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NOTES

Thermophilic Iron-Oxidizing Bacteria Found in Copper Leaching Dumps

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Rod-shaped bacteria capable of oxidizing ferrous iron at 55°C were cultured from samples of a copper mine leach dump. Yeast extract or cysteine was required by these *Thiobacillus*-like bacteria for growth, using ferrous iron as an energy source.

Thermophilic thiobacilli have been cultured from samples collected from thermal springs (5, 7, 8) and a simulated copper leaching system (3). It has been speculated that thermophilic sulfur- and iron-oxidizing thiobacilli may be present in the copper leaching operations in which mesophilic thiobacilli are found because there are regions in leach dumps in which temperatures as high as 60 to 80°C have been measured (1).

A limited number of samples have been collected from leach dump cores, leach solutions, blast hole material from pyritic ore bodies, and pyritic tailings from copper leaching operations in the southwestern United States. Growth of apparent iron-oxidizing thiobacilli occurred when these samples were incubated in acid ferrous sulfate medium at 45°C, but no growth was observed at 60°C incubation (C. L. Brierley, Crit. Rev. Microbiol., in press). Further studies, however, were not performed at intermediate temperatures between 45 and 60°C, and it was concluded that thermophilic thiobacilli were not present. However, the enrichment culturing of an iron-oxidizing, moderately thermophilic *Thiobacillus*-like microbe from a test leach system (3) using mine dump material and leach solution obtained from the Chino Mine dump of the Kennecott Copper Corp., Hurley, N. Mex., suggested that these microbes might be present in the mine dump. It is the purpose of this report to show that thermophilic iron-oxidizing bacteria are present in copper dump leaching operations.

Samples of mine waste material were collected from the surface of an actively leached area of the Chino Mine leach dump. This leaching operation has zones within the dump of at least 63°C (D. Reese, personal communication). Enrichment cultures of the samples were prepared in a nutrient solution (4) at pH 2.6 containing 36 mM $FeSO_4 \cdot 7H_2O$ and 0.02% (wt/vol) yeast extract, the latter added because other thermophilic *Thiobacillus*-like microbes require yeast extract for growth, using ferrous iron as an energy source (2). Incubation was in water baths at 50 and 60°C. Growth of gram-negative, rod-shaped microorganisms and iron oxidation, noted by formation of the red-yellow ochre of basic ferric sulfates and ferric hydroxide, occurred only in the medium incubated at 50°C. No growth or iron oxidation occurred at 50°C in the absence of yeast extract. To date, it has not been possible to culture these microbes on a solidified medium with either agar or silica gel and the ferrous iron-containing nutrient solution.

This physiological group of microbes (designated TH3) selected from a copper leach dump operation, capable of oxidizing ferrous iron at 50°C, has characteristics in common with the other thermophilic, iron-oxidizing bacteria (designated TH1) obtained from an Icelandic thermal spring (7) and those obtained from a test copper leach system (3) (designated TH2). All three strains require yeast extract, do not grow at 60°C, and are capable of using either ferrous iron or pyrite as a source of energy. During growth on pyrite (1 g/100 ml of medium) with 0.02% yeast extract, strain TH3 will decrease the pH from 2.4 to 1.9 during 4 days of incubation at 50°C. This indicates sulfide oxidation. Some differences in cell size occur among these strains of microbes (Table 1). TH3, from the mine dump, is similar in size to *Thiobacillus ferrooxidans* ATCC 19859. However, TH3 is observed to occasionally form very long chains of cells.

TABLE 1. *Comparative size of strains of thermophilic, iron-oxidizing bacteria and T. ferrooxidans*

Strain	Length (μm)[a]	Range of length (μm)	Width (μm)[a]
TH1	2.5	1.6–3.2	0.8
TH2	2.5	1.6–4.2	0.8
TH3	1.2	1.1–1.6	0.5
T. ferrooxidans	1.2	0.8–2.0	0.5

[a] Mean values from measurement of 30 separate cells stained with crystal violet.

