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DNA sequence analysis of the tellurite-resistance determinant from clinical strain of *Escherichia Coli* and identification of essential genes

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Abstract

The tellurite-resistant *Escherichia coli* strain KL53 was found during testing of the group of clinical isolates for antibiotics and heavy metal ion resistance (Burian *et al.* 1990). Determinant of the tellurite resistance of the strain was located on the large conjugative plasmid pTE53 and cloned into pACYC184. Three different Te^r clones harboring pLK2, pLK18 and pLK20 were isolated (Burian *et al.* 1998). The smallest functional Te^r clone harboring pLK18 was chosen for further analysis. Plasmid pLK18 have been subcloned to obtain convenient DNA fragments for sequencing of tellurite-resistance determinant. Sequencing of this DNA fragments provided complete DNA sequence of the determinant, 5250 bp in size. The sequence has been compared with nucleotide and protein databank (BLAST programs) and significant homology with the three known operons coding for tellurite resistance has been found (determinat on plasmid pR478 from *Serratia marcescens*, on plasmid pMER610 from *Alcaligenes* sp. and chromosomal tellurite resistance genes from *Proteus mirabilis*). We identified 5 ORFs coding for 5 genes named *terB* to *terF*. The clone harboring pLK18 was subjected to the transposition with Tn1737Km to disrupt determinant of the tellurite resistance. Plasmid DNA of several clones containing pLK18 with Tn1737Km was isolated to locate the target site of Tn1737Km. Analyses showed, the genes *terB*, *terC*, *terD* and *terE* are essential for conservation of the resistance whereas the gene *terF* is not important in this respect.

Introduction

The toxicity of potassium tellurite to microorganisms, particularly Gram-negative bacteria, is well established. However, some of the Gram-positive bacteria are naturally resistant against tellurite anions (Hoeprich *et al.* 1960; Jacoby 1979; Silver *et al.* 1981). Resistance to tellurite is mostly conferred by highly specific genetic determinants (Walter & Taylor 1992). A number of plasmid-associated tellurite anion resistance determinants have been studied in detail by now. In general, they can be divided into 5 basic classes on the basis of DNA homology and/or hybridization experiments and knowledge of their mechanism of resistance (Turner *et al.* 1995). One class includes the Te^r determinants from the plasmid incompatibility groups HI2, HI3 and HII. A second class includes the cryptic Te^r within the IncPα plasmids. A third class includes the oxyanion efflux pump of the *arsRDABC* of the IncFI plasmid. The fourth class includes chromosomally encoded Te^r. The final group includes the chromosomal genes *tehAtehB* from *Escherichia coli* that requires overexpression to mediate resistance towards tellurite (Taylor *et al.* 1994). In spite of the fact that several tellurite-resistance determinants have been characterized, little is known about the mechanisms responsible for this resistance.

The clinical isolate of *E. coli* strain KL53 was discovered to be resistant against potassium tellurite (Burian *et al.* 1990). The strain formed typical black colonies on solid LB medium with tellurite. The tellurite-resistance determinant of the strain was located on conjugable plasmid pTE53. The strain KL53 has MIC (minimal inhibition concentration) to tellurite ions on solid media 150 μg ml^{-1}. A gradual increase in tellurite concentration resulted in increased resis-

tance with the MIC value of 1500 μg ml^{-1}. The corresponding value for the wild type of *E. coli* on solid media is 0.125 μg ml^{-1}. The potassium tellurite resistance determinant of plasmid pTE53 was cloned into high-copy-number and medium-copy-number plasmids. Cloning into high copy plasmid was generally not successful. Recombinant Te^{r} clones were obtained only as a result of cloning into medium-copy-number vector pACYC184 (Burian *et al.* 1998). The MIC's for these clones were comparable to the parent strain KL53.

In this paper, we have provided the DNA sequence analysis of the novel tellurite-resistance determinant from the *Escherichia coli* strain KL53 discovered by Burian *et al.* (1990) as well as identification of essential genes of the determinant.

Materials and methods

Bacterial strains and plasmids

The strains of *E. coli* and plasmids used are listed in Table 1.

