The transcriptome analysis of *Escherichia coli* responding to tellurite

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Research Article

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Abstract

Tellurite is a strong antimicrobial agent highly toxic to many microorganisms, while its toxicity mechanism is still unclear. In this study, the comparative transcriptome analysis of E. coli MG1655 responding to the stress of tellurite was performed and the differentially transcribed genes were analyzed, to understand toxicity mechanisms of tellurite preliminaryly and uncover metabolism processes changes resulted from tellurite globally. After treated with 10 μg/mL tellurite for 1 h, high concentration and long time, the cells exhibited an obvious adaptive reaction and many metabolic processes were influenced. The transcription of the genes involved in the ribosome metabolism and the flagella assembly were changed significantly, implying they might be the major pathway affected by tellurite. The transcription of the genes encoding the transcriptional factors and small RNAs, and the genes functioned in the cell motility, metal ion metabolism and membrane function were also varied, which may participate in the metabolism adjustment and damage repair to resist the toxicity of tellurite. This work can facilitate the study of the toxicity mechanism of tellurite and promote the clinical application of this chemical.

Introduction

Tellurium is one of the metalloid elements in the same family of oxygen, sulfur and selenium. In natural environments, except of the elemental tellurium (Te^0), it normally exists in several stable oxidation states, including VI (tellurate, TeO_4^{2-}), IV (tellurite, TeO_3^{2-}) and II (telluride, Te^{2-}); the tellurate and tellurite are the most common forms (Yurkov et al. 1996). Although tellurium and its oxidation states are low in nature (10^{-2} to 10^{-8} ppm) and unevenly distributed, the tellurite is toxic to vast majority of microorganisms and can inhibit cell growth with concentrations of up to 1 μg/mL (Yurkov et al. 1996). The constant emerging of antibiotic resistance related pathogens has become one of the most concerned health problems (Dantas et al. 2008). Sub-lethal concentrations of tellurite has been found to be able to improve the effect of antibiotics like ampicillin, tetracycline, chloramphenicol and cefotaxime on *E. coli* and *Pseudomonas aeruginosa*, implying its potential as a powerful synergistic antimicrobial agent (Molina-Quiroz et al. 2012). Understanding of the toxic mechanisms of tellurite to bacteria may shed light on the current dilemma of antibiotic resistance.

Previous research indicated that tellurite is toxic to cells as it can cause severe oxidation stress (Elías et al. 2012; Turner et al. 1999). After entering the cell through phosphate transport protein PitA, tellurite can be reduced into the elemental tellurium, causing the oxidation of intracellular thiol and glutathione, producing reactive oxygen and free radicals (O_2^-) (Elías et al. 2012; Turner et al. 1999; Turner et al. 2001). The balance of the intracellular redox state is disrupted and the produced superoxides can act on the metabolic enzyme containing the iron sulfur center cluster, causing the loss of Fe^{2+}, the enzyme inactivation and the damage of central metabolism (Calderón et al. 2009). Meanwhile, the produced superoxides and the released Fe^{2+} can also act on nucleic acids and membrane lipids, generating the lipid peroxides and affecting the cell process (Refsgaard et al. 2000). Proteomics analysis showed that under the tellurite stress, the expression of antioxidase, superoxide dismutase, catalase, and

oxidoreductase in cells enhanced significantly (Aradská et al. 2013). Tellurite can also inhibit the heme synthesis under aerobic and anaerobic conditions and produce the toxic hydroxyl radicals (OH), damaging the biomolecules and leading the cell death (Morales et al. 2017). However, there are still some contradictions in the proposed hypothesis and the molecular mechanism of tellurite toxicity is still not very clear. The excess reactive oxygen is considered as the main toxicant, while tellurite is still toxic to cells in anaerobic conditions (Tantaleán et al. 2003). Selenite can also cause a higher level of reactive oxygen in the cell, its toxicity is much less than that of tellurite (Vrionis et al. 2015). Moreover, *Deinococcus radiodurans*, strongly resistant to reactive oxygen, is still sensitive to tellurite (Anaganti et al. 2015).

Up to now, the understanding of the toxicity mechanism to microorganisms by tellurite mainly points to oxidative stress and other side-effects, which seems to be same to chemical oxidizing agents like hydrogen peroxide. Comparative transcriptome analysis can find the differentially transcribed genes in different conditions and study the metabolic variation in microorganisms under different stress (Evans 2015). After treated with tellurite of 0.5 μg/mL for 15 min, the minimal inhibition concentration (MIC) of *E. coli* and short time, cells showed an obvious decrease in the oxygen consumption and converted into the anaerobic breathing (Molina-Quiroz et al. 2014). In this work, the comparative transcriptome analysis of the genes in *E. coli* MG1655 treated with high concentration of tellurite for long time was detected and analyzed to reveal the fitness mechanism of cells to the tellurite toxicity. The results could be partially explained by the previous hypothesis and some findings implying other stress response pathways were also found. This work can help the understanding of the tellurite effect on cell metabolism, facilitate the toxicity mechanism study, and promote its potential clinical application as an antimicrobial agent.

