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NOTES

Flagella and Pili of Iron-Oxidizing Thiobacilli Isolated from a Uranium Mine in Northern Ontario, Canada

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Five strains of *Thiobacillus ferrooxidans*, which included three recent isolates from a uranium mine, possessed flagella. Three of the strains had several pili per cell. The dimensions, fine structure, and orientation of the flagella were different. Both polar and peritrichous flagella were observed, indicating strain-dependent ultrastructural variation in acidophilic thiobacilli. Neither flagella nor pili were detected in eight other strains of *T. ferrooxidans* and two strains of *Thiobacillus acidophilus* by electron microscopy, although all of the cultures contained motile cells.

Thiobacillus ferrooxidans, a motile acidophilic autotroph, is reported to possess a single polar flagellum characteristic of the *Pseudomonadaceae* (21), the presence of which has not been confirmed by electron microscopy. Flagella have been studied in sulfur-grown *Thiobacillus thiooxidans* (5), a closely related acidophile which does not oxidize ferrous iron. Gromova et al. (6) reported the presence of pili in sulfur-grown *T. ferrooxidans*, but did not detect these structures when ferrous iron was used as growth substrate. Pili have also been found in *Thiobacillus kabobis* (14) and in thermophilic sulfur-oxidizing bacteria (2, 22). Fimbriae have been detected in *Thiobacillus* sp. strain A2 (10).

Recently, the hypothesis has been proposed that the iron-oxidizing thiobacilli may not be one distinct species but, rather, a group of metabolically similar bacteria (16). This premise is based on the differences in the physiological characteristics (17) and guanine and cytosine contents of the DNA (8) of different isolates of *T. ferrooxidans*. This report extends the examination of different strains of these bacteria by investigating the presence and external morphology of flagella and pili in a number of different cultures of acidophilic thiobacilli, including iron oxidizers which are currently being used in the commercial extraction of uranium at Agnew Lake, Ontario. The physical and chemical characteristics and the concentrations of iron-oxidizing and heterotrophic bacteria in the leach liquors from various points in the extraction circuit have been reported (20). Several isolates of the iron-oxidizing thiobacilli from this mine were found to possess plasmids; they were also characterized (20).

All of the cultures were grown at room temperature (22°C) in unagitated 20-ml test tubes containing 10-ml samples of media. The ferrous iron (pH 1.5), tetrathionate (pH 4.5), and glucose (pH 2.0) media have been described elsewhere (15, 18, 19). The strains were subcultured at least four times before examination.

Strains TFI-1, TFI-4, TFI-6, TFI-7, TFI-9, TFI-10, and TFI-13 of *T. ferrooxidans* were isolated from various locations of the Agnew Lake uranium mine of northern Ontario (20). *T. ferrooxidans* strain TFI-17 was obtained as a derivative of *T. ferrooxidans* ATCC 19859 from A. P. Harrison (University of Missouri, Columbia), TFI-23 was obtained from G. J. Olson (National Bureau of Standards, Washington, D.C.), TFI-27 and TFI-29 were obtained from A. E. Torma (New Mexico Institute of Mining and Technology, Socorro), and strain TFI-30 was obtained from E. J. Brown (University of Alaska, Fairbanks); strain TFI-35 is a derivative of the original isolation of *Ferrobacillus ferrooxidans* (11), which has been continually maintained on ferrous iron (18). All of the *T. ferrooxidans* strains were grown on ferrous iron, and strains TFI-7 and TFI-35 were also grown on tetrathionate. *Thiobacillus acidophilus* strains AFG-2 and AFG-3 were derived from the original culture of *T. acidophilus* (7) and were grown on glucose and on tetrathionate.

Samples for electron microscopy were taken from the surfaces of the static liquid cultures. Under these conditions, glucose-grown thioba-

TABLE 1. Chemical analysis and bacterial enumeration of leach liquor samples from the Agnew Lake uranium mine[a]

