VIỆN KHOA HỌC VÀ CÔNG NGHỆ VIỆT NAM
VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

ISSN 1811-4989

TẠP CHÍ
CÔNG NGHỆ SINH HỌC
JOURNAL OF BIOTECHNOLOGY

Tập (Volume) 10 Số (Number) 1 2012

VIỆN KHOA HỌC VÀ CÔNG NGHỆ VIỆT NAM

TẠP CHÍ CÔNG NGHỆ SINH HỌC TẬP 10, SỐ 1 - 2012

MỤC LỤC

CONTENTS

Tạp chí Công nghệ Sinh học **10**(1): 1-6, 2012

CHARACTERIZATION OF *Ter*E PROTEIN – AN ESSENTIAL PART OF TELLURITE RESISTANCE DETERMINANT IN *ESCHERICHIA COLI*

Hoang Vinh Phu[1], Silvia Vavrova[2], Nguyen Thi Giang An[1], Jan Turna[2]

[1]*Vinh University, Nghe An, Vietnam*
[2]*Comenius University, Bratislava, Slovakia*

SUMMARY

Tellurite (TeO_3^{2-}) resistance serves as a selection trait of several pathogenic bacteria and is conferred by various determinants including ter gene cluster formed by two associated parts (*terWXYZ* and *terZABCDEF*). Mechanism of tellurite resistance in microorganism is not fully understood. In bacterial cells, tellurite was reduced to black metallic tellurium to form a black colony. The tellurite resistance operon consists of four essential genes named *terBCDE*. The role of individual proteins in tellurite resistance operon is still mysterious. In the present study, *terE* gene was amplified by PCR and cloned into the expression vector pET21a(+) resulting in pEterE. The TerE protein was successfully expressed in *E. coli* strain BL21 (DE3) and purified by Ni-NTA agarose affinity column. The complementation test was performed to verify biological function of recombinant protein. The pKLdelE plasmid-the deletion of *terE* gene was transformed into cells containing pEter*E* plasmid. The growth of cells containing two plasmids on LB media with K_2TeO_3 and formed black colonies indicated that biological function of recombinant protein was not damaged. The co-expression of two proteins in cells was achieved by using two-plasmid systems and through that we have identified interactions among tellurite resistance proteins (Ter). The interaction among TerE protein and Ter proteins was also identified. Results showed that TerE protein has interacted with TerB and TerD protein. The dimeric form of TerE protein has been also detected. The initial results of the interaction between protein TerE with other essential proteins of Te^R operon provide initial suggestions to understand mechanisms of tellurite resistance of bacteria.

Keywords: *expression, protein-protein interaction, purification, tellurite resistance determinant, tellurite resistance genes*

INTRODUCTION

Tellurium(Te) compounds have a long history as antimicrobial and therapeutic agents. It has been suggested that potassium tellurite toxicity stems from its strong oxidizing ability, which might interfere with many cellular enzyme processes, but an alternative suggestion is that tellurite could replace sulfur in various cellular functions with catastrophic consequences (Taylor, 1999). The specific mechanism underlying tellurite toxicity is not fully understood. Once inside the cell, tellurite (Te^{4+}) is reduced, enzymatically or non-enzymatically, to Te^0. Some enzymes as nitrate reductase (Avazeri *et al.*, 1997), oxidases from the electron transport chain (Trutko *et al.*, 2000), catalase (Calderon *et al.*, 2006) and lately dihydrolipoamide dehydrogenase (Castro *et al.*, 2008) have been shown to be proficient in Te^{4+} to Te^0 conversion. The genetic determinants of tellurite resistance were encoded by the *ter* genes that have been widely found in microbial flora, mostly within pathogenic microorganisms. It has been detected on the larger conjugative plasmids of *Serratia marcescens* (Whelan *et al.*, 1995), *Alcaligenes* sp (Jobling, Ritchie 1988), *Klebsiella pneumoniae* (Chen *et al.*, 2004) and was also incorporated into the chromosome of *Proteus mirabilis* (Toptchieva *et al.*, 2003) and *Escherichia coli* O157:H7 (Perna *et al.*, 1998). The tellurite resistance determinant found on a large conjugative plasmid pTE53, which is isolated from uropathogenic *E. coli* KL53, was previously studied as Burian *et al.* (1998), Kormutakova *et al.* (2000), Nguyen *et al.* (2001), Vavrova *et al.* (2006), Valkova *et al.* (2007). This cluster consisting of two parts *ter*WXYZ and *terZABCDEF*, but the *terBCDE* genes are the essential genes required to maintain the resistance (Kormutakova *et al.*, 2000). The pKL18 plasmid, which was prepared by *in vitro* cloning from pTE53 plasmid (Burian *et al.*, 1998) containing five genes *terBCDEF*, was sequenced and terBCDE genes are essential to maintain the tellurite resistance (Kormutakova *et al.*, 2000). Bioinformatic analysis

