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# Resistance of *Thermus* spp. to Potassium Tellurite

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**Two members of the genus *Thermus* were examined for their resistance to toxic inorganic compounds. They both proved to be fairly resistant to tellurite and selenite and to many other heavy metal salts. Cell extracts of *Thermus thermophilus* HB8 and of *T. flavus* AT-62 catalyze the reduction of $K_2TeO_3$ in a reaction which is dependent on NADH oxidation.**

Extreme thermophiles of the genus *Thermus* are defined as aerobic, nonmotile, pigmented, nonsporeforming, gram-negative rods which are able to grow at temperatures over 75°C (3, 4, 13, 14, 25). Genetic studies within this genus have been hampered because of the lack of an adequate system for gene transfer and expression in these microorganisms. Even when a transformation protocol for *Thermus* spp. was recently described (10), a suitable cloning vector was lacking. Plasmids isolated from different *Thermus* strains are potential candidates to be used as cloning vehicles. However, efforts to ascribe a phenotypic trait to these genetic elements have been unsuccessful to date (1, 2, 5, 8, 12, 22–24). We are interested in finding an easily selected gene or gene product which can be used as a marker in genetic experiments. In this sense, an attractive approach is to study the resistance of these cells to toxic inorganic compounds, as well as the biochemical basis of that resistance. In this communication, we report results of studies on the resistance of *Thermus thermophilus* HB8 (ATCC 27634) (13) and *T. flavus* AT-62 (ATCC 33923) (14) to several heavy metal salts and toxic ions.

Cells were grown in ATCC media (0.6% tryptone [Difco Laboratories], 0.4% yeast extract [Difco], 0.3% NaCl) supplemented with appropriate concentrations of the salts under study at 75°C for 18 to 24 h with vigorous agitation. When solid media were used, agar was added to a final concentration of 2%.

Both *Thermus* strains were resistant to many toxic ions. In addition, to the previously reported MICs of some metals (23, 24), we have also tested both bacterial strains for their resistance to other toxic compounds. MICs of $Ce^{2+}$, $Ni^{2+}$, $V^{5+}$, and $Mn^{2+}$ fell in the range of 0.1 to 0.5 mM, while those for $Cr^{3+}$ and $F^-$ were 2 to 10 mM. Concentrations higher than 10 mM for $AsO_4^{3-}$, $S_2O_3^{2-}$, $Li^+$, and $S^{2-}$ or 1 mM for $Fe^{2+}$ and $Cd^{2+}$ could not be tested because of problems with the solubility of these compounds in the culture media. The concentrations of some of the salts allowing growth of these bacteria were in the range of those present in some geysers of Yellowstone National Park, a natural habitat of thermophiles belonging to the genus *Thermus* (3). This fact would probably reflect some kind of evolutionary adaptation of these cells to those environments. All of the MICs which we determined were the same for both *Thermus* strains, irrespective of the presence of plasmids. Therefore, it was not possible to correlate these resistances with the presence of plasmids pTT8 (5) and pTF62 (23).

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During the course of these studies, we noticed that both *Thermus* strains were also resistant to oxyanions of tellurium and selenium in the form of potassium tellurite and sodium selenite, as are some strains of *Pseudomonas* spp. (16, 18), but *Escherichia coli* strains are not resistant (7). In general, these compounds are toxic for most microorganisms and animals (6, 9, 15, 16, 17). This toxicity has been exploited for many years as the basis of certain diagnostic tests in clinical microbiology. Many microbes produce black intracellular deposits when they are grown in solid or liquid media supplemented with tellurite (11, 16, 18–21). Tucker et al. (20, 21) have shown that the black precipitate is metallic tellurium.

When *T. thermophilus* HB8 and *T. flavus* AT-62 are grown in potassium tellurite-containing media, a strong garlic odor is produced, and the bacteria form black intracellular deposits, like some other organisms do (16, 18) (see Fig. 1). Indeed, the black deposit dissolves in bromine water, as reported previously for metallic tellurium (11). A similar behavior is observed when these cells are grown in the presence of sodium selenite, where they turn pinkish red, probably because of an intracellular deposit of elemental selenium (6). The study of the factor(s) responsible for tellurite reduction and the possible association of reduction with the resistance to this salt shown by these cells was particularly interesting to us. Since this phenotype is easily recognizable (Fig. 1) and could be used as a potential genetic marker, we decided to analyze it further.

The viable cell numbers determined for *T. thermophilus*

TABLE 1. Resistance of *T. thermophilus* HB8 and *T. flavus* AT-62 to potassium tellurite and sodium selenite

| Salt concn (M) | $10^4$ Viable cells per ml | | | |
|---|---|---|---|---|
| | *T. thermophilus* HB8 with: | | *T. flavus* AT-62 with: | |
| | $K_2TeO_3$ | $Na_2SeO_3$ | $K_2TeO_3$ | $Na_2SeO_3$ |
| 0 | 1,380 | 948 | 3,000 | 1,260 |
| $3 \times 10^{-7}$ | 1,420 | 950 | 2,800 | 1,300 |
| $1 \times 10^{-6}$ | 1,350 | 930 | 2,867 | 1,280 |
| $3 \times 10^{-6}$ | 1,320 | 962 | 2,890 | 1,252 |
| $1 \times 10^{-5}$ | 1,240 | 630 | 2,600 | 948 |
| $3 \times 10^{-5}$ | 1,190 | 460 | 1,867 | 382 |
| $1 \times 10^{-4}$ | 1,368 | 3 | 867 | 12 |
| $3 \times 10^{-4}$ | 900 | 1 | 667 | 1 |
| $1 \times 10^{-3}$ | 450 | 1 | 468 | 2 |
| $3 \times 10^{-3}$ | ND[a] | 0.8 | ND | 1 |

[a] ND, Not determined.