Mine location[b]	pH	Eh (mV)	U (g/liter)	Th (g/liter)	^{226}Ra (pCi/liter)	SO_4-S (g/liter)	Fe^{2+} (g/liter)	Total Fe (g/liter)	Iron oxidizers (cells/ml)	Glucose oxidizers (cells/ml)
700-5-E	1.75	570	0.030	1.18	230	10.7	0.73	4.37	0.7	4
1,000-5-E	1.72	550	0.038	1.20	330	10.8	0.74	4.31	4×10^1	9
1,070-5-E	1.72	590	0.081	1.23	200	10.5	1.19	4.42	2×10^0	7×10^2
1,250-5-W	1.76	480	0.085	1.17	45	10.4	0.82	4.48	1×10^4	8×10^1
1,180-3-W	1.77	450	0.085	1.13	140	10.4	1.28	4.48	0.4	1×10^4
1,500-3-W	1.76	516	0.087	1.23	100	10.4	0.42	4.46	8×10^4	8
1,500-3-E	1.82	532	0.110	1.06	90	0.92	0.40	4.35	4×10^4	2×10^1
Pregnant solution	1.82	450	0.104	1.21	90	9.94	0.34	4.33	1×10^0	4×10^2
Barren solution	1.69	473	0.0024	1.14	75	10.4	0.98	4.15	1×10^4	4
Pile discharge	1.52	474	0.0034	1.23	130	11.9	0.91	4.11	<0.3	8×10^1
Tailings pond	6.65	450	<0.0001	<0.0001	<25	0.48	<0.001	0.00008	<0.3	<0.3
Solution to pile	1.72	470	0.0020	1.13	110	10.4	0.99	4.18	4×10^3	2×10^2

[a] The chemical methods and viable count technique (MPN) are explained in reference 20.

[b] The stopes of the mine are numbered in the following manner: depth (feet)-ore zone-East/West. Uranothorite, the main uranium mineral, occurs in two separate areas of the ore body designated zones 3 and 4. The solution flow in the mine was described previously (20).

cilli form a surface pellicle. A light surface film formed in tetrathionate-grown cultures. The iron-grown thiobacilli developed a white, thin surface film, with pellicle formation at the solution-glass interface. At pH values greater than 1.8, this pellicle formation becomes indistinguishable owing to precipitates formed by the hydrolysis of ferric iron. Samples were placed on Parlodion and carbon-coated grids and were negative stained with 2% (wt/vol) uranyl acetate for 2 to 5 min. A negative stain with 1% phosphotungstic acid was also tested, but it was excluded because of its inferior resolution in the present procedure. The grids were examined and photographed under a Zeiss model 9A electron microscope. The length measurements of flagella and pili were determined from electron micrographs, using a Numonics digitizer.

The Agnew Lake uranium mine, the environment from which iron-oxidizing strains of thiobacilli TFI-1 to TFI-13 were isolated, currently employs surface heap leaching and flood leaching of the underground stopes for the recovery of uranium (20). Table 1 shows the chemical analysis and numbers of iron-oxidizing and heterotrophic bacteria of the leach solutions in January, 1981. These solutions contained up to 0.11 g of uranium per liter and up to 1.23 g of thorium per liter. Iron is present at concentrations between 4 and 5 g/liter, mostly in the trivalent state, and acidophilic thiobacilli are present in all parts of the leach circuit with the exception of the tailings pond. The concentrations of iron-oxidizing thiobacilli and heterotrophic bacteria are similar to those found in other mining operations (1, 3, 4, 23) or in the Agnew Lake mine at different times of the year (20).

All of the cultures contained cells that were motile, but the percentage of motile bacteria in each culture varied. Electron microscopic examination showed the presence of flagella in only five of the strains when grown on ferrous iron, TFI-1, TFI-9, TFI-10, TFI-27, and TFI-35. The inability to detect flagella in the other cultures is probably due to the low percentage of flagellated cells and to their tendency to detach from the cell upon sample preparation.

Strain TFI-35 (Fig. 1) has a single polar flagellum, with a maximum length of 5.4 μm and an average diameter of 18.9 nm. These cells have up to five pili, with a maximum length of 3.6 μm and an average diameter of 5.4 nm. Only a small proportion of these bacteria retained their flagella after a number of subcultures. Strain TFI-1 was similar to strain TFI-35, with a maximum flagellum length of 9 μm and an average diameter of 19.9 nm. Cells of strain TFI-1 also contained up to five pili, with a maximum length of 1.5 μm and an average diameter of 8.9 nm. Figures 2 and 3 show that cells of *T. ferrooxidans* TFI-10 have at least four peritrichous flagella (average diameter, 18.2 nm). These flagella are extremely fragile, detaching easily from the cell and breaking into small segments despite all precautions taken during the preparation for electron microscopy. This strain also has pili (Fig. 4), with an average diameter of 4.9 nm. Strains TFI-9 and TFI-27 also possessed peritrichous flagella. The maximum flagellum lengths of TFI-9 and TFI-27 were 3.9 and 4.6 μm, respectively, with average diameters of 19.3 and 16.9 nm. Pili were not, however, observed in either strain TFI-9 or TFI-27.