characterized that *terB* codes 151 amino acids, *terC* codes 346 amino acids, *terD* codes 192 amino acids and *terE* codes 191 amino acids. In which TerC protein was characterized as transmembrane protein. The role of *terF* was not determined (Kormutakova *et al.*, 2000; Valkova *et al.*, 2007; Hoang *et al.*, 2009; Valkovicova *et al.*, 2011). In this paper, we report the some additional successful studies of TerE protein in *E. coli* bacterial expression system.

MATERIALS AND METHODS

Bacterial strains, plasmids, vectors and media

All bacterial strains used in this study were from collection of the Department of Molecular Biology, Comenius University in Bratislava, Slovakia. Escherichia coli cells were cultivated overnight at 37˚C in Luria-Bertani Broth (LB) media, with addition of the appropriate selection agent. Antibacterial agent concentrations were used as: K_2TeO_3 (Biomark Laboratories) in range 0.1 mM - 4 mM, ampicillin 100 μg/ml and chloramphenicol 34μg/ml for recombinant cells selection. Plasmid construction and manipulations were carried out with the standard laboratory *E. coli* strain DH5α, controlled expression of TerE was done in host strain *E. coli* BL21(DE3) (Novagen, German). Vector pJET 1.2/blunt (from Clone JET^{TM}PCR Cloning kit, Fermentas, Lithuania) was used for PCR cloning and vector pET21a (+) (Novagen, German) was used for expression assay. The pKLdelE (pKL18 plasmid with truncated *terE* gene) and pCDF duet-terE plasmids (coding the TerE protein with N-terminal His.tag) were presented from the Department of Molecular Biology, Comenius University. The pGEX-B, pGEX-C, pGEX-D and pGEX-E plasmids coding for proteins TerBCDE with N-terminal GST fusion were our constructs in other work.

Construction of pETerE plasmid

The gene *terE* was amplified by PCR from *E.coli* KL53 genomic DNA with two primers **E21F** TG*GCTAGC*ATGGCAGTTTCTCTCGTAAAAGG CGGCA and **E21R** TCGGATCCGATGTTAACG CCGTGCTGGG. The standard PCR procedure using thermocycler of BioRad was 30 cycles of 94˚C/30s, 50˚C/30s, 72˚C/45s. The PCR products were analyzed in 1% agarose gel, stained with ethidium bromide and visualized on UV lamp. The PCR products was subcloned into the vector pJET 1.2/blunt resulting in pJTerE. The recombinants were transformed into *E. coli* strain DH5α. DNA samples were isolated and purified by QIAprep Spin Miniprep Kit (QIAGEN, Germany). Sequence was verified by the automatic sequencer ABI 3100-Avant Genetic Analyser using pJET1.2/blunt forward and reserve primers. The obtained nucleotide sequences were aligned with nucleotide database, using the BLAST tools at the National Centre for Biotechnology Information(NCBI) of the United States National Institute of Health. The *Nhe*I-*Bam*HI fragment from pJTerE was cloned into the expression vector pET21a(+), resulting in pETerE and afterwards the ligation was transformed into strain BL21(DE3).

Preparation of two-plasmid systems and complementary test

The *E.coli* BL21(DE3) cells containing pETerE were prepared as competent cells and then used to transform a pLKdelE plasmid. Transformants were cultivated on media containing Ap and Cm antibiotics. Two plasmids were isolated to prove the presence of both plasmids in cells. Transformants were cultivated on media containing 0.1 mmol.l^{-1} of K_2TeO_3 to verify biological function of the protein TerE.