Materials And Methods

Strains and materials

The *E. coli* MG1655 strain (F^- λ^- *ilvG*$^-$ *rfb*$^-$ *rph*$^{-1}$) was the laboratory storage. Luria-Bertani (LB) medium (tryptone 10.0 g, yeast extract 5.0 g, NaCl 10 g/L, pH 7.0) and the MM minimal medium (Na_2HPO_4 6.0 g, KH_2PO_4 3.0 g, NH_4Cl 1.0 g, NaCl 0.5 g, $MgCl_2$ 1 mM, $CaCl_2$ 100 μM, $FeSO_4$ 1 μM, $CuSO_4$ 1 μM, glucose 0.2%, pH 7.2) were used for strain culture. The Total RNA Extraction Reagents, the PrimeScript Reverse Transcriptase Kit and the Premix Ex Taq (Probe qPCR) were purchased from TaKaRa (Dalian, China). All other reagents were of analytical reagent grade purchased from Sigma-Aldrich (Saint Louis, USA). The oligonucleotides used for reverse transcription and quantitative PCR (RT-qPCR) were synthesized at Sangon Ltd. (Shanghai, China).

Strain culture, library construction and sequencing

The single colony of *E. coli* MG1655 strain was inoculated in 3 mL LB medium overnight in a shaker at 37℃ for 200 rpm. The cells were collected and the inoculum was transferred into the fresh M9 medium with an initial OD_{600} about 0.05, and cultured in a shaker at 37℃ for 200 rpm to about 0.5 OD_{600}. The

cells were centrifugated and resolved with the fresh M9 medium or the M9 medium containing 10 μg/mL of tellurite, and then cultured for 1 h at 37℃. The experimental groups were marked as BLA_Te_1/BLA_Te_2 and the control group were recorded as BLA_1/BLA_2; two parallels were set for each sample. All the samples were sent to Beijing Novogene Company for RNA-seq sequencing analysis, and the Illumina HiSeq^{TM}2500/Miseq^{TM} was performed after the library was qualified.

Analysis of the differentially transcribed genes

The transcription levels of the genes in *E. coli* MG1655 cells under the tellurite stress were analyzed by comparing with those of the control group, cells in the glucose condition. The readcount data were standardized by DESeq method and the probability (*p* value) was calculated, according to the negative binomial distribution of the model; the FDR (false discovery rate) value was adjusted by multiple hypothesis test. Screening criteria of significant differences in transcription gene was padj < 0.05. After screening the differential transcription genes, the effects of tellurite on gene function in *E. coli* are expounded with GO (Gene Ontology) database for the enrichment analysis and the distribution study of differential transcription genes. The main biochemical metabolic pathways and the signal transduction pathways of the differentially transcribed genes under the tellurite stress and their cooperation networks were predicted by the KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis.

RNA extraction and RT-qPCR

The *E. coli* MG1655 cells were cultured as above and the total RNA was extracted; the RNA yield was determined using a Nanodrop UV spectrometer (Thermo Scientific, DE, USA). The reverse transcription was achieved with the PrimeScript Reverse Transcriptase Kit with 1 μg RNA and 20 ng random primers. The quantitative PCR was performed in the qTOWER3G touch Real-time PCR System (Analytik Jena AG, DE), using the Premix Ex Taq (Probe qPCR) and gene-specific primers (Table S1). The cycling conditions were 95 ℃ for 3 min, and 40 cycles of 95 ℃ for 10 s, 55℃ for 20 s and 72℃ for 20 s. A final melting analysis was obtained by slow heating with 10 s increments of 0.5℃ from 54℃ to 95℃. The threshold cycle (Cq) value of each sample was determined and the relative fold change in mRNA quantity was calculated using the DDCt method (Livak et al. 2001). Three independent experiments were performed for each sample and the average values with the standard errors were calculated.