Preparing partial DNA fragments for sequencing

Determinant of tellurite resistance from *E. coli* strain KL53 was cloned into plasmid pACYC184 (Burian *et al.* 1998). Plasmid carrying tellurite-resistance determinant, named pLK18, was subcloned subsequently to obtain convenient DNA fragments for sequencing of tellurite-resistance determinant. The plasmid was digested separately with enzymes EcoRI, SmaI, SalI, HindIII, EcoRV (Amersham) and simultaneously with SalI/EcoRV and EcoRI/HindIII. Partial DNA segments of tellurite-resistance determinant obtained after digestion of pLK18 have been cloned into pTZ18R and pBSKS^{+} vectors and transformed into strains XL1-Blue and JM109.

DNA sequencing

DNA samples were sequenced on Vistra DNA sequenator (Amersham) using standard reverse and forward M13 primers. Consensus sequence was obtained using DNA assembly program Gap4 from Staden Package software (Staden 1996).

Homology search and coding regions identification

5250 bp long sequence from pTE53 (submited to EMBL, ID ECO238043) was searched against nucleotide databank at NCBI by BLAST program (Altschul 1990). Coding regions were identified by DNA analysis program nip4 from Staden Package software (Staden 1996) as well as by comparison with homologous sequences coding for tellurite resistance.

Transposition of the transposon Tn1737Km

Plasmid pRU887 carrying Tn1737Km was transformed into strain MC4100 with plasmid pLK18. Transformants were selected on LB plates containing chloramphenicol (Cm) 30 μg ml^{-1} and kanamycin (Km) 25 μg ml^{-1}. Single transformants growing on plates with Cm and Km were inoculated into LB media with Cm and Km and subsequently cultivated overnight at 37 °C. Overnight culture was plated on LB plates containing Cm and Km and incubated overnight at 42 °C. Due to the thermosensitive replication of pRU887, the growth of bacteria at 42 °C on LB plates with Km is conditioned by tranposition of Tn1737Km into either chromosome or plasmid pLK18. All colonies obtained so far were harvested and subjected to the total plasmid DNA isolation.

A total plasmid DNA isolated after transposition of Tn1737Km into pLK18 was used to transform *E. coli* MC4100 strain. Transformants were selected on LB plates with Cm and Km. The plasmids from single transformants were isolated and compared electrophoretically (using 1% agarose gel and TAE buffer) with plasmid pLK18 as a standard.

Determination of tellurite resistance of the clones MC4100 (pLK18::Tn1737Km)

Tellurite resistance of the clones MC4100 (pLK18::Tn 1737Km) was determined on a solid medium with potassium tellurite 12.7 μg ml^{-1}. Following overnight incubation at 37 °C, the bacterial growth was inspected visually.

Localization of the target site for Tn1737Km insertion

Selected clones of the *E. coli* MC4100 (pLK18:: Tn1737Km) sharing the potential to grow on plates with potassium tellurite together with several additional ones lacking tellurite resistance were picked up to isolate their plasmid DNA. Plasmid DNAs were subjected to the digestion with EcoRI, SalI, HindIII

Table 1. Bacterial strains and plasmids used in this study.

Strain, plasmid	Genotype	Source/reference
Strains		
MC4100	F$^-$ *ara*D139 Δ(*lac*IPOZIA) U169*str*A	Stratagene
XL1 Blue	*end*A1 *hsd*R17 (m_k^-, r_k^+) *sup*E44 *thi*-1 *rec*A1 *gyr*A96 *rel*A1 *lac*$^-$ [F′ *tra*D36 *pro*AB *lac*I^{q}ZΔM15 Tn10(Tc^r)]	Stratagene
JM109	el4$^-$ (*mcrA*) *rec*A1 *end*A1 gyrA96 *thi*-1 *hsd*R17 (r_k^-, m_k^+) *supE*44 *rel*A1 Δ(*lac-pro*AB) [F′ *traD*36 *pro*AB *lac*I^{q}ZΔM15]	Stratagene
Plasmids		
pRU887	pME305::Tn1737Km [*lac*Z]^c Km^{r}Ap^r Tc^r t*rf*A *ts tra*$^+$ IncP	Ubben and Schmitt, 1987
pLK18	*Te^r Cm^r	Burian *et al.*, 1998
pBSKS$^+$	Ap^r	Stratagene
pTZ18R	Ap^r	Pharmacia

*Te^r – tellurite resistance

and EcoRV restriction enzymes. Digestion products were separated electrophoretically using 1% agarose gel and TAE buffer.

