Tellurite Resistance and Reduction by Obligately Aerobic Photosynthetic Bacteria

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Seven species of obligately aerobic photosynthetic bacteria of the genera *Erythromicrobium*, *Erythrobacter*, and *Roseococcus* demonstrated high-level resistance to tellurite and accumulation of metallic tellurium crystals. High-level resistance without tellurite reduction was observed for *Roseococcus thiosulfatophilus* and *Erythromicrobium ezovicum* grown with certain organic carbon sources, implying that tellurite reduction is not essential to confer tellurite resistance.

Tellurium compounds can be found in high concentrations in land and water near sites of waste discharge of industrial manufacturing processes (5). Potassium tellurite (K_2TeO_3) is toxic to many microorganisms at concentrations as low as 1 $\mu\text{g/ml}$ (13, 14, 19). Intrinsic low-level resistance to TeO_3^{2-} has been reported for a few gram-positive organisms (e.g., *Corynebacterium diphtheriae*, *Streptococcus faecalis*, and some *Staphylococcus aureus* strains), but little is known about the mechanisms responsible for this resistance (14). Resistance to tellurite of some gram-negative bacteria is associated with the presence of plasmids and has been studied extensively (2, 5, 13, 16, 18–20).

A constitutive high-level resistance (HLR) to rare earth oxides and oxyanions at concentrations approaching 1,000 $\mu\text{g/ml}$ was recently described for photosynthetic purple nonsulfur bacteria of the alpha subclass of *Proteobacteria* (9, 10). Obligate aerobic anoxygenic phototrophs, a new physiological group of the same subclass (4, 6, 12, 21, 28), produce bacteriochlorophyll *a* but are unable to grow photosynthetically under anaerobic conditions.

In this study we describe HLR to tellurite among freshwater

species of obligate aerobic phototrophs and their ability to reduce TeO_3^{2-} to metallic Te in very large amounts.

Seven species, *Erythrobacter litoralis* T4, *Roseococcus thiosulfatophilus* RB3, *Erythromicrobium ramosum* E5 (28), *Erythromicrobium sibiricum* RB16-17, *Erythromicrobium ursincola* KR99, *Erythromicrobium ezovicum* E1, and *Erythromicrobium hydrolyticum* E4(1) (23–27) (personal collection of Vladimir Yurkov), have been studied.

Figure 1 shows examples of growth of *Erythromicrobium ezovicum* (A), *Erythromicrobium ramosum* (B), *Erythrobacter litoralis* (C) and *Roseococcus thiosulfatophilus* (D) supplemented with different K_2TeO_3 concentrations in rich organic (RO) medium (6, 28). With the exception of the highly tellurite-sensitive *Erythromicrobium ezovicum*, for which no growth occurred at 5.0 μg of K_2TeO_3 per ml, six other species presented HLR (with MICs between 500 and 2,000 μg of K_2TeO_3 per ml) in RO medium or minimal salts (MS) medium containing yeast extract (3) (Table 1). However, these MICs depend upon the carbon source. This is summarized in Table 1 for the different species studied, grown in MS medium in the presence of trace elements (6), supplemented with different

TABLE 1. Reduction of K_2TeO_3 by different species of obligate aerobic phototrophs depending on organic carbon source

C source ^a	<i>Erythrobacter litoralis</i>			<i>Erythromicrobium hydrolyticum</i>			<i>Erythromicrobium ursincola</i>			<i>Erythromicrobium ramosum</i>			<i>Erythromicrobium sibiricum</i>			<i>Roseococcus thiosulfatophilus</i>			<i>Erythromicrobium ezovicum</i>		
	Grow- th ^b	Te concn ^c	MIC ^d	Grow- th	Te concn	MIC	Grow- th	Te concn	MIC	Grow- th	Te concn	MIC	Grow- th	Te concn	MIC	Grow- th	Te concn	MIC	Grow- th	Te concn	MIC
RO	+	500	1,200	+	500	1,200	+	500	1,000	+	50	750	+	50	750	+	100	500	+	NR	5
Yeast extract	+	250	1,200	+	500	2,000	+	500	2,000	+	250	1,500	+	500	1,200	+	250	1,000	+	NR	5
L-Glutamine	+	500	1,500	+	750	1,200	+	1,000	2,000	+	250	1,200	+	100	1,000	+	NR	1,200	+	1,000	2,000
Succinate	+	750	1,200	+	750	1,200	+	750	1,200	+	500	1,200	–	+	NR	1,200	+	750	2,000		
Malate	–			+	750	1,200	+	500	1,200	+	250	1,200	–	+	NR	1,200	+	500	1,200		
Tartrate	–			±	NR	5	–			±	100	500	–	+	NR	1,200	±	NR	5		
Acetate	+	1,000	1,200	+	1,000	2,500	+	250	2,700	+	1,000	2,300	+	500	1,200	+	NR	1,200	+	NR	500
Ethanol	±	250	750	+	100	250	–			+	250	1,000	–			–			+	NR	5

^a The bacteria were grown on RO medium, MS medium with yeast extract, or MS medium with the indicated organic carbon source.

^b Possibility to grow without tellurite addition. Symbols: +, good growth; ±, weak growth; –, substrate cannot be utilized.

^c Concentration of tellurite (in micrograms per milliliter) with the highest amount of reduction observed (the tellurite reduction has been estimated by the level of A_{950}).