Results

Quality analysis of the transcriptome sequencing data

The data quality of the transcriptome sequencing was the basis of effective analysis. After the high-throughput sequencing, the number of raw reads in the cDNA libraries of the BLA_1, BLA_2, BLA_Te_1 and BLA_Te_2 samples were 15,260,680, 18,802,972, 21,718,822, 19,398,942, respectively; the number of clear reads was 14,634,334, 17,166,478, 21,053,516, and 18,834,606. The clean bases were 2.2G, 2.57G, 3.16G, 2.83G, respectively; the Q20 percent (%) reached 98.22, 97.90, 97.89, 98.05, and the Q30 percent (%) were 94.72, 93.96, 93.97, 94.28, respectively. The combined number of base G and base C in the four groups

accounted for 52.94, 52.82, 53.52, 53.45, respectively, meaning that there was not GC/AT separation. These results showed that the cDNA libraries obtained by the transcriptome sequencing was of high quality and could fit the requirement of the subsequent bioinformatics analysis. In addition, the reference sequences comparison showed that the sequence number of the control *E. coli* MG1655 samples and the tellurite treated samples were 11,471,540, 13,868,777, 12,381,688, and 13,866,787; the percentage was 78.39, 80.79, 58.81, 80.79, respectively. Besides, the percentage of the multiple positioning sequences to the total was 3.74, 4.47, 2.61, 2.8. All these results meant that the reference genome was appropriate and no contamination in these experiments.

The RNA-seq correlation test showed that the correlation coefficient R^2 between the control samples of BLA_1 and BLA_2 was 0.968, and the R^2 between the tellurite treated samples of BLA_Te_1 and BLA_Te_2 was 0.978. It confirmed the high similarity of two biological repeat samples in each group and the reliability of the transcriptome sequencing data. Furthermore, the coefficient of sample correlation between different groups were relatively small, indicating that the stress of tellurite exactly affected the gene transcription of *E. coli* MG1655. The gene transcription analysis results showed when the four samples were at FPKM >60, the transcription abundance were 42.43%, 40.00%, 34.88% and 33.39%, respectively; it meant that the transcription abundance and the transcription level of the samples were high.

Summary of the differentially transcribed genes

With the comparison of the transcriptome sequencing data of the control groups, the transcription levels of 1629 genes in *E. coli* MG1655 cells under tellurite stress changed significantly. Therein, the transcription levels of 805 genes were significantly increased ($P<0.05$), and those of 633 genes were increased strikingly ($P <0.01$); the transcription of 824 genes showed a significant reduction ($P<0.05$), and those of 630 genes decreased extremely significantly ($P<0.01$) (Table S2). The distinct distribution of the differentially transcribed genes was shown with the volcanic map (Fig. 1). Little difference was observed in the number of the up-regulated genes and the down-regulated genes in these *E. coli* MG1655 cells with different treatments, implying that the high-concentration and long-time treatment of tellurite affected the transcription of certain genes, not the indiscriminate transcription inhibition. The *E. coli* cells adopted an adaptive reaction by modifying certain metabolic pathways to repair the damage and maintain cell survival.

The GO enrichment analysis of the differentially transcribed genes

The GO (Gene Ontology) enrichment analysis can help the classification of genes and gene products into three parts: Molecular Function (MF), Biological Process (BP), and Cellular Composition (CC). Thus, GO functional enrichment analysis of the 1629 differentially transcribed genes of *E. coli* MG1655 cells under the tellurite stress was performed. Results indicated that the up-regulated genes were mainly enriched to 1940 GO terms, and 46 GO terms showed the significant enrichment ($p<0.05$). As to the down-regulated

genes, 1805 GO terms were enriched and 50 GO terms of them showed the significant enrichment (p<0.05).

For the up-regulated genes, 24 GO terms of the 46 enriched GO terms were related to biological process, 8 GO terms were linked to molecular function and 14 GO terms were belonged to the cellular component. In the terms of biological process, most of the differentially transcribed genes (369 genes) were enriched into the cellular process, and the organic substrate metabolic process followed with the enrichment of 346 genes. In terms of molecular function, the largest number of the differentially transcribed genes were enriched into the molecular function (557 genes), followed by the genes enriched into the organic cyclic compound binding and heterocyclic compound binding (both 257 genes). As to the terms of cell composition, the differentially transcribed genes were mostly enriched into the cell components (304 genes), the genes enriched into the cell components (144 genes) and cell parts (144 genes) followed (Table S3). These results demonstrated that with the treatment of tellurite in high concentration for long time, the genes related to molecular function, cell process and the organic substrate metabolism were the significantly up-regulated, meaning the molecular synthesis and central metabolism pathways were strengthened to resist the tellurite toxicity.

