 14, 17, 20, 23, 25 ^b
	Thiosulfate	A,B,C,D	6, 17, 23, 24, 25 ^b
<i>F. sulfooxidans</i> NCIB 9275 ATCC 14119	Sulfur or thiosulfate	A,B	6, 9

^a A, Growth in liquid; B, growth on agar; C, growth on membrane filters; D, oxidation and coupled CO₂ fixation by cell suspensions.

^b Unpublished observations by the present authors.

bacilli from *T. ferrooxidans* are invalid. All three "species" will grow on sulfur or thiosulfate as well as on iron (Table 2). Kinsel (9) concluded that *F. sulfooxidans* produced colonies on thiosulfate-agar medium only because of sulfur production from thiosulfate. She reported, however, that no precipitated material was observed in or around the colonies, and in our experience thiosulfate does not decompose significantly in this medium. Our own observations using thiosulfate-agar (Difco Thiobacillus-Agar) have been made by placing membrane filters carrying the bacteria on the surface of the agar. Colonies of *T. ferrooxidans* and *F. ferrooxidans* developed on the membranes at the expense of thiosulfate diffusing to the bacteria. No sulfur formation occurred. Hutchinson, Johnstone, and White (6) grew all three species in liquid media with sulfur or thiosulfate and reported that, for growth to be successful with thiosulfate, prior culture on sulfur was necessary. We have also experienced difficulties in transferring from iron to thiosulfate medium, but we do confirm that both *T. ferrooxidans* and ferrobacilli will use thiosulfate.

The observations we have summarized demonstrate that the characters presently used to distinguish ferrobacilli from thiobacilli cannot be confirmed on close examination of representative cultures of the three species. No other features, morphological, ultrastructural, or biochemical, distinguish the species from each other. All three are incapable of heterotrophic growth when inoculated onto organic

nutrient medium after autotrophic culture, and, although some strains at least can be adapted to glucose (16), this phenomenon is variable even with different cultures of a single strain (C. A. Jones and D. P. Kelly, unpublished data).

It is urged therefore, that all the ferrous-iron-oxidizing members of the suborder *Pseudomonadineae* be regarded as examples of *Thiobacillus ferrooxidans* and that, in the next edition of *Bergey's Manual*, the names *Ferrobacillus ferrooxidans* and *F. sulfooxidans* be cited as subjective synonyms of *T. ferrooxidans*.

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