^d In micrograms per milliliter. NR, not reduced.

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FIG. 1. Appearance of cultures of obligately aerobic photosynthetic bacteria growing in RO medium supplemented with K_2TeO_3 . (A) *Erythromicrobium ezovicum*; (B) *Erythromicrobium ramosum*; (C) *Erythrobacter litoralis*; (D) *Roseococcus thiosulfatophilus*. The amounts of K_2TeO_3 (in micrograms per milliliter) in the flasks are as follows: no tellurite addition (flasks 1), 1,000 (cell-free control) (flasks 2), 50 (flasks 3), 100 (flasks 4), 500 (flasks 5), 750 (flasks 6), and 1,000 (flasks 7). In control experiments with 1,000 $\mu\text{g/ml}$ K_2TeO_3 added to growth medium in the absence of cells (flasks 2), no darkening was observed, indicating that tellurite reduction was caused by bacterial activities and not by the direct chemical reaction with the compounds in the medium.

carbon sources (1 g/liter). The highest level of resistance to tellurite (MICs between 2,300 and 2,700 μg of K_2TeO_3 per ml) was observed for cells of *Erythromicrobium hydrolyticum*, *Erythromicrobium ursincola*, or *Erythromicrobium ramosum* grown in the presence of acetate. This is 2 to 3 times higher than the highest MICs of tellurite (800 and 1,200 $\mu\text{g/ml}$) described by Moore and Kaplan for *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* (9, 10).

In most cases, a black coloration appears during the growth in the presence of tellurite (Fig. 1). This is due to the reduction of tellurite in metallic tellurium with its intracellular accumulation (8, 9, 17). Intracellular deposits appear as electron-dense

crystals in electron microscopy (Fig. 2) and have been shown to consist of elemental tellurium in several strains of bacteria (1, 7–10, 15, 17). Excluding *Roseococcus thiosulfatophilus* grown in the presence of L-glutamine, succinate, malate, tartrate, or acetate, and *Erythromicrobium ezovicum* grown with acetate, all species examined in the present work were able to reduce TeO_3^{2-} to elemental Te in combination with HLR to this compound (Table 1 and Fig. 1). Most of the tellurite was biotransformed to metallic Te in 24 h, with the exception of *Roseococcus thiosulfatophilus* for which the tellurite reduction occurred only after 72 to 96 h of growth. The amount of reduced tellurite depended upon the species and the carbon source (Table 1). Transmission electron microscopy pictures, obtained as previously described (11) show very abundant and large black crystals which sometimes occupied about 20 to 30% of the cell volume (see, as an example, the case of *Erythromicrobium ursincola* in Fig. 2A). The size and total amount of the Te crystals in the species studied in this study are usually greater than those observed in *Escherichia coli*, or *Pseudomonas* or *Rhodobacter* species. The only exception was *Roseococcus thiosulfatophilus*, which formed and accumulated relatively small deposits (Fig. 2B).

The strong oxidant property of tellurite confers its toxic character for microorganisms (13). In most cases analyzed in this study, HLR to tellurite is correlated to its reduction into Te. However, HLR without tellurite reduction was observed for *Roseococcus thiosulfatophilus* grown with L-glutamine, succinate, malate, tartrate, or acetate, and *Erythromicrobium ezovicum* grown with acetate as the organic carbon source. These results imply that tellurite reduction is not essential to confer tellurite resistance and some other mechanisms, such as continuous tellurite efflux or tellurite complexing or methylation could play an important role in the resistance character.

Previous work has demonstrated that the extent of tellurite reduction in *Rhodobacter sphaeroides* was inversely related to the oxidation state of the carbon source present in the growth medium (9, 10). In addition to its detoxification effect, reduction of metal oxide could be a way to dispose of excess electrons by the reoxidation of NADH, $FADH_2$, or quinones and, therefore, for maintenance of an optimal redox poise in vivo (9, 10). A clear correlation between the tellurite reduction and the oxidation state of the carbon source is not evident in the case of obligate aerobic photosynthetic bacteria (Table 1) with the exception of *Roseococcus thiosulfatophilus*. Indeed this species can reduce $Te(IV)$ to $Te(0)$ in MS medium with yeast extract and RO medium but not in MS medium containing L-glutamine, succinate, malate, tartrate, or acetate as the sole organic carbon source. These results are consistent with the idea that during growth in RO medium *Roseococcus thiosulfatophilus* cells presented with an excess of reducing power, and electron carriers could be oxidized by giving electrons to tellurite, thus equilibrating the redox poise in vivo.

Industrial activity has an enormous influence on the geochemical migration processes of some toxic heavy metals by their dispersion in water, soil, and atmosphere, or their concentration in specific areas. These processes contribute to serious pollution problems (22). The reduction of soluble $Te(IV)$ to solid $Te(0)$ could be an important mechanism for the removal of this element from polluted places. In this context, the

FIG. 2. Intracellular localization of accumulated tellurium (A) *Erythromicrobium ursincola*; (B) *Roseococcus thiosulfatophilus*. Bar in panel A, 100 nm; bar in panel B, 200 nm.

development of microbiological methods for environmental cleaning systems for tellurium oxides is of interest. Obligate aerobic photosynthetic bacteria, being able to transform Te(IV) to Te(0) in very high concentrations, are promising candidates for such a process.

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