J. Indian Inst. Sci., July–Aug 1999, **79**, 287–293.

Molybdenum-resistant *Thiobacillus ferrooxidans*

C. D. VENKATAKRISHNAN AND T. S. CHANDRA*
Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Madras (IITM), Chennai 600 036, India.
email: chandra@acer.iitm.ernet.in

Abstract

Thiobacillus ferrooxidans TfK1, isolated from acid mine water of Kolar Gold Mines, was grown in the presence of sodium molybdate (2.6 mM Mo^{6+}). Its resistance to molybdenum (Mo^{6+}) is higher compared to all other *Thiobacillus* strains reported so far. *Thiobacillus ferrooxidans* TfCh1, for example, resisted only 2.0 mM Mo^{6+}. Mo adapted cells and unexposed cells did not show difference in resistance to other metals (153 mM Zn^{2+}; 170 mM Ni^{2+}). Copper sulphate (157 mM Cu^{2+}) was slightly inhibitory. Mo-adapted TfK1 oxidised iron (II) in 0.9-K medium in the presence of 0.1% and 1% of molybdenite concentrate from Jaduguda mines.

Keywords: *Thiobacillus ferrooxidans*, adaptation, molybdenum resistance, metal resistance, iron oxidation, bioleaching.

1. Introduction

Thiobacillus ferrooxidans, a Gram-negative mesophilic chemolithotrophic bacterium, oxidises iron(II) and various reduced forms of sulphur as a source of energy. *Thiobacilli* have been employed for bioleaching of a variety of ores like iron pyrite[1], chalcopyrite[2], arsenopyrite[3], etc. However, they could not be used for molybdenite leaching.[4] *T. ferrooxidans* is highly resistant to some heavy metal cations while relatively sensitive to some other metals. The most toxic metal was molybdenum (supplied as molybdate anion) which completely inhibited iron oxidation of concentrations above 0.0521-mM Mo^{6+}[4, 9]. Varying reasons have been given to explain the resistance of *Thiobacillus*[5,6] to molybdenum. Molybdenum is a strategic metal for a developing country like India and is imported in large quantities for application in steel, electrochemical and other industries. Hence it is important to evaluate whether molybdenum can also be obtained by bioleaching apart from chemical means.[4]

The objective of the present study is to compare and improve the resistance of two different strains of *Thiobacillus* towards molybdenum and to test the growth in low iron medium with added molybdenite concentrate.

2. Materials and methods

2.1. *Organisms*

Thiobacillus ferrooxidans strain TfK1 was isolated from acid mine water of Kolar Gold Mines by enrichment in 9 K medium.[7] Strain TfCh1 isolated from Chitradurga Mine water was a

*Author for correspondence.

kind gift from Prof. K. A. Natarajan, Indian Institute of Science, Bangalore. The cultures were maintained by repeated transfers in fresh 9 K medium every fortnight.

2.2. *Adaptation to molybdenum*

Erlenmeyer flasks containing 90 ml of 9 K medium and varying concentrations of sodium molybdate (1.00, 2.00 and 3.00 mM of Mo^{6+}) were inoculated with 10 ml of 36-h-grown culture of *Thiobacillus ferrooxidans*. The growth and resistance to Mo was monitored by determining the iron oxidation at 12-h intervals. Residual iron was estimated titrimetrically using 0.1N $K_2Cr_2O_7$ and diphenylamine as indicator.[8] When the organism showed a rate of iron oxidation equivalent to that of unexposed cells, serial transfers were made to the next higher concentration. The sequence of transfers is shown in Figs 1 and 2.

2.3. *Resistance to other metals*

100 ml of 9 K medium containing 153 mM Zn^{2+}, 170 mM Ni^{2+}, and 157 mM Cu^{2+} as $ZnSO_4.7H_2O$, $NiSO_4.6H_2O$, $CuSO_4.5H_2O$, respectively, was inoculated with unexposed and 2.0 mM Mo-adapted cells of TfK1. Growth was monitored by measuring iron oxidation.

