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Isolation of thermophilic iron-oxidising bacteria from sulfidic waste rock

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SUMMARY

A thermophilic, rod-shaped, iron-oxidising bacterium was isolated by enrichment culture of rock samples from an overburden dump at the Rum Jungle mine site in Australia's Northern Territory. Oxidation of ferrous iron and sulfur occurred at 50–55°C, with a temperature maximum of 60°C. The isolate required yeast extract for growth. The pH optimum for iron oxidation at 50°C was 1.4. Rapid iron-oxidation occurred at a pH as low as 0.35, but little or no oxidation occurred at or above pH 2.2.

INTRODUCTION

The majority of work in the field of microbial leaching of minerals has concentrated on mesophilic autotrophs such as *Thiobacillus ferrooxidans, T. thiooxidans* and, more recently, *Leptospirillum ferrooxidans*. However, there is now increasing interest in acidophilic, thermophilic iron- and sulfur-oxidising bacteria. Examples of these organisms have typically been isolated from thermal springs, especially those of the genus *Sulfolobus* [2,6,11,15]. A *Thiobacillus*-like thermophile (TH1) has also been isolated from hot springs [10], as have bacteria which oxidise sulfur at weakly acidic or neutral pH [18].

Thermophilic microorganisms in sulfidic ore deposits have not been widely studied. Brierley and Lockwood [4] isolated a *Thiobacillus*-like organism (TH2) from a simulated copper leach heap, Brierley [3] isolated TH3 from an active copper leaching operation, and Golovacheva and Karavaiko [7] isolated a new genus, *Sulfobacillus thermosulfidooxidans*, from hot zones in sulfidic ore deposits.

This paper reports the isolation of an iron- and sulfur-oxidising thermophile from an overburden dump at the Rum Jungle mine site in the Northern Territory, Australia. During operation of the mine, and since its closure in 1971, the leaching of sulfidic waste rock dumps has caused considerable sulfuric acid and heavy metal pollution of the water systems in the area. Previous work [8] has shown the pres-

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ence of a wide range of mesophilic autotrophs and heterotrophs within the dump. Extensive heat generation and the high temperatures previously attained within the dump [9] suggested a significant role for acidophilic, thermophilic microorganisms capable of oxidising iron and sulphur.

METHODS

During 1985–86, the dump underwent extensive earthmoving and rehabilitation, allowing in situ sampling of the dump in several locations to a depth of 10 meters. Samples taken in the field were stored at 4°C before analysis.

Enrichment was carried out in 9K medium [16], containing 4 g/l Fe^{2+}, at pH 2.0. The medium also included yeast extract at 0.02% (w/v), as other workers have shown a requirement for organic carbon and reduced sulfur compounds by thermophilic thiobacilli [3,11]. The medium for sulfur oxidation was that of Brierley et al. [5] at pH 3.5, and included 10 g/l precipitated sulfur (sterilised by gamma irradiation). Enrichment cultures were kept stationary, at 55°C, whereas later growth studies were at 50°C, using an orbital shaker at 180 rev/min. Inocula were prepared by harvesting cells at late growth/early stationary phase from medium at pH 2.0 containing ferrous sulfate. These cells were suspended in dilute sulfuric acid (pH 2.0) and stored at 4°C.

Growth in ferrous sulfate medium was monitored by the depletion of Fe^{2+}, as measured by the *o*-phenanthroline assay [17]. The rate of depletion of Fe^{2+} has been demonstrated to be a valid indicator of growth rate in similar organisms [11,12]. Therefore the growth rate of this organism (doubling time) was calculated indirectly as the rate of depletion of Fe^{2+}. Growth on elemental sulfur was followed as production of sulfuric acid, as indicated by pH measurement.

RESULTS AND DISCUSSION

Of 15 samples enriched in Fe^{2+} medium, 12 yielded iron-oxidising bacteria at 55°C. Sites for the 12 samples ranged from the surface layers of the dump through to 10 meters depth. Two morphologically distinct cell types were visible. Both were gram-negative motile rods, existing predominantly as single cells, although some doubles were visible, especially during active growth. The larger of the two cell types was approximately 2–2.5 μm long and 0.8 μm wide, whereas the smaller cells were approximately 1–1.5 μm long and 0.5 μm wide (similar in size to *T. ferrooxidans*). An example of this smaller cell type was selected for further investigation. This organism, designated J-8, was isolated from a depth of 1.8 meters, where the temperature of the dump was 35°C, and the pH was 2.75.

The optimum growth temperature of this organism is 50–55°C, and is similar to that for thermophiles isolated by other workers [4,7,10,13]. The maximum temperature for growth is approximately 60°C, at which growth proceeds slowly. The isolate was unable to grow appreciably in 9K medium without the addition of yeast extract. Under strictly autotrophic conditions, iron oxidation proceeded at a rate only marginally faster than chemical oxidation. Supplementation with trace elements failed to enhance growth in the presence or absence of yeast extract.