On the other hand, in the 50 enriched GO terms of the down-regulated genes, 43 GO terms of them were related to biological process and 7 GO terms were linked to molecular function. In the terms of biological process, the largest number of the differentially transcribed genes were enriched into the oxidation reduction process (109 genes), and the small molecule biosynthetic process followed with the enrichment of 47 genes. In terms of molecular function, most of the differentially transcribed genes were enriched into the oxidoreductase activity (116 genes), followed by the genes enriched into the cofactor binding (85 genes) (Table S3). These results indicated that in the condition of high-concentration of tellurite for long time, the oxidoreductase activity, the oxidation reduction process and the cofactors binding process were inhibited significantly.

To further clarify the distribution of the differentially transcribed genes, the top 30 significantly enriched GO terms in biological process, molecular function and cellular component were shown (Fig. 2). Results indicated that most of the significant differentially-transcribed genes were enriched into biological process (18 GO terms), followed by cellular component (4 GO terms) and molecular function (2 GO terms). In almost all the GO terms, the number of up-regulated genes was higher than that of down-regulated genes. It meant that the treatment of high-concentration of tellurite for long period damaged the cell composition and molecular function and severely affected the fundamental physiological processes. Cells must activate the biological process to repair these damages, maintain the central metabolism and eliminate the toxic effect of tellurite.

The KEGG functional enrichment analysis of the differentially transcribed genes

KEGG functional enrichment analysis can systematically predict the gene functions metabolic pathways, and function network. The KEGG analysis of the 1629 differentially transcribed genes of *E. coli* MG1655 cells was performed to explore the metabolic pathways affected by tellurite stress. Results indicated the

up-regulated genes were enriched into 77 metabolic pathways, and down-regulated genes were enriched into 82 metabolic pathways. To clarify the enriched metabolic pathways of the differentially transcribed genes, the top 20 enrichment pathways of the up-regulated genes and the down-regulated genes were shown (Fig. 3).

The top five enriched metabolic pathways of the up-regulated genes were Geraniol degradation, Ribosome, Citrate cycle, Fatty acid metabolism, and Protein export; therein, the significantly enriched five pathways were Ribosome, Citrate cycle, Fatty acid metabolism, Protein export, and Arginine and proline metabolism. Among them, the ribosome pathway was strikingly enriched with the Q value of 5.17E-06. There were 78 annotated genes in this pathway, and the transcription of 44 genes changed with the tellurite treatment. The rich factor, the ratio of the number of differentially transcribed genes to the number of annotated genes in this pathway, was 0.56. The ribosome genes with the highest fold change were *rpmD*, *rplD*, *rpmC, rpsP*, *rplS*, and the up-regulated fold reached 3.3861, 2.9156, 2.709, 2.6068, 2.6048, respectively, implying these genes and this pathway were activated obviously under the tellurite stress (Fig. 3a). Meanwhile, the top five enriched metabolic pathways of the down-regulated genes were Flagellar assembly, Folate biosynthesis, Nitrotoluene degradation, vitamin B6 metabolism, Glycolysis/Gluconeogenesis; and the significantly enriched five pathways were Flagellar assembly, Glycolysis/Gluconeogenesis, Amino sugar and nucleotide sugar metabolism, Folate biosynthesis, Starch and Sucrose metabolism. Therein, the flagellar assembly pathway was most significantly enriched with the Q value of 0.048239876; 23 genes of the 36 annotated genes in this pathway showed differential transcription and the rich factor was 0.64. The genes in the flagella assembly metabolic pathway with the highest fold change were *flhC*, *flhD*, *flgH*, *fliS*, and *fliE*, and the down-regulated fold were -3.488, -3.3376, -3.2018, -3.1097, and -3.064, respectively, implying these genes and this pathway were inhibited greatly under the tellurite stress (Fig. 3b). All these results demonstrated that the treatment of high-concentration of tellurite for long period severely affected the basic cellular biomolecular metabolic pathways and energy metabolism, including amino acids, nucleotides, polysaccharides, lipids, vitamins, etc. The great influence on the function of ribosome and the assembly of flagella indicated the cells was in an adaptive state to strengthen the ribosome activity and reduce the cell motility to ensure enough energy for core metabolism.