2.4. *Growth in the presence of molybdenite concentrate*

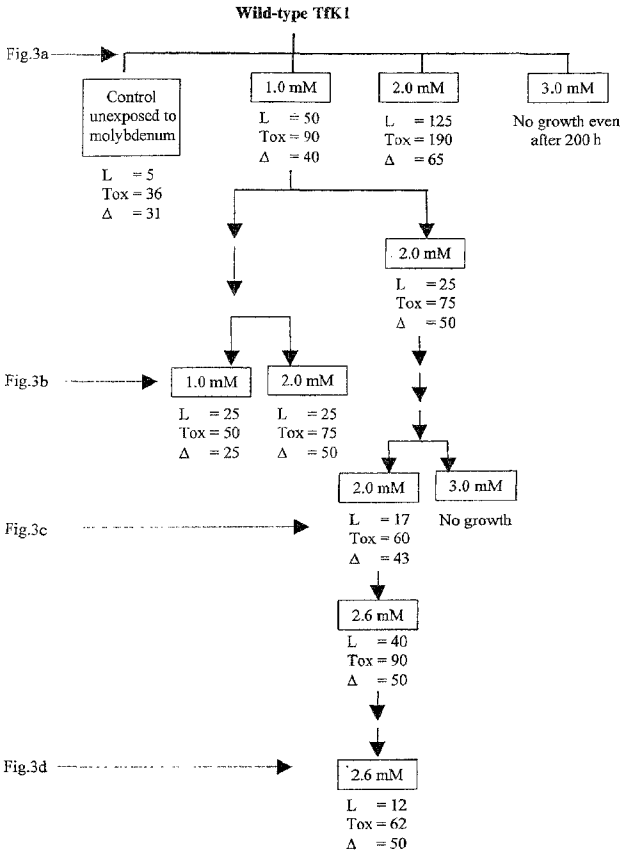
The molybdenite concentrate was procured from the Uranium Corporation India Limited (UCIL), Jaduguda, Bihar. The percentage of molybdenum, copper, nickel and iron in the concentrate was determined by ICP-AES (ARL 3410, Bruker). The concentrate was subjected to X-ray diffraction (Seifert) to know the form of molybdenum present. The average particle size was determined by laser diffraction particle size analyser (Malvern Instruments Master Particle Size analyser M5.4)

For growth studies, 0.1% and 1% (w/v) of the concentrate were added to 90 ml of 0.9 K medium and inoculated with 10 ml of 60-h-grown cells of 2.6 mM Mo-adapted cells of TfK1. Iron oxidation was followed once every two days.