Construction of two-plasmid system to idetify protein interactions

The plasmids pSDFduet-E, pGEX-B, pGEX-C, pGEX-D and pGEX-E were used for construction of two-plasmid systems. *E. coli* BL21/pSDFduet-E cells were prepared as competent cells to transform additionally plasmids pGEX-B, pGEX-C, pGEX-D and pGEX-E. The presence of both plasmids in transformants was verified by cultivation on media containing Ap and Sm antibiotics and two plasmids were isolated. From that, partners of E-B, E-C, E-D and E-E were created.

Expression and Purification of proteins

The BL21 (DE3) cells containing expression vectors harboring the target gene were re-inoculated using 1 ml ovenight culture and kept growing up to OD_{600} of 0.5. One ml of culture was havested for preparing the non-induced sample. The expression of the desired protein was achieved by adding IPTG inducer at the final concentration of 1 mM. Cells were further incubated on a rotary shaker at 37^{0}C for 3 h. After that 1 ml of the culture was collected for preparing the induced sample. The rest was havested for purification by centrifugation at 6000 rpm for

7 min. The pellet was suspended in 5 ml equilibration solution and sonicated from 10 to 15 times according to the protocol: 15 s sonicating and 30 s cooling. The suspension was centrifuged at 6000 rpm for 7 min to remove intact cells and debris. Streptomycin phosphate at the final concentration of 1% (w/v) was added into collected supernatant for precipitation of genomic DNA. The clarified crude extract was harvested by centrifugation at 6000 rpm for 7 min. Ni-NTA agarose affinity column was used for purification of the target protein. The proteins fused with His.tag were washed by wash solutions 1 (0.3M NaCl; 0.05M Phosphate buffer pH=7.6; 0.01M imidazole) and wash solution 2 (0.3M NaCl; 0.05M Phosphate buffer pH=7.6; 0.02M imidazole). The target protein was eluted by solution containing 0.25M imidazole. After that, the purified protein was verified by SDS-PAGE and Western blot.

RESULTS AND DISCUSSION

Expression and purification of TerE

The *terE* gene is one of four essential genes of Te^R determinant encoded by the *ter* genes. The protein TerE consists of 191 amino acids (aa) with a calculated molecular mass of 20.474 kDa and predicted as a cytoplasmic protein (Kormutakova *et al.*, 2000). It was cloned into pET21a(+) vector to yield C-terminally His.tagged protein, thereby it has more 27 amino acids in C-terminus and its molecular weight is 23.67 kDa (Fig 1). Protein TerE was expressed by 1 mM IPTG (Fig 1A) and purified with 0.25 mM of imidazole (Fig 1B). This result confirmed that terE gene has been successfully cloned into the expression vector pET21a(+). The most purified protein was in the second elution fragment (Fig 1B).

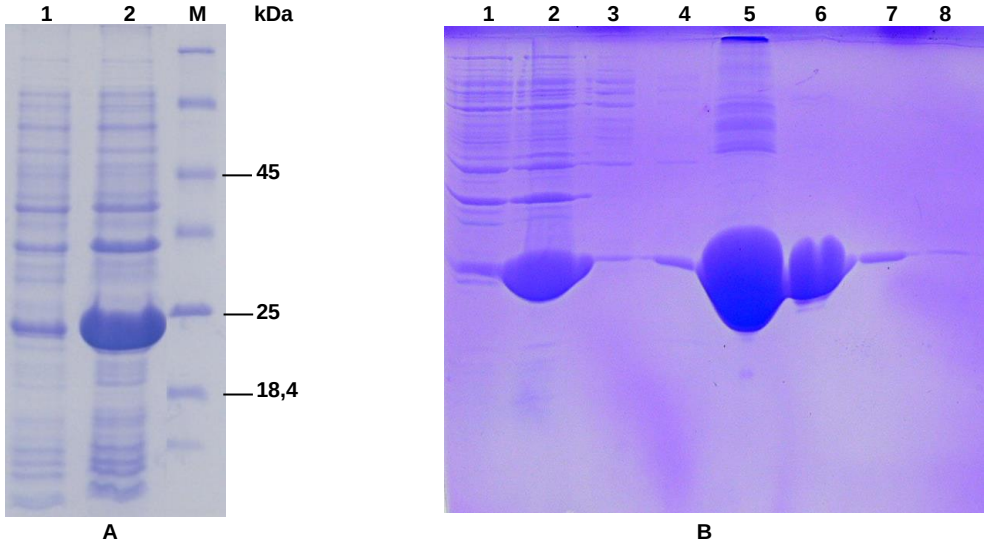

Figure 1. SDS-PAGE of the C-terminal His.tag expressed and purified protein TerE. Above lands were description of samples. The ladder is unstaining protein markers (SM0431, Fermentas). M. . Ladder; 1. Non-induced; 2. Induced; 3: First washed; 4. Second washed washed; 5. First elution; 6. Second elution; 7. Third elution; 8. Fourth elution.