The RT-qPCR verification of the differentially transcribed genes

To verify the transcriptome results, the transcription levels of several genes in *E. coli* MG1655 with the same treatment of tellurite were analyzed by RT-qPCR detection. The target genes were the genes probably involved in the tellurite response, including small RNA genes (*oxyS*, *glmZ*, *glmY*, *tff*, *sraB*, and *rygD*), the metal resistance-related genes (*marR* and *arsR*), the oxidative stress-related genes (*dnaK* and *ydeI*), and the metal ion metabolism-related genes (*mgtS*). In addition, in order to gain insight into the response mechanism of cells to tellurite stress, the gene *tehB* annotated as the tellurite resistance-related genes, were also selected. RT-qPCR results showed that the transcription of genes *glmZ*, *glmY*, *tff*, *marR*, *arsR*, *dnaK*, *mgtS* were up-regulated, and those of genes *oxyS*, *ydeI*, *tehB* were down-regulated; no significant changes was observed in the transcription of gene *sraB* and *rygD* (Fig. 4). These genes were

related to the oxidative stress resistance and the metal ion metabolism, similar to the KEGG analysis. Comparison of the two methods indicated that the transcription change of most of the functional genes could both be detected and in the similar trend; the detection of small RNA existed difference (Table 1).

Discussion

The tellurite stress activated the ribosome metabolism in *E. coli*

Ribosomes are important places where mRNA are translated into amino acids, producing proteins to maintain cellular structure and cell survival (Schmeing et al. 2009). After the tellurite treatment, the ribosome metabolism pathway was the significantly enriched; 44 genes were up-regulated and 2 genes were down-regulated (Fig. 3 and Table S3). The top six up-regulated genes were *rpmD*, *rplD*, *rpmC, rplS*, *rpmD* and *rpsP*, encoding the 50S ribosome protein L30, L4, L29 and L19, and the 30S ribosome protein S16 (Cerretti et al. 1983; Li et al. 1996; Zurawski et al. 1985; Oberto et al. 1996; Persson et al. 1995). Ribosome protein L4 can not only regulate the synthesis of its own protein and inhibit the translation of other ribosme proteins, but also can regulate the RNase E activity to enhance the expression level of stress response proteins and sustain normal cellular metabolism in adverse conditions (Yates et al. 1980; Zengel et al. 1980; Singh et al. 2009). The ribosome protein S16 participates in the assembly of 30S ribosome, and possesses the endonuclease activity dependent on Mg^{2+} and Mn^{2+} (Oberto et al. 1996; Jagannathan et al. 2003). Ribosome protein L19 is responsible for assembly of 50S ribosomes, connecting the large subunit to the small subunit (Oberto et al. 1996; Vannice et al. 2016). Under the treatment of high-concentration tellurite for long time, the ribosome metabolism pathway was enhanced to promote the protein synthesis by restoring the assembly of ribosome subunit and improving the ribosome activity. Meanwhile, the intracellular RNase E and endonuclease activity were also increased to strengthen the stress response and damage repair processes.

The tellurite stress inhibited flagella assembly and cell motility in *E. coli*

Flagella is responsible for ell motility and chemotaxis, and its assembly is an energy consuming process (Yonekura et al. 2003). Under the nutritional deficient conditions, cells will lose flagella (Zhuang et al. 2020). After the tellurite treatment, the flagella assembly pathway was the significantly enriched; 23 genes were down-regulated and only 4 genes were up-regulated (Fig. 3 and Table S3). The top five down-regulated genes were *flhC*, *flhD*, *flgH*, *fliS* and *fliE*, encoding proteins FlhC, FlhD, FlgH, FliS and FliE respectively. FlhCD is a transcription activator responsible for the transcriptional activation of gene *fliA* and involved in the regulation of the flagella synthesis (Mudge et al. 2021; Fitzgerald et al. 2014). FlgH is the component of the L-ring located on the outer membrane layer, forming a molecular tube with the P-ring (Akiba et al. 1991; Minamino et al. 2015). And FliS is the regulator of flagella synthesis and the component of flagella hook and substrate complex (Müller et al. 1992). Therefore, after the treatment of high-concentration tellurite for long time, the energy generation was inhibited; cells tended to reduce the flagella synthesis and assembly to save the cellular energy to maintain the core metabolism and conduct the damage repair.