The pH range of this organism appears to be somewhat lower than that reported for related bacteria. *T. ferrooxidans* is able to grow on ferrous iron over a pH range 1.0–4.8, with an optimum between pH 2 and 3 [18]. The lower limit of pH 1.0 has been reported as the limiting value in pyrite oxidation by *T. ferrooxidans* [1]. Similar pH ranges have been recorded for thermophilic iron-oxidisers [14], although the upper limit is lower than that for *T. ferrooxidans*, with a published range of pH 1.0–4.0, and optima around pH 2–2.5. However, the organ-

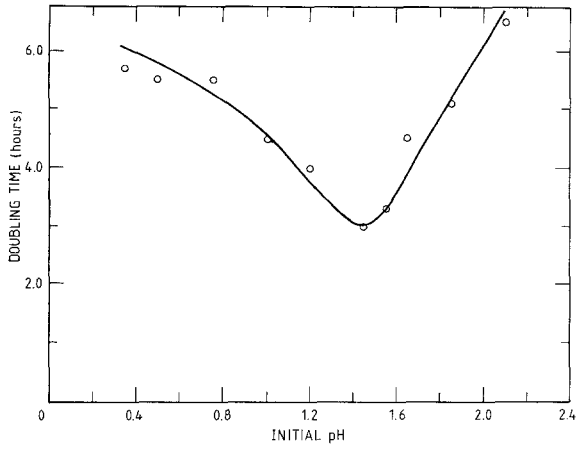

Fig. 1. Changes in minimum cell doubling time with medium pH.

ism reported here grew optimally at pH 1.4 at 50°C (Fig. 1), with little or no growth at or above pH 2.2. Growth occurred as low as pH 0.35. However, at this pH the maximum growth rate occurred after a prolonged lag phase (Fig. 2) and was short-lived.

Despite the increasing lag time with decreasing pH, the cells were able to adapt to the extreme acidity. The transfer of growing cells at pH 0.4 into fresh medium resulted in rapid growth after 30 h, with no ferrous iron remaining after approximately 90 h.

Above pH 2.2, the organism remained viable, and cell numbers remained constant, but no rapid oxidation of Fe^{2+} occurred. At pH as low as 0.25, very slow oxidation of Fe^{2+} took place after an extended lag phase. After 10 days, approximately 25% of the ferrous iron had been oxidised, but no significant oxidation occurred in the following days.

On completion of ferrous iron oxidation, final pH levels varied little from the initial values. Near the upper limit for growth (pH 2–2.2) the culture pH decreased somewhat with time, while at lower levels, pH generally remained within 0.1 unit of the initial value.

The isolate J-8 oxidised elemental sulfur for growth, with the production of sulfuric acid and the consequent drop in pH of the medium. Significant growth occurred when the initial pH was 2.4, reaching a value of 2.1 after 21 days. More rapid growth was evident when an initial pH of 3.4 was used, dropping to pH 2.2 after 21 days (Table 1). An increase in cell number, revealed by microscopic examination, was evidence of growth using sulfur as the energy source.

This is the first report of the isolation of this type of microorganism from Australian mine sites, although extensive work with mesophilic iron- and sulfur-oxidisers has been carried out on samples from the same source [8]. This strain appears to be similar to TH1 [10], TH2 [4] and TH3 [3]. However, the comparatively rapid growth rate as low as pH 0.35 (>0.4 M H^+) has not previously been reported. Similarly, the lack of growth on ferrous iron at pH $\geqslant 2.2$ was not anticipated.

The role of this microorganism within the waste rock dump is not clear. It appears that the extremely low pH required by the isolate for rapid Fe^{2+}

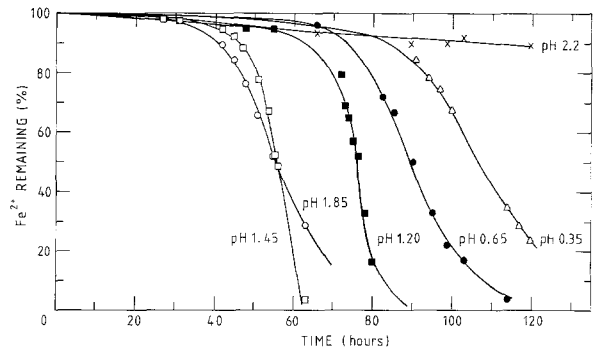

Fig. 2. Growth response of the thermophilic isolate over a range of initial medium pH.

Table 1

Oxidation of elemental sulfur by the thermophilic isolate at different initial pH values

Flasks were incubated at 50°C without shaking.

Days	pH	
At inoculation	2.40	3.40
4	2.30	3.25
15	2.15	2.45
21	2.10	2.20

oxidation precludes significant activity of this kind. However, it is possible that generation of microenvironments in the immediate vicinity of the bacteria may allow localised pH conditions that are more conducive to ferrous iron oxidation. This point is yet to be resolved. The organism does grow well on elemental sulphur at the pH generally found in the dump (between pH 2.5 and 3.5). This variability in pH requirement will be pursued further, particularly in relation to pyritic oxidation. Continuing effort is required to clarify the association of the thermophiles with moderate-temperature organisms, and any interaction in the oxidation of pyrite and sulfide ores within the dump. Increasing the knowledge of these organisms will contribute to a greater understanding of the complex oxidation processes involved in this and similar environmentally sensitive areas.

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