Functional expression of TerE using two-plasmid system

The biological function of expressed protein was verified by complementation test using two-plasmid system (Fig. 2). The pLKdelE plasmid was created by truncation of *terE* gene in plasmid pKL18 (Burian *et al.*, 1998) containing four essential genes (*terB, terC, terD, terE*) of Ter operon (Kormutakova *et al.*, 2000). The cells containing pLKdelE or pETerE were not grown on medium containing no antibiotics but K_2TeO_3 at a low concentration. The complementation containing pLKdelE + pETerE grew on this medium with black colonies as standard tellurite resistant determinants (Fig.2) (Taylor, 1999). This confirmed that the presence of 27 amino acids at C-terminus did not show a strong influence on the biological function of the protein TerE.

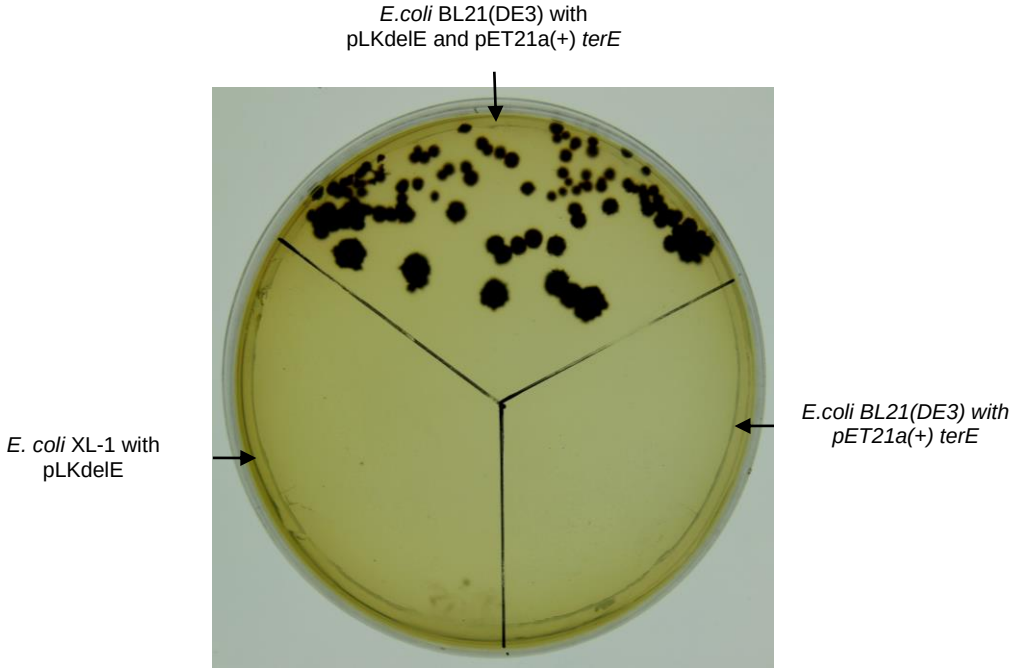

Figure 2. Growth of *E. coli* BL21/ pLKdelE + pETerE, XL1/pLKdelE, BL21/pETerE on medium containing 0.1 mM of K_2TeO_3 and 0.5 mM IPTG

Interaction among Ter proteins by *in vivo* assay

In this case, the protein TerE fused with N-terminal His.tag was expressed together with other Ter proteins as TerB, or TerC, or TerD fused with the N-terminal GST fusion. When purifying, the protein TerE with His.tag was attached on Ni-NTA column and captured the interacted proteins. The samples were analyzed on SDS-PAGE and verified by Western blot with the anti-His.tag and anti-GST monoclonal antibodies (Fig. 3).