The fine structures of the flagella from strains TFI-35 and TFI-10 (Fig. 5) appear different. The flagella of strain TFI-35 stained evenly, whereas

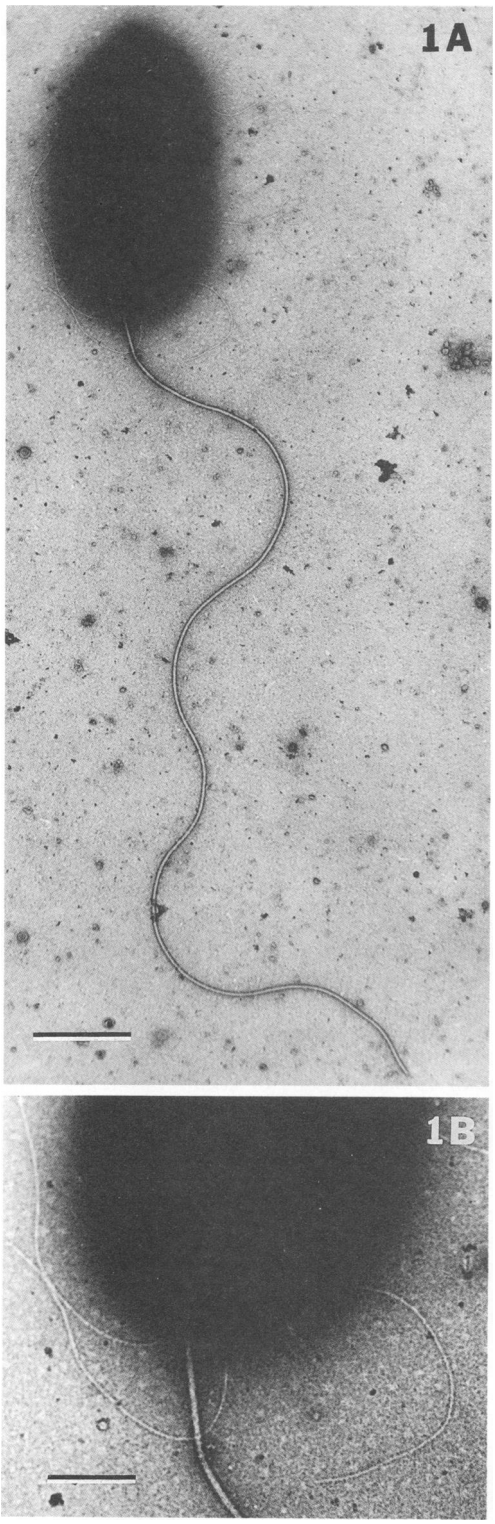

FIG. 1. A, Flagellum and pili of negatively stained *T. ferrooxidans* TFI-35. Bar, 0.5 μm. B, Pili of *T. ferrooxidans* TFI-35. Bar, 0.2 μm.

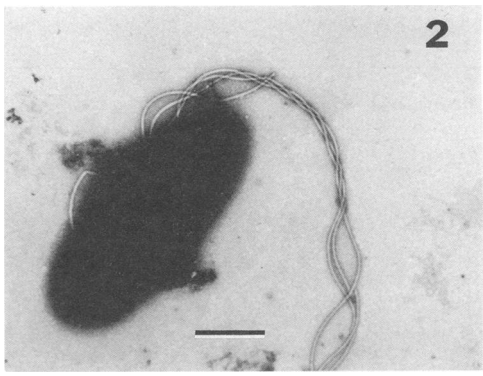

FIG. 2. Flagella of negatively stained *T. ferrooxidans* TFI-10. Bar, 0.5 μm.

those of TFI-10 showed alternating light and dark regions of approximately 3 nm each. Flagella and pili were not detected by electron microscopy in any isolate of *T. ferrooxidans* grown on tetrathionate or in *T. acidophilus* grown on either glucose or tetrathionate despite their motility under phase-contrast microscopy.