Results and discussion

In vitro cloning of the large conjugative plasmid pTE53 from *E. coli* strain KL53 resulted in obtaining of 5 kb fragment with fully functional tellurite resistance. This was also the minimal functional DNA fragment obtained so far (Burian *et al.* 1998). Sequencing of this DNA insert provided complete DNA sequence of the determinant, 5250 bp in size. We identified 5 ORFs coding for 5 genes which have been named *terB* to *terF*. The first gene, *terB*, codes for 151 amino acids (aa) long protein with MW of 17 kDa. The *terC* gene codes for 346 aa long protein with MW 38 kDa. We identified 9 regions with a high hydrophobicity in TerC protein, the putative membrane-spanning domains. Proteins coded by genes *terD* (192 aa, MW 20 kDa) and *terE* (191 aa, MW 20 kDa) show a high level of sequence homology (65% identity, 77% similarity). The gene *terF* codes for 413 aa protein with MW of 45 kDa. We also observed a relatively high level of homology (14% identity, 20% similarity) between TerF protein and TerD/TerE proteins.

The BLAST search of 5250 bp fragment from pTE53 against nucleotide database showed significant homology with 3 known operons coding for tellurite resistance genes. The first is carried by the plasmid pR478 from *Serratia marcescens* (EMBL ID P47TERZ, Whelan 1997). The next is from *Alcaligenes* sp. plasmid pMJ606 (EMBL ID PLMTEAD, Jobling & Ritchie 1988). The third is a chromosomal tellurite resistance operon from *Proteus mirabilis* (EMBL ID AF168355, Toptchieva *et al.* 1999). The overall alignment of 4 homologous tellurite resistance operons is shown in Figure 1. Only four of the seven genes are present in all the four operons. They are *terB*, *terC*, *terD* and *terE*, respectively, whose role in tellurite resistance mechanism is probably essential. Since TerD and TerE proteins are very similar in primary structure, we suppose that only three functionally separated protein products are involved in tellurite resistance mechanism, the first being coded by the *terB* gene, the second by the *terC* gene and the third being coded by both *terD* and *terE* genes. This theory is also supported by our transpozon – disruption assay (described below). Table 2 shows sequences homology between proteins TerB-TerF from pTE53 and corresponding proteins from pR478, pMER610 and chr. DNA from *P. mirabilis*.

Sequence homology search of TerB protein revealed no homology with any other proteins. On the contrary, the *terC* gene showed significant homology with several membrane proteins (data not shown). In addition, the hydrophobicity analysis indicates that the gene most likely codes for the integral membrane protein. The TerC protein may play one of the key roles in tellurite resistance mechanism. Turner *et al.* (1995) showed that tellurite resistance determinant from pR478 (homologous with pTE53 determinant)