The basic metabolism in *E. coli* was affected *by* tellurite

With the stress of tellurite, the transcription of genes involved in the metabolism of the core biomacromolecules changed significantly, including those of amino acids, nucleotides, polysaccharides, lipids and vitamins. The transcription levels of genes involved in the degradation pathways of amino acids changed more significantly than those of genes in the synthesis pathways. For example, valine, leucine and isoleucine, the amino acids with branch chain and large hydrophobic side chains, function as the internal support structure of water-soluble proteins and promote synthesis of ATP through the synthesis of nicotinamide adenine dinucleotide and reduction of flavin adenine dinucleotide. The transcription of five genes in their degrading pathways were up-regulated, and the change of *fadB* gene was the most significant, whose product with isomerase, hydrolase and dehydrogenase activities (Yang et al. 1983). In their biosynthesis pathways, only one gene was up-regulated, and four genes were down-regulated; the highest change was observed in the transcription of *tdcB*, encoding enzyme for threonine dehydration (Goss et al. 1984). In the lysine biosynthesis pathway, three genes were up-regulated, and five genes were down-regulated; the transcription of gene *dapD* was repressed most (Richaud et al. 1984). In arginine and proline metabolism pathway, the transcription levels of fifteen genes was enhanced and those of five genes were reduced; the putrescine metabolic gene, *puuD* was up-regulated significantly (Kurihara et al. 2006). As to the amino sugar and nucleotide metabolism, twenty genes were decreased and gene *nanA* participating in the degradation of sialic acids changed significantly (Vimr et al. 1985). Nine genes in the protein export pathway were up-regulated and four genes were down-regulated; gene *ffh* responsible for protein transport and necessary for viability was increased most significantly (Phillips et al. 1992). These results showed that after the treatment of high-concentrated tellurite for long time, the protein synthesis in *E. coli* was repressed and the protein degradation process was accelerated; therefore, the fundamental protein metabolism was affected and cells were hard to survive.

The glycolysis/gluconeogenesis pathway is the main route of sugar metabolism to maintain the cellular balance of sugar (Xiong et al. 2011). The transcription of most of the genes (21 genes) in this pathway were reduced, and gene *adhE* encoding the ethanol/acetaldehyde dehydrogenase was repressed significantly (Aristarkhov et al. 1996). Tricarboxylic acid (TCA) cycle is the important aerobic metabolism center, not only the final metabolic pathway of amino acids, lipids and sugars for complete oxidization, but also the metabolism junction of amino acids, lipids, sugar and even nucleic acid (Maulucci et al. 2016). Thirteen genes in this pathway was up-regulated and seven genes were down-regulated; gene *ybhJ* encoding a hydratase was with the highest change. These meant that tellurite affected the basic and core sugar metabolism, and cells need to catabolize more sugar to produce more energy for cell survival. Besides, fatty acids supply energy and produce lipids as the main component of membrane structure and scaffold. In addition to genes involved in fatty acid metabolism, genes related to acetone metabolism, carbon and nitrogen metabolism, starch and sucrose metabolism, fructose and mannose, methane metabolism pathways showed differential transcription. According to this result, under the stress of tellurite, the transcriptional levels of the most genes related to energy metabolism and material metabolism have been up-regulated of down-regulated, indicating the genes and pathways involved in the response of cells to tellurite toxicity are numerous and complex.

Tellurite treatment influenced the cellular oxidation-reduction balance

Precious work showed that tellurite are toxic to cells due to their oxidation, including the thiol consumption, the alteration of reduction potential and the production of reactive oxygen, and the expression of reactive oxygen detoxifying enzymes in cells was increased with the addition of tellurite (Turner et al. 2001; Anaganti et al. 2015; Tremaroli et al. 2007; Pérez et al. 2007). In this work, after the treatment of tellurite, the transcription levels of the genes involved in the metabolic pathways of some antioxidant products like ubiquinone and terpenoid-quinone, ascorbic acid and aldehydic acid, cysteine and methionine were significantly up-regulated. The number of the up-regulated genes in the three pathways were 5, 5 and 9, and the number of the up-regulated genes is 3, 1 and 6 respectively. The UbiA (the transferase in the biosynthesis pathway of ubiquinone and terpenoid-quinone), the UlaF (the epimase in ascorbic acid and aldehydic acid metabolism pathway) and the MetB (the lyase in cysteine and methionine metabolism pathway) showed the most significant change in its transcription (Lilley et al. 1993; Campos et al. 2007; Kirby et al. 1986). In addition, the transcription of SodB (the superoxide dismutase Fe-SOD) and SoxS (the transcription activator) also increased significantly (Niederhoffer et al. 1990; Wu et al. 1991). These results meant that under the stress of high-concentration tellurite, the reactive oxygen was produced due to the redox unbalance, cells need to produce large amount of antioxidant products to repair the damage by excess reactive oxygen and the genes responsible for energy metabolism and oxidiation-reduction system.