TerE did not interact with TerC (Fig. 3B) and maybe weekly interact with TerB (Fig.3A). This result is identical with result of Valkovicova's *in vitro* pull-down assay (still unpublished). The proteins coded by the genes terD and terE showed high level of sequence homology (Kormutakova *et al.,* 2000). Otherwise, the interaction of TerE and TerD was clearly visible and existing of the protein-dimer TerE to predict that TerE and TerD have similar functions in Ter operon.

CONCLUSION

Tellurium compounds were used commonly as antimicrobial agents prior the antibiotics era and are nowadays used in media for isolation of emergent pathogens. Several different gene loci encoding for tellurite resistance were identified, but the highest MIC is devoted to the so called *ter* genes operon. The protein TerE was supposed to be one of the crucial proteins providing the host cell the selective advantage – the resistance to potassium tellurite, but for many pathogens this means also resistance to oxidative stress and primary non-specific immune control, as it was published in previous paper (Valkova *et al.*, 2007). However, the mechanism of this resistance still remains unclearly. In this work we reported its expression in pET system, with conserved biological function. This gives us the tool to study its role in resistance phenotype and hopefully will help us to expound this puzzle. The interaction of the protein has important roles in cellular functions. The knowledge of interactions between Ter proteins helps us to understand the mechanisms of this resistance. The initial results of protein-protein interaction can be directing for next studies. We would like study interaction of all Ter proteins by various methods.

