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Cloning, purification and characterization of *Geobacillus stearothermophilus* V uroporphyrinogen-III C-methyltransferase: evaluation of its role in resistance to potassium tellurite in *Escherichia coli*

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Abstract

The *Geobacillus stearothermophilus* V *cobA* gene encoding uroporphyrinogen-III C-methyltransferase (also referred to as SUMT) was cloned into *Escherichia coli* and the recombinant enzyme was overexpressed and purified to homogeneity. The enzyme binds S-adenosyl-L-methionine and catalyzes the production of III methyl uroporphyrinogen in vitro. *E. coli* cells expressing the *G. stearothermophilus* V *cobA* gene exhibited increased resistance to potassium tellurite and potassium tellurate. Site-directed mutagenesis of *cobA* abolished tellurite resistance of the mesophilic, heterologous host and SUMT activity in vitro. No methylated, volatile derivatives of tellurium were found in the headspace of tellurite-exposed *cobA*-expressing *E. coli*, suggesting that the role of SUMT methyltransferase in tellurite(ate) detoxification is not related to tellurium volatilization.

Keywords: cobA; Tellurite; Uroporphyrinogen; SUMT; S-adenosyl-L-methionine; Methyltransferase

1. Introduction

Tellurium (Te), a scarce element in the earth crust, is not an essential element for living organisms. Te belongs to Group 16 (VIA) of the periodic table and shares chemical properties with biologically important elements such as oxygen, sulfur and selenium. It is rarely found in the non-toxic, elemental state (Te^0), and soluble oxyanions, tellurite (TeO_3^{2-}) and tellurate (TeO_4^{2-}), are toxic for most forms of life [35].

Te was considered as a somewhat exotic element until its intensive use in the chemical industry converted it into an important environmental pollutant [24]. The geochemical cycle of tellurium has not yet been elucidated, and hence the role of microorganisms, if any, is not fully understood.

Tellurite has proven to be highly toxic for most microorganisms at concentrations as low as 1 μg/ml [35], although some bacteria exhibit natural resistance to K_2TeO_3 [39]. This tellurite toxicity has been extensively exploited as a selective agent in diverse microbiological culture media.

A few bacterial tellurite resistance mechanisms have been proposed [17,39,50]. While the genetic, biochemical and/or physiological bases underlying TeO_3^{2-} resistance are still

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poorly understood, in recent years there has been some progress in understanding the reasons for tellurite toxicity [5,6,18,22,23,28,42,46,47,51].

One of our strategies when studying bacterial resistance to TeO_3^{2-} has been the characterization of genes from the tellurite-resistant, thermotolerant, Gram-positive rod *Geobacillus stearothermophilus* V [48] that confers tellurite resistance when expressed in *Escherichia coli*. Using this experimental approach we have previously shown that *cysK* [49], *iscS* [38] and *ubiE* [2] genes of this bacterium mediate tellurite resistance in *E. coli*. In this work, we show that expression of a fourth gene of *G. stearothermophilus* V, the *cobA* gene, encoding uroporphyrinogen-III C-methyltransferase (also referred to as SUMT), confers resistance upon tellurite and tellurate in *E. coli*.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

E. coli JM109 and JM109(DE3) were grown in LB medium at 37 °C with shaking. Growth in the presence of defined tellurite concentrations used appropriate volumes of sterile stock solutions of K_2TeO_3 that were amended to culture media. When required, isopropyl thio-β-D-galactopyranoside (IPTG, 1 mM), ampicillin (100 μg/ml), tetracycline (10 μg/ml), kanamycin (40 μg/ml) or chloramphenicol (50 μg/ml) was added to the medium. The specifications and characteristics of plasmids utilized in this work are shown in Table 1.

The minimal inhibitory concentration (MIC) of K_2TeO_3 or K_2TeO_4 was determined in liquid cultures (2 ml) as described previously [9]. Optical density was read after 24 h of tellurite(ate) exposure at 37 °C with shaking. All tests were carried out at least in triplicate.

2.2. Molecular biology procedures

Standard molecular biology procedures such as plasmid and chromosomal DNA purification, restriction assays and DNA ligation, were carried out as described by Sambrook et al. [30].