3. Results and discussion

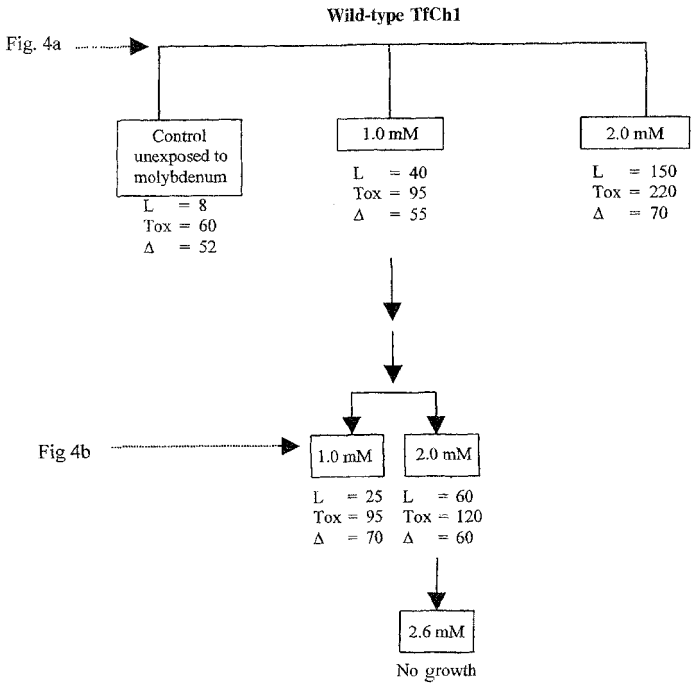
3.1. *Adaptation to molybdenum*

TfK1 was grown on different levels of molybdenum (Figs 1 and 3). In the absence of Mo^{6+}, TfK1 oxidised iron totally in 36 h. With increasing concentration of Mo^{6+} (Figs 1 and 3a), there was increase in lag period and finally no growth at 3.0 mM Mo^{6+}. Though repeated transfers of TfK1 on 1.0 mM (Fig. 1) reduced the total iron oxidation time, lag phase did not decrease from 25 to 5 h as in unexposed (Fig. 3). After four tranfers on 2.0 mM, the lag period reduced significantly to nearly 12–17 h (Figs 1 and 3). On three transfers on 2.6 mM Mo^{6+}, the lag phase reduced to 12 h, while the total oxidation time reduced to 62 h resembling 2.0 mM adapted cells (Figs 1 and 3). It failed to grow on 3.0 mM Mo^{6+} even on repeated transfers. This clearly indicates that 2.6 mM Mo^{6+} is the upper limit of molybdenum resistance for this strain.



L = Lag period (h); Tox = Total iron oxidation (95–98%) time taken (h); Δ = (Tox–L)h.
The growth patterns are represented in Fig. 3a-d at the transfer points indicated.
Number of arrows indicates the number of transfers made.
Concentration of Mo^{6+} in mM is shown in boxes.

FIG. 1. Flowchart for adaptation of *Thiobacillus ferrooxidans* TfK1 to sodium molybdate (Mo^{6+}).



L = Lag period (h); Tox = Total iron oxidation (95–98%) time taken (h); Δ = (Tox-L) h
The growth patterns are represented in Figs 4a and b at the transfer points indicated
Number of arrows indicates the numbers of transfers made.
Concentration of Mo^{6+} in mM is shown in boxes

FIG. 2. Flowchart for adaptation of *Thiobacillus ferrooxidans* TfCh1 to sodium molybdate (Mo^{6+}).

In contrast, the strain TfCh1 showed a slower growth pattern on 9 K medium without Mo^{6+} (Figs 2 and 4). The lag period on 1.0 or 2.0 mM did not reduce to level of unadapted cells even after repeated transfer. Moreover, its total iron oxidation time remained over 100 h compared to unexposed showing only 60 h. The strain failed to grow on 2.6 mM Mo^{6+} concentration.

Resistance to metal cations is based on their ability to exclude these from their internal structures. On this basis, two hypotheses have been proposed to explain the prolonged lag phase of iron oxidation caused by heavy metal cations.[9]

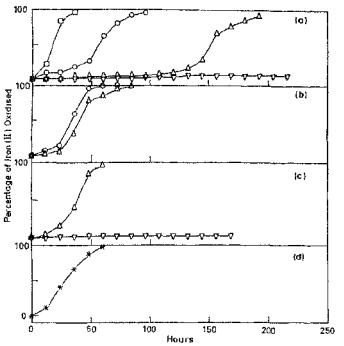


FIG. 3. Growth pattern of *Thiobacillus ferrooxidans* (TfK1) in the presence of varying concentrations of sodium molybdate (Mo^{6+}) in 9 K medium. □: No molybdenum (control), o: 1.00 mM Mo^{6+}, Δ: 2.00 mM Mo^{6+}, ∗: 2.6 mM Mo^{6+}, V: 3.0 mM Mo^{6+}.