The regulatory network in *E. coli* was affected *by* tellurite

Under the stress of tellurite, the transcription of transcriptional regulators and small RNAs in *E. coli* showed significant changes. The transcription levels of genes related to DNA replication, homologous recombination and mismatch repair pathway changed significantly. The transcription of DnaK protein increased which acts as molecular partners to aid protein folding and maintain the protein function (Kim et al. 2013; Bhandari et al. 2015). MarR, the transcriptional regulator promoting the transcription of drug efflux protein MarRAB, was also up-regulated significantly with the treatment of tellurite, which may increase the resistance of *E. coli* to tellurite (Cohen et al. 1988; Martin et al. 1995). Small RNA (sRNA) is a class of non-coding RNA, widely existing in organisms from bacteria to mammals and functioning as important regulatory factors of many cellular processes like metabolism, stress tolerance and detoxification reaction (Holmqvist et al. 2013). The transcription of several sRNAs including *glmZ*, *glmY*, *tff* and *rygD* were sown-regulated, while that of sRNA *oxyS* showed a significant decrease trend under the stress of tellurite. sRNA *oxyS* is a global regulator induced by oxidative stress, and sRNA *glmZ* and *glmY* can increase the stability of *glmS* mRNA and facilitate the synthesis of outer membrane peptidoglycan. These results implied that with the treatment of high-concentration tellurite for long time, the cellular regulatory network in *E. coli* was influenced significantly and the primary task of the cells was to maintain the core and basic function of macromolecules. Cells not only increased the transcription of some transcriptional regulators to promote biofilm formation for resistance, but also regulated the transcription of some sRNAs to cope with the imbalance of intracellular redox. Thus, cells could maintain a period of growth stagnation to reduce the energy consumption and repair the cellular damage.

Tellurite influenced the metal ion metabolism in *E. coli*

According to the effect on cells, metal ions can be divided into heavy metals with high toxicity to cells, functional divalent metal ions, univalency metal ions controlling membrane potential and other ions. Heavy metals can damage DNA, induce bacterial SOS responses, and accelerate genetic mutations after entering cells (Foster 2005). Arsenic is a highly toxic metal to cells that replaces phosphates and binds to the protein thiol group. ArsR, encoded by gene *arsR*, is a trans-acting factor with high affinity with arsenic and can improve the tolerance of strains to arsenic (Xu et al. 1996). The transcription of gene *arsR* was increased significantly under stress of tellurite, meaning the ArsR may participated in the reduction process of tellurite and improve the cell resistance. Meanwhile, the transcription of protein ChaA responsible for the reverse transport of Ca^{2+}/H^{+} and ZntA responsible for the efflux of Zn^{2+} also showed an up-regulation trend (Ohyama et al. 1994; Sharma et al. 2000). It has been reported that tellurite resistance gene is often located in the heavy metal island and adjacent to many heavy metal metabolism related genes. It could be speculated that under the stress of tellurite, cells need to pump out the tellurite and its reduced product, in which a synergistic function against heavy metals may be also achieved by increasing the efflux transportation of heavy metal ion. The tellurite anion and metal cations could be discharged into the environment and its effect on cell growth could be eliminated. As to the functional divalent metal ions like magnesium and manganese, the genes involved in their transportation also changed under the stress of tellurite. Magnesium is vital for ribosome stability; ribosome can bind more than 170 magnesium ions and keep its stable (Schuwirth et al. 2005). The ribosome metabolism pathway is one of the most significantly up-regulated pathway in *E. coli* MG1655 after the treatment of tellurite. The intracellular concentration of magnesium ions was also important for the flagella motor power (Imazawa et al. 2016). When the cellular Mg^{2+} is low, protein MgtS encoded by gene *mgtS* acts on the protein MgtA promoting the influx of Mg^{2+} and improve the sensitivity of antimicrobial peptides (Wang et al. 2017; Moon et al. 2013). MgtS can combine with small RNA MgrR and modulate the PitA phosphate symporter, involved in the entrance of tellurite, to boost intracellular magnesium levels (Yin et al. 2019). The treatment of tellurite induced the up-regulation in the transcription level of gene *mgtS*, implying a deficiency trend of intracellular Mg^{2+} concentration. On the contrary, the transcription of gene *mntP*, encoding protein MntP responsible for Mn^{2+} efflux, showed an down-regulation trend (Martin et al. 2015). These results indicated that the ribosome pathway was probably the main functioning pathway of tellurite toxicity. Tellurite entered into cells through PitA transporter with the pump-out of the intracellular Mg^{2+}, resulting in the decrease of cellular magnesium ions. Then cells activated the transcription of gene *mgtS* and *mgtA* to improve the influx of Mg^{2+}, enhance the free Mg^{2+} concentration, activate ribosome activity and maintain the cell metabolism. Besides, Calcium is helpful for cells adapt the stress environment and the genes for calcium transportation were also up-regulated with the treatment of tellurite (King et al. 2020). These results demonstrated that tellurite could decrease the intracellular concentration of magnesium ions, which can further affect the stability of ribosome, the movement of flagella and the activities of enzymes. The destroy of metal ions balance caused by the treatment of tellurite can also affect the cell respiration and the membrane potential, resulting the growth inhibition and cell death.