PCR reactions consisted of DNA template (4 ng), 200 μM dNTPs, primers (1 μM each), PCR buffer and 2.5 U of Taq polymerase (Perkin–Elmer, USA) in a final volume of 100 μl. Following an initial denaturation step (94 °C for 5 min), the amplification reaction consisted of 30 cycles (94 °C for 1 min, 37 °C for 1 min, 72 °C for 1 min) followed by a final extension at 72 °C for 10 min to ensure complete amplification.

The oligonucleotides used in this work were: P1 (*cobA*, direct) GGAATTC**CATATG**ACAAATGGAAAAGTATATATCGTCG, P2 (*cobA*, reverse) ACCC**AAGCTT**TTATGTGGTGGCAACCGTCT-3′; P3 (*cobA*−60 nt, reverse) ACCC**AAGCTT**ATCTGGCCATTTTATTGATCCTGTAT, P4 (*cobA*−120 nt, reverse) ACCC**AAGCTT**ATTCTATGATAATATGGTTTTCTTCCAAA, P5 (*cobA*−180 nt, reverse) ACCC**AAGCTT**ATACATTAAAAGGAATGATGGCCAGTC, P6 (*cobA*G12A, direct) GGAATTC**CATATG**ACAAATGGAAAAGTATATATCGGTGCCGCTCCCGGCGATG, and P7 (*cobA* + promoter, direct) ACCC**AAGCT**TAAAACCGGGAATGGCATTCAC. Restriction sites are shown in bold. The codon representing the $G^{12}A$ substitution in SUMT is underlined.

2.3. Cloning, overexpression and purification of wild-type and mutant SUMTs

Plasmid p1VH [2] was used as a template to amplify the *cobA* gene by PCR using P1, P2 and P7 primers (see above). *Nde*I and *Hin*dIII sequences at their 5′ and 3′ ends, respectively, were used to clone the amplified fragments into pET21b(+) expression vector. Deletions of 60, 120 and 180 bp from the 3′ end of the *G. stearothermophilus* V *cobA* gene were obtained by PCR using P1/P3, P1/P4 and P1/P5 primers generating truncated SUMT proteins M1-R239, M1-D219 and M1-I199, respectively (Fig. 2). Similarly, a *cobA* gene encoding SUMT with a $G^{12}A$ amino acid change was generated by PCR using P6 and P2 primers. All mutations and plasmid constructs were checked by DNA sequencing.

Overexpression and purification of wild-type SUMT was carried out as described earlier for IscS desulfurase [38]. *E. coli* JM109(DE3) cells carrying the *G. stearothermophilus* V *cobA* gene were grown in LB-ampicillin medium (1 l) to an OD ∼0.6 and IPTG (1 mM) was added. Cells were induced for 5 h, sedimented at 4500 × *g* for 10 min at 4 °C and washed with buffer A (10 mM Tris–HCl pH 7.5, 1 mM EDTA, 0.1 mM NaCl). After suspending in the same buffer (5 ml/g cells, wet weight) cells were subjected to sonic

Table 1
Plasmids used in this study.

Plasmid	Relevant data	Source or reference
pSP72	Cloning vector	Promega
p1VH	Derivative of pSP72 carrying a 3824 bp of *G. stearothermophilus* V chromosomal DNA	[2]
pET21b(+)	Expression vector Amp^R	Novagen
pBluescript SK^-	Cloning vector	Stratagene
p*cobA*	*cobA* gene of *G. stearothermophilus* V cloned into the *Nde*I/*Hin*dIII sites of pET21b(+)	This work
p*cobAr*	*cobA* gene of *G. stearothermophilus* V along with its own promoter cloned into the *Eco*RI site of pBluescript SK^-	This work
p*cobA*(Δ60)	*cobA* gene of *G. stearothermophilus* V lacking 60 bp from its 3′ end cloned into the *Nde*I/*Hin*dIII sites of pET21b(+)	This work
p*cobA*(Δ120)	*cobA* gene of *G. stearothermophilus* V lacking 120 bp from its 3′ end cloned into the *Nde*I/*Hin*dIII sites of pET21b(+)	This work
p*cobA*(Δ180)	*cobA* gene of *G. stearothermophilus* V lacking 180 bp from its 3′ end cloned into the *Nde*I/*Hin*dIII sites of pET21b(+)	This work
p*cobA*$G^{12}A$	*cobA* gene of *G. stearothermophilus* V with a point mutation ($G^{12}A$) cloned into the *Nde*I/*Hin*dIII sites of pET21b(+)	This work