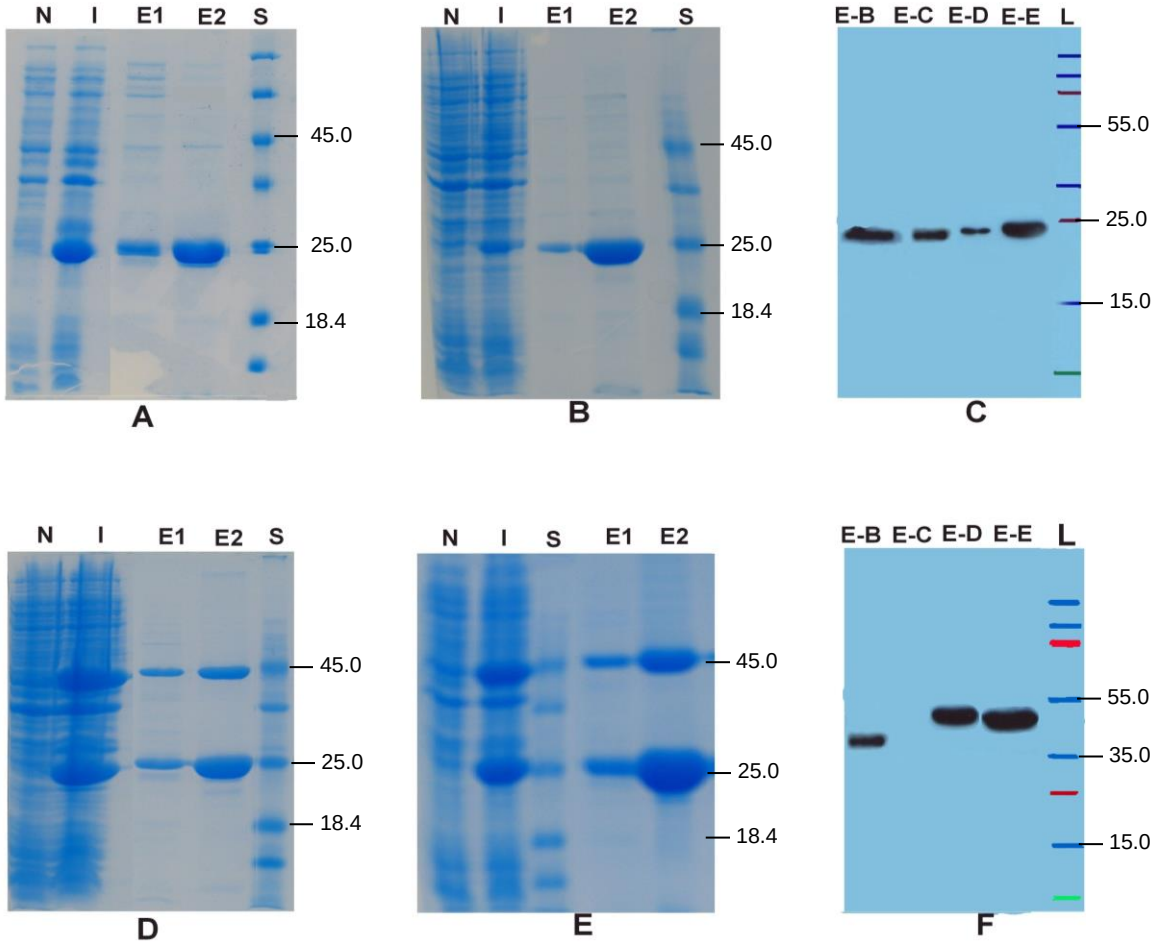

Figure 3. SDS PAGE and western blot analysis of *in vivo* coexpressed tagged proteins. SDS PAGE of His.tagged TerE protein with GST.tagged TerB (**A**), TerC (**B**), TerD (**D**) and TerE(**E**) proteins. In this, the uninduced (N), induced (I) samples of cell expressed both tagged proteins were loaded.The E1 is eluate after first elution, E2 is eluate after second elution and S is Unstained Protein Molecular Weight Marker (Fermentas SM0431). Western blot analysis of eluates by anti-His (**C**) and anti-GST (**F**) monoclonal antibodies. The first letter of mark at the top of figure indicates particular Ter protein which was His tagged and was captured at column and second letter is examined specific GST tagged Ter protein. L is PageRulerTMPrestained Protein ladder (Fermentas SM 1811).

Acknowledgement: *This work was supported by the VEGA grant No.1/0346/10 from the Ministry of Education of the Slovak Republic, the Comenius University grant No. UK/560/2010 and by the project APVV-20-054005 of Slovak Research and Development Agency.*

REFERENCES

Avazeri C, Turner RJ, Pommier J, Weiner JH, Giordano G, Vermeglio A. (1997) Tellurite reductase activity of nitrate reductase is responsible for the basal resistance of *Escherichia coli* to tellurite. *Microbiology* 143: 1181-1189.

Burian J, Nguyen Tu, Klučar L, Guller L, Lloyd-Jones G, Stuchlik S, Fejdi P, Siekel P, Turňa J (1998) *In vivo* and *in vitro* cloning and phenotype characterization of tellurite resistance determinant conferred by plasmid pTE53 of clinical isolates of *Escherichia coli*. *Folia Microbiol* 43: 589-599.

Calderón IL, Arenas FA, Pérez JM, Fuentes DE, Araya MA, Saavedra CP, Tantaleán JC, Pichuantes SE, Youderian PA, Vásquez CC (2006) Catalases Are NAD(P)H-Dependent Tellurite Reductases . *PLoS ONE* 1, e70.

Castro ME, Molina RC, Díaz W, Pichuantes SE, Vasquez CC (2008) The dihydrolipoamide dehydrogenase of *Aeromonas caviae* ST exhibits NADH-dependent tellurite reductase activity. *Biochem. Biophys Res Commun* 375: 91-94.

Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL (2004) Sequencing and analysis of the large virulence plasmid pLVK of *Klebsiella pneumoniae* CG43. *Gene.* 337: 189-198.

Jobling MG, a Ritchie DA (1998) The nucleotide sequence of a plasmid determinant for resistance to tellurium anions.*Gene* 66: 245-258.

Kormuťáková R., Kľučár L, Turňa J (2000) DNA sequence analysis of the tellurite resistance determinant from clinical isolate of Escherichia coli and identification of essential genes, *Biometals* 13: 135-139.

Nguyen Tu, Burian J, Stuchlík S, Kormuťáková R, Kľučár Ľ, Lackovičová D, Turňa J (2001) In vivo cloning of tellurite resistance genes with mini-Mu derivate of bacteriophage Mu, *Biologia* 56/3: 251-255.

Perna NT, Mayhew GF, Posfai G (1998) Molecular evoluion of pathogenicity island from entorehemorrhagic *Escherichia coli* O157:H7. *Infect Immun* 66: 3810-3817.

Taylor DE (1999) Bacterial tellurite resistance: Widespread determinants for resistance to a rare oxyanion *Trends Microbiol.* 7: 111-115.