Tellurite affected some membrane proteins in *E. coli*

The transportation and reduction of tellurite was driven by some membrane proteins, and results showed that the transcription of some conserved membrane proteins involved in toxicity were changed with the treatment of tellurite. Protein YdeI is a conservative membrane protein of BOF protein family, which regulates bacterial toxicity and responds to stressful environments in *Salmonella* (Arunima et al. 2020). Under the stress of tellurite, the transcription of gene *ydeI* was decreased. Interestingly, the transcription of gene *tehB* as a tellurite resistance-related gene in the genome of *E. coli* MG1655 was also decreased. It implied that these membrane proteins repressed in the treatment of tellurite may participate in the transport and detoxication of tellurite in *E. coli*.

To promote to revealing the specific toxic mechanism of tellurite, this work tried to analyze the global transcriptional change of genes in *E. coli* MG1655 treated by tellurite of high-concentration for long time through the transcriptomic analysis. Some results of this work was consistent with those of previous transcriptomic and proteomics reports that the *E. coli* cells were treated with low-concentration of tellurite for short period (Aradská et al. 2013; Molina-Quiroz et al. 2014). The transcription of genes related to basic metabolic pathways, oxidative stress and resistance pathways changed significantly, implying that the basic metabolism for proteins, lipids, sugars, nucleic acids and other molecules was impaired and the oxidizing-reducing process was also stimulated by oxidative stress. It was similar to the effect of the oxidative stress induced by H_2O_2. Therefore, the resistance related genes were also induced to implement the non-specific defense protection. Interestingly, some genes responsible for ribosome metabolism, metal ion metabolism, and resistance-related membrane proteins, some transcriptional factors and small RNAs, were firstly observed with great enrichment, implying the ribosome metabolism and flagella assembly pathways may be the main functioning pathways of tellurite toxicity. However, different from the change in the oxidative stress induced by H_2O_2, proteins responsible for DNA breaks including protein RecFJQOR and RuvA, failed to show significant enrichments (Demple et al. 1994; Giroux et al. 2017). Under the treatment of high-concentration tellurite for long-time, cells have been in an adaptive reaction and began to activate multiple pathways to deal with stress and repair damage through a specific mechanism. It can be speculated that the ribosome suffering from oxidative damage was repaired later to maintain the basic metabolism, while cells were likely to decompose and discard the flagella to reduce cell movement and save energy. The transport system of heavy metal ions may assist in the exclusion of the tellurite anion in the cells, and several membrane proteins may also participate in the transfer process. Some regulatory factors and small RNAs regulating the stress resistance and toxicity detoxification also participated in the resistance to tellurite toxicity and restoration of cellular basic metabolism. This work can premilinaryly help to reveal the main and specific toxicity mechanism of tellurite to cells and the molecular mechanism of cell resistance. And even it may be beneficial to deliver us a spiration that the difference of toxicity mechanisms between tellurite and other oxidizing agents like hydrogen peroxide, further promoting the bioremediation for environmental pollution control and clinical response to drug-resistant pathogens.

Declarations

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Tables

Table 1. The transcription levels of the target genes detected by transcriptome sequencing and qRT-PCR

Genes	Function	Transcriptome sequencing (log_2FoldChange±padj)	qRT-PCR (log_2FoldChange±SD)
oxyS	sRNA, in response to oxidative stress	/	-0.955±0.865
glmZ	sRNA responsible for synthesis of the outer membrane peptidoglycan	/	4.434±0.785
glmY	sRNA, protecting glmZ from degradation	/	2.948±0.669
tff	sRNA	/	2.177±0.933
sraB	sRNA	/	0.131±1.164
rygD	sRNA	/	0.349±0.267
marR	Stress response regulator	4.166 ±1.3612E-30	3.480±0.274
arsR	arsenic efflux	2.3526 ±0.0001758	4.451±0.659
dnaK	molecular chaperones, in response to heat shock and protein aggregation	2.4857 ±3.11E-15	2.587±0.127
ydeI	molecular chaperones, in response to oxidative stress	-3.3778 ±0.01872	-5.924±0.082
mgtS	Mg^{2+} transport	4.7063 ±2.64E-21	6.091±0.190
tehB	tellurite resistance	-1.1855 ±0.034073	-4.247±1.837

Padj: The corrected statistical significance test index *p*-value; under normal circumstances. SD Standard Deviation, the square root of the arithmetic mean from the square of the mean (variance).

Figures