The presence of polar flagella of strains TFI-1 and TFI-35 confirms the present classification character of *T. ferrooxidans* (21). In contrast, the presence of multiple flagella in a peritrichous arrangement in *T. ferrooxidans* strains TFI-9,

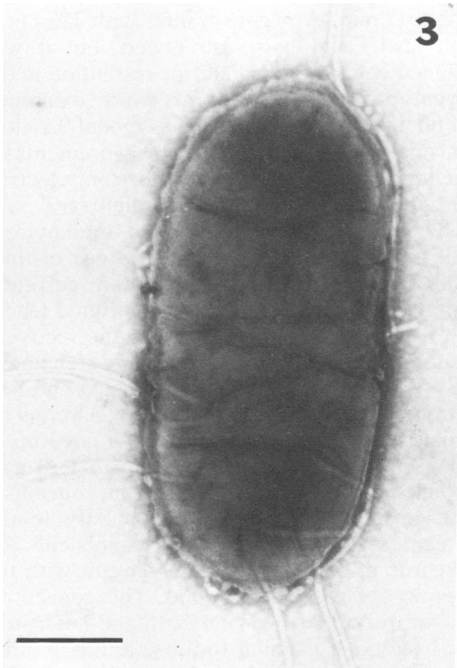

FIG. 3. Flagella of negatively stained *T. ferrooxidans* TFI-10; sites of attachment can be seen on the cell surface. Bar, 0.2 μm.

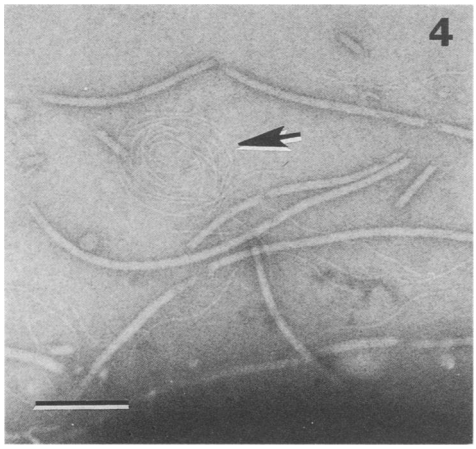

FIG. 4. Pili (arrow) of negatively stained *T. ferrooxidans* TFI-10. Bar, 0.2 μm.

TFI-10, and TFI-27 shows that there is structural variation in different strains of the iron-oxidizing thiobacilli. Pili and flagella are involved in the attachment of bacteria to surfaces. These organelles of the Agnew Lake uranium mine isolates TFI-1, TFI-9, and TFI-10 are of significance, as are those of strains TFI-27 and TFI-35, as the interaction of the iron-oxidizing thiobacilli with metal sulfide minerals requires a close contact and even attachment of the bacteria with the crystal surface (9). Although the occurrence of pili and flagella on iron-oxidizing bacteria attached to ore particles has not been demonstrated, these organelles are now shown to be present on *T. ferrooxidans* isolates from an environment in which these bacteria are known to oxidize sulfide minerals (20). The concentrations of thiobacilli associated with mineral particles are at least equal to or greater than those in the bulk solution phase (3, 4, 13).

The pili of *T. ferrooxidans* may be involved in the transfer of DNA, but unlike those of many other bacteria, this function has not been demonstrated in the thiobacilli. Many different plasmids have been detected in a variety of strains from the Agnew Lake uranium mine (20), including one of about 13 megadaltons which may encode resistance to uranium. The structure-function relationships of both flagella and pili of these organisms warrant further study in view of their ability to function in conditions of extreme acidity.

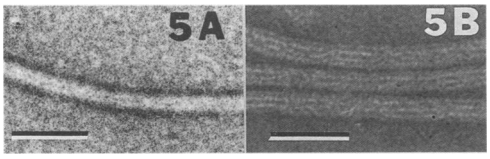

FIG. 5. A, Flagellum of negatively stained *T. ferrooxidans* TFI-35. Bar, 0.1 μm. B, Flagella of *T. ferrooxidans* TFI-10 showing light and dark staining regions. Bar, 0.1 μm.

Variations in physiological and genetic characteristics of the iron-oxidizing thiobacilli have been previously demonstrated (8, 12, 16, 17, 20). In this report, we have demonstrated that morphological variations of taxonomic importance also exist in different isolates of these bacteria. This supports the hypothesis that the iron-oxidizing thiobacilli may not belong to one distinct species, but may comprise a group of metabolically similar, but taxonomically distinct, bacteria.

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