Toptchieva, Sisson GA, Bryden LJ, Taylor DE, Hoffman PS (2003) An inducible tellurite-resistance operon in Proteus mirabilis. *Microbiology*. 149: 1285-1295.

Trutko SM, Akimenko VK, Suzina NE, Anisimova LA, Shlyapnikov MG, Baskunov BP, Duda VI, Boronin AM. (2000) Involvement of the respiratory chain of gram-negative bacteria in the reduction of tellurite *Arch Microbiol* 173: 178-186.

Valková D, Valkovičová L, Vávrová S, Kováčová E, Mravec J, a Turňa J (2007) The contribution of tellurite resistance genes to the fitness of Escherichia coli uropathogenic strains, *Cent Eur J Biol* 2(2): 182-191.

Vávrová S, Valková D, Drahovská H, Kokavec J, Tuňa J (2006) Analysis of the tellurite resistance determinant on the pNT3B derivate of the pTE53 plasmid from uropathogenic *Escherichia coli. BioMetals* 19: 453-460.

Whelan KF, Colleran E, a Taylor D (1997) Characterization of a Region of the IncHI2 Plasmid R478 Which Protects Escherichia Coli from Toxic Effects Specified by Components of the Tellurite, Phage, and Colicin Resistance Cluster. *J Bacteriol* 177(17): 63-71.

MÔ TẢ PROTEIN TerE - MỘT PHẦN CƠ BẢN CỦA YẾU TỐ KHÁNG TELLURITE Ở VI KHUẨN *ESCHERICHIA COLI*

Hoàng Vĩnh Phú*, Silvia Vávrová, Nguyễn Thị Giang An, Ján Turňa

TÓM TẮT

Sự kháng tellurite (TeO_3^{2-}) cung cấp đặc điểm chọn lọc của nhiều vi khuẩn gây bệnh và được quy định bởi nhiều yếu tố kháng bao gồm các nhóm *ter* gen và được định dạng bởi 2 phần liên hệ với nhau (*terWXYZ* and *terZABCDEF*). Cơ chế kháng tellurite ở vi sinh vật chưa được hiểu đầy đủ. Trong tế bào vi khuẩn, tellurite được khử thành dạng nguyên tử telu và hình thành các khuẩn lạc màu đen. Operon kháng tellurite chứa đựng bốn gen kháng cơ bản được đặt tên là *terBCDE*. Vai trò của các protein riêng biệt trong operon kháng tellurite vẫn còn là điều bí ẩn. Trong nghiên cứu này, gen *terE* đã được kích hoạt bằng PCR và được nhân dòng vào vector biểu hiện pET21a(+) tạo ra plasmid tái tổ hợp pEterE. Protein TerE đã được biểu hiện thành công trong chủng *E. coli* BL21(DE3) và được tinh sạch bởi cột ái lực Ni-NTA agarose. Chức năng sinh học của protein tái tổ hợp đã được kiểm chứng bằng test bổ sung. Plasmid pKLdelE chứa gen terE không hoàn chỉnh đã được biến nạp vào trong tế bào chứa đựng plasmid pEterE. Sự phát triển của các tế bào chứa đựng 2 plasmid trên môi trường LB chứa đựng K_2TeO_3 và hình thành các khuẩn lạc màu đen chứng tỏ chức năng sinh học của protein tái tổ hợp không bị phá vỡ. Sự biểu hiên đồng thời của 2 protein trong cùng tế bào đạt được bởi việc sử dụng hệ thống 2 plazmid và thông qua đó chúng tôi đã nhận diện được các tương tác giữa các protein kháng tellurite. Sự tương tác giữa protein TerE và các protein kháng tellurite kháng cũng đã được nhận diện. Kết quả thu được cho thấy protein TerE tương tác với các protein TerB và TerD. Dạng dimer của protein TerE cũng đã được phát hiện. Những kết quả ban đầu về sự tương tác giữa protein TerE với các protein thiết yếu khác của operon kháng tellurite cung cấp những gợi ý ban đầu để tìm hiểu cơ chế kháng tellurite của vi khuẩn.

Từ khoá: *Biểu hiện, tương tác protein, tinh sạch, yếu tố kháng tellurite, các gen kháng tellurite*

* *Author for correspondence:* Tel: *+84-917705777;* E-mail: *phu_khoasinh@yahoo.com*