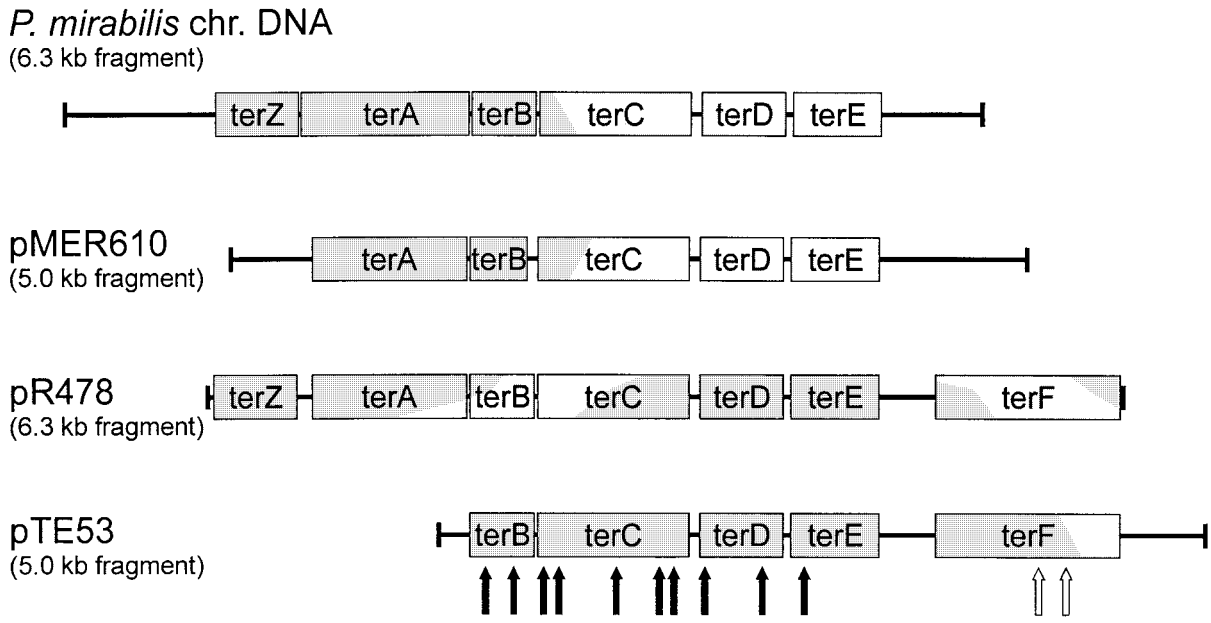

Figure 1. Overal alignment of four homologous operons coding tellurite resistance. Chromosomal tellurite resistance operon from *Proteus mirabilis* (Toptchieva *et al.* 1999), plasmid pMJ606 from *Alcaligenes* sp. (Jobling & Ritchie 1988), plasmid pR478 from *Serratia marcescens* (Whelan 1997) and plasmid pTE53 from *E. coli* (this paper). Arrows indicate position of Tn1737Km inserted into pTE53 operon (black arrows represent essential and white arrows non-essential positions, see text for details).

Table 2. Homologies of TerB-TerF proteins from pTE53 with their counterparts found in pR478 (*Serratia marcescens*), pMER610 (*Alcaligenes* sp.) and chromosomal DNA form *Proteus mirabilis*.

Source protein	pR478	pMER610 identity (similarity) [%]	*P. mirabilis* chr. DNA
TerB	99 (99)	71 (75)	81 (90)
TerC	100 (100)	91 (94)	87 (92)
TerD	96 (98)	95 (96)	92 (95)
TerE	99 (100)	89 (95)	88 (82)
TerF	98 (99)	(not present)	(not present)

does not mediate neither reduced uptake nor increased efflux. Thus, the TerC protein seems to have different function than postulated transmembrane transport of TeO_3^{2-} ions. Both, TerD and TerE protein sequences showed significant homology with a well characterized cAMP binding protein CABP1 from cellular slime mould *Dictyostelium discoideum* which is composed of two units (Grant & Tsang 1990). Their analysis suggests that both CABP1 transcripts are derived from a single gene by an alternative splicing. High sequence similarity between *terD* and *terE* genes may result in a gene dosage effect with increased resistance level or, alternatively, the two genes can code for a functional dimmer protein with two similar but distinct domains.

Several clones with plasmid pLK18 carrying Tn1737Km have been obtained. The clones have been selected on solid media with potassium tellurite. Plasmid DNA from five clones sharing the potential to grow on plates with potassium tellurite and plasmid DNA from ten clones lacking tellurite resistance were subjected to the restriction analyses to localize insertion site of Tn1737Km. Analyses showed that disruption of *terB*, *terC*, *terD* and *terE* genes resulted in abolishing of tellurite resistance, and that the gene *terF* is not essential for conservation of tellurite resistance (Figure 1). Also, a larger distance between *terF* and other genes together with the presence of transcription terminator 15 bp downstream from the STOP codon of *terE* gene suggest that the product of *terF* gene is not a part of the tellurite resistance pathway. Results of the experiment can be interpreted in two different ways. The first explanation is that only products of the genes *terB*, *terC*, *terD* and *terE* are involved in the tellurite resistance mechanism. An alternative possibility is that transcription of the genes of the determinant is proceeded from a common transcription regulation region localised upstream of the

gene *terB*. In such case, the lost of tellurite resistance after transposon insertion can be caused by the transcription interruption. However, to confirm one of these hypotheses, it is necessary to involve into study the promoters, the regulation of expression of the determinant, and other possible regulation elements.

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