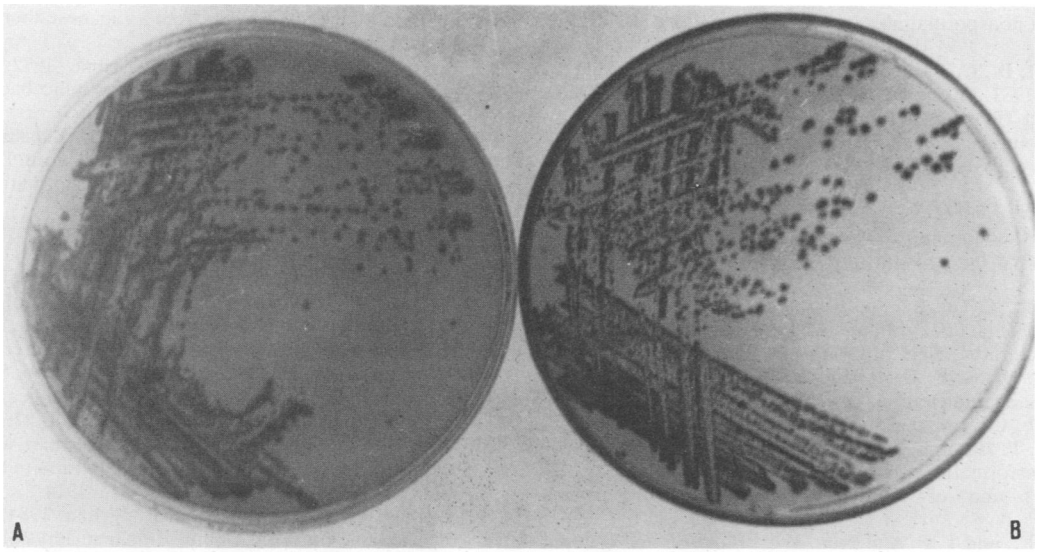


FIG. 1. In vivo reduction of potassium tellurite by *T. thermophilus* HB8. (A) Cells grown in ATCC medium. (B) Cells grown in the presence of 0.1 mM $K_2TeO_3$.

HB8 and *T. flavus* AT-62 grown at the indicated concentrations of tellurite and selenite are shown in Table 1. After growing the cells in the presence of the salts, appropriate dilutions were plated in ATCC media containing 2% agar without $K_2TeO_3$ or $Na_2SeO_3$ and incubated at 75°C for 18 h. From these data, it can be concluded that selenite is more toxic than tellurite for these bacteria. Although the mechanism of tellurite resistance is not well understood, it has been reported that in some genera, it is plasmid mediated (16–18). This is not the case for *Thermus* spp. however, since both cured and wild-type cells exhibited the same behavior and level of resistance towards this salt.

It seems to us that there is a relationship between the ability of these extreme thermophiles to reduce $K_2TeO_3$ and their resistance to this salt, since upon increasing the potassium tellurite concentration in the medium, its reduction is also enhanced. However, additional experimental evidence is required to determine whether the latter is the mechanism underlying tellurite resistance.

Terai et al. (19) reported that tellurite reduction also occurs in *Mycobacterium avium*, and they demonstrated that a protein fraction could reduce this salt. Since then however, no other report has appeared in the literature regarding the in vitro reduction of potassium tellurite. We have tested the ability of crude cell extracts (prepared by sonic disruption) of these *Thermus* strains to reduce $K_2TeO_3$. The results of experiments done with extracts from *T. thermophilus* which relate the reduction to a cellular factor are shown in Table 2. Assays were carried out at 75°C in a final volume of 250 μl and halted with an equal volume of a 2 M NaCl solution. One unit was defined as the amount of enzyme which caused an increase in $A_{500}$ of 0.001 U $min^{-1}ml^{-1}$. The system was absolutely dependent on the addition of exogenous NADH. This activity, which was dialyzable and destroyed by proteases, was also abolished by boiling or by detergent treatment. These observations suggest that this reaction is enzymatic and thermostable. Further biochemical and physicochemical characterization of this protein(s) is now in progress.

Taken together, these results suggest that the activity responsible for tellurite reduction (or resistance) could be a good marker for developing a cloning vehicle for *Thermus* spp. In addition to *T. thermophilus* HB8 and *T. flavus* AT-62, we have observed tellurite resistance in some *Thermus*-like laboratory-derived strains. This natural tolerance may be a generalized phenotypic property among *Thermus* spp. Since taxonomic criteria for these microbes are rather scarce, the ability to grow in the presence of (or to reduce) $K_2TeO_3$ could be used as an additional biochemical test to identify *Thermus* spp., as well as *Thermus*-like isolates.

TABLE 2. Tellurite-reducing activity of cell extracts of *T. thermophilus* HB8[a]

| Assay conditions[b] | Activity (U) |
|---|---|
| A | None |
| A + crude extract | <1 |
| B | <1 |
| B + crude extract | 320 ± 40 |
| B + crude extract (heat treated, 75°C for 15 min) | 310 ± 36 |
| B + crude extract (heat treated, 100°C for 15 s) | 94 ± 21 |
| B + crude extract (heat treated, 100°C for 2 min) | <1 |
| B + crude extract + 1% sodium dodecyl sulfate | <1 |

[a] Values are the arithmetic mean of seven independent determinations ± their standard deviations.

[b] Medium A contained 10 mM Tris hydrochloride (pH 7.5) and 0.1 mM $K_2TeO_3$; medium B contained the contents of A and 1 mM NADH.

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