The oxidation of ferrous iron by the thermophilic *Thiobacillus*-like strain TH3, supplemented with yeast extract, was monitored at several temperatures (Table 2). This experiment was carried out in 250-ml Erlenmeyer flasks containing 100 ml of acidic ferrous iron nutrient solution. Ferrous iron in the medium was measured by titration with 0.01 N ceric sulfate with 1,10-phenanthroline ferrous sulfate complex as the indicator. The upper temperature limit for growth and iron oxidation by the organism was observed to be 55°C. There is a broad range for growth on iron, with active oxidation occurring between 37 and 55°C. A similar observation was reported for the iron and pyrite oxidation capabilities of the Icelandic thermal spring strain TH1 (2).

The yeast extract requirement for growth of strain TH3 could be replaced by supplementing the medium with cysteine at 10 mg/100 ml (Table 3). Cysteine will also substitute for the yeast extract requirement for the strains TH1 and TH2 (Brierley, unpublished data). Glutathione has also been found to replace yeast extract for growth of strain TH1 (D. P. Kelly, personal communication). The basis of the need for this requirement is currently under investigation.

A similar thermophilic, iron-oxidizing microbe was reportedly discovered in Russia in locations where spontaneous ignition of a copper-zinc-pyrite deposit had occurred (R. S. Golovacheva and G. I. Karaviko, Abstr. Int. Symp. Microbial Growth on C_1-Compounds, Pushchino, U.S.S.R., p. 108–109, 1977). This microbe has a temperature optimum for activity at 50°C; it oxidizes ferrous iron, sulfur, pyrite, and other minerals in the presence of yeast extract and is capable of heterotrophic growth on glucose or sucrose "after a short period of adaptation." It was suggested by these authors that this is a new species, and they proposed the name *Thiobacillus thermosulfidooxidans*. The strains of thermophilic, iron-oxidizing bacteria from the thermal spring (strain TH1) and the metal leaching environments (strains TH2 and TH3) could be of the same type identified by the Russian investigators. These strains are clearly different from other thermophilic *Thiobacillus* species (5, 8) based on cell physiology and growth requirements. Additional study is not only desirable but necessary (e.g., DNA base composition, obtaining "pure" cultures) before establishing the taxonomic status of the thermophilic, iron-oxidizing *Thiobacillus*-like microbes. These studies are in progress and will be reported elsewhere.

The presence of thermophilic *Thiobacillus*-like microbes may not have been reported previously, because these organisms require an organic supplement (either yeast extract, cysteine, or glutathione) and do not grow above 55°C. This requirement prevents development of these microbes in purely inorganic enrichment cultures which have been used to search for thermophilic *Thiobacillus* species.

The mine waste leach dumps are extremely complex ecosystems containing *Thiobacillus* species, yeasts, flagellates, amoebae (6), and also thermophilic, iron-oxidizing, *Thiobacillus*-like microorganisms. The association among these microorganisms and the system which provides the thermophilic TH3 strains with their organic requirements has not been defined.

TABLE 2. *Effect of temperature on the oxidation of ferrous iron by the thermophilic Thiobacillus strain TH3 in the presence of 0.02% yeast extract*

Time (days)	Temp (°C)	Fe^{2+} (mM)		% Fe^{2+} biologically oxidized
		Inoculated	Control	
4	25	30.0	34.0	12
	37	17.5	33.5	48
	44	18.5	35.0	47
	55	17.5	28.5	39
	60	28.5	30.0	5
7	25	23.8	35.0	32
	37	3.0	33.5	91
	44	2.0	32.0	94
	55	13.0	27.0	52
	60	27.2	28.0	3

TABLE 3. *Oxidation of ferrous iron by thermophilic Thiobacillus TH3 in the presence of cysteine at 50°C*

Time (days)	Inoculated		Uninoculated	
	Fe^{2+} (mM)	% Fe^{2+} oxidized	Fe^{2+} (mM)	% Fe^{2+} oxidized
0	34	0	30.5	0
1	34.5	0	30.5	0
2	33.5	1.5	30.8	0
3	31.8	6.5	30.2	0.9
4	21.5	36.8	29.5	3.3
5	6.8	80.0	29.0	4.9

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