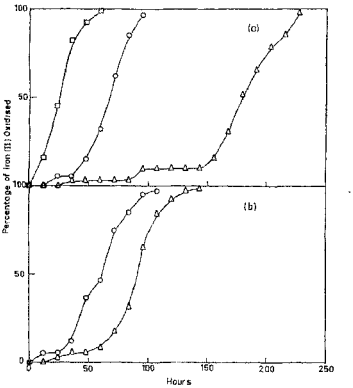


FIG 4. Growth pattern of TfCh1 in the presence of varying concentrations of sodium molybdate (Mo^{6+}) in 9 K medium □. No molybdenum (control), o 1.00 mM Mo^{6+}, Δ· 2.00 mM Mo^{6+}.

First, a long lag period may be needed during which the membrane-associated enzyme protection system develops, which enables the cells to oxidise ferrous iron for their energy. The second hypothesis favours selection of metal-resistant cells. Heavy metal resistance could be achieved therefore by adding a larger inoculum.[9]

For molybdenum resistance, a very early paper of Karavaiko *et al.*[6] favoured selection theory by showing the effect of inoculum in overcoming metal toxicity, possibly by the excretion of exo-metabolites which predominantly contain amino acids. Dicarboxylic amino acids and amino acids containing additional hydroxyl groups chelate the metal ion, thereby reducing the metal toxicity. Yong *et al.*[5] by extensive biochemical studies proved that membrane-bound cytochrome C oxidase was involved in Mo resistance in the soil isolate *Thiobacillus* Funis 2-1.

Yong *et al.*[5] screened nearly 75 strains of *Thiobacillus* and found one strain resistant to Mo^{6+} at a maximum level of 1.25 mM. The present observation that TfK1 resisted Mo^{6+} up to 2.6 mM indicates the unique feature of this strain and is the first report on such a high level of molybdenum resistance. This strain therefore is promising for more intense studies on the biochemical mechanism of molybdenum resistance. TfCh1 will serve as a good strain for comparative studies on these aspects in view of its lower resistance to Mo^{6+} up to 2.0 mM.

As Mo-resistance level of TfK1 appeared to be promising for molybdenite leaching, we undertook further investigation on its ability to resist multi-metals[10] likely to be present in the concentrate.

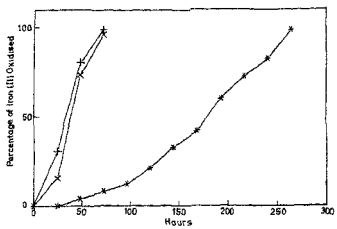


FIG. 5. Growth pattern of 2.0-mM Mo-adapted cells of TfK1 in the presence of nickel, zinc, copper in 9 K medium. +: 170 mM Ni^{2+}, x: 153 mM Zn^{2+}, *: 157 mM Cu^{2+}.

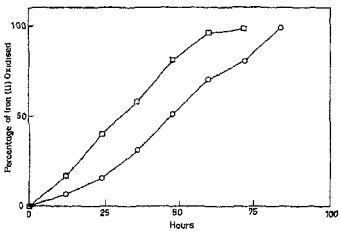


FIG. 6. Iron oxidation of 2.6 mM Mo-adapted cells of TfK1 in the presence of molybdenite concentrate in 0.9 K medium. □: 0.1% concentrate, o. 1.0% concentrate.

Molybdenite concentrate was found to have a composition of 38% Mo, 0.79% copper, 0.76% nickel and 10% iron. This analysis is more or less in agreement with the stated composition of the concentrate as given by UCIL. The concentrate was fine black in colour with the presence of a number of shiny crystals. X-ray diffraction studies showed the existence of molybdenum as MoS_2. The average particle size of the concentrate was 44 μm by laser particle diffraction analyser studies.

Based on the compositional analysis of the concentrate, 10% levels of 153 mM Zn^{2+}, 170 mM Ni^{2+}, and 157 mM Cu^{2+} were added to 9 K medium. Both unexposed and Mo-adapted cells (2.0 mM) of TfK1 oxidised iron similarly in the presence of nickel and zinc (Fig. 5), while copper was slightly inhibitory.

Initial studies were done on the rate of iron oxidation in the presence of 0.1 and 1.0% (w/v) levels of molybdenite concentrate in 0.9 K medium with 2.6 mM Mo-adapted cells of TfK1. These cells could oxidise iron completely although the total oxidation time taken was 80–90 h (Fig. 6). In contrast, the total iron oxidation in 9 K medium with unexposed cells takes place within 36 h (Fig. 3a). More investigations are underway to determine the status of Mo in solution, and on the uptake of molybdenum by the cells.

4. Conclusion

TfK1 showed resistance to 2.6 mM Mo^{6+} as sodium molybdate which is higher than any earlier reports with soluble molybdenum salt. TfCh1 could resist up to 2.0 mM Mo^{6+}, but showed slow iron oxidation rate compared to TfK1. TfK1 exhibited multi-metal resistance to 153 mM Zn^{2+}, 170 mM Ni^{2+}, and 157 mM Cu^{2+}.

These metal concentrations are higher than that of Jaduguda molybdenite concentrate which has 38% molybdenum, 0.79% copper, 0.76% nickel and 10% iron. Mo-adapted TfK1 could oxidise iron completely when grown in the presence of 0.1 and 1.0% of molybdenite concentrate in a 0.9 K medium.

Acknowledgement

We thank the Uranium Corporation of India Limited (UCIL), Jaduguda, Bihar, for supplying the molybdenite concentrate; RSIC, IITMadras, Chennai, for ICP-AES analysis of the concentrate; MSRC, IITMadras, Chennai, for XRD data of the concentrate; and K. Sankaranarayanane of Tata Refractories Limited for particle size analysis. We thank Mr. Digambara Patra for help in graphs. CDV thanks IITMadras, Chennai, for fellowship.

References

1. SILVERMAN, M. P. — Mechanism of bacterial pyrite oxidation, *J. Bact.*, 1967, **94**, 1046–1051.

2. SAKAGUCHI, H AND SILVER, M. — Microbiological leaching of a chalcopyrite concentrate by *Thiobacillus ferrooxidans*, *Biotechnol. Bioengng*, 1976, **18**, 1091–1101.

3. TUOVINEN, O H. *et al* — Oxidative dissolution of arsenopyrite by mesophilic and moderately thermophilic acidophiles, *Appl. Environ. Microbiol.*, 1994, **51**, 3268–3274

4. BHAPPU, R. B., REYNOLDS, D. H. AND ROMAN, R. J. — Molybdenum recovery from sulphide and oxide ores, *J. Metals*, 1965, **17**, 1199–1205.

5. YONG, K. N., OSHIMA, M., BLAKE, R. C II AND SUGIO, T. — Isolation and some properties of an iron-oxidising bacterium *Thiobacillus ferrooxidans* resistant to molybdenum ion, *Biosci. Biotech. Biochem.*, 1997, **61**, 1523–1526.

6. KARAVAIKO, G. I., PIVOVAROVA, T A. AND DZHANSUGUROVA, R. S. — *Thiobacillus ferrooxidans* resistance to molybdenum, *Biohydrometallurgy*, 1989, 461–473.

7. SILVERMAN, M. P. AND LUNDGREN, D G. — Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans* I. An improved medium and a harvesting procedure for securing high cell yields, *J. Bact.*, 1959, **77**, 642–647.

8. VOGEL, A. I. — *A textbook of quantitative inorganic analysis including elementary instrumental analysis*, Third Edition, ELBS, 1962, pp. 450–452.

9. TUOVINEN, O. H., NIEMELA, S. I. AND GYLLENBERG, H. G. — Tolerance of *Thiobacillus ferrooxidans* to some metals, *Antonie van Leeuwenhoek*, 1971, **37**, 489–496.

10. DAS, A., MODAK, J. M. AND NATARAJAN, K. A. — Studies on multi-metal tolerance of *Thiobacillus ferrooxidans*, *Miner. Engng*, 1997, **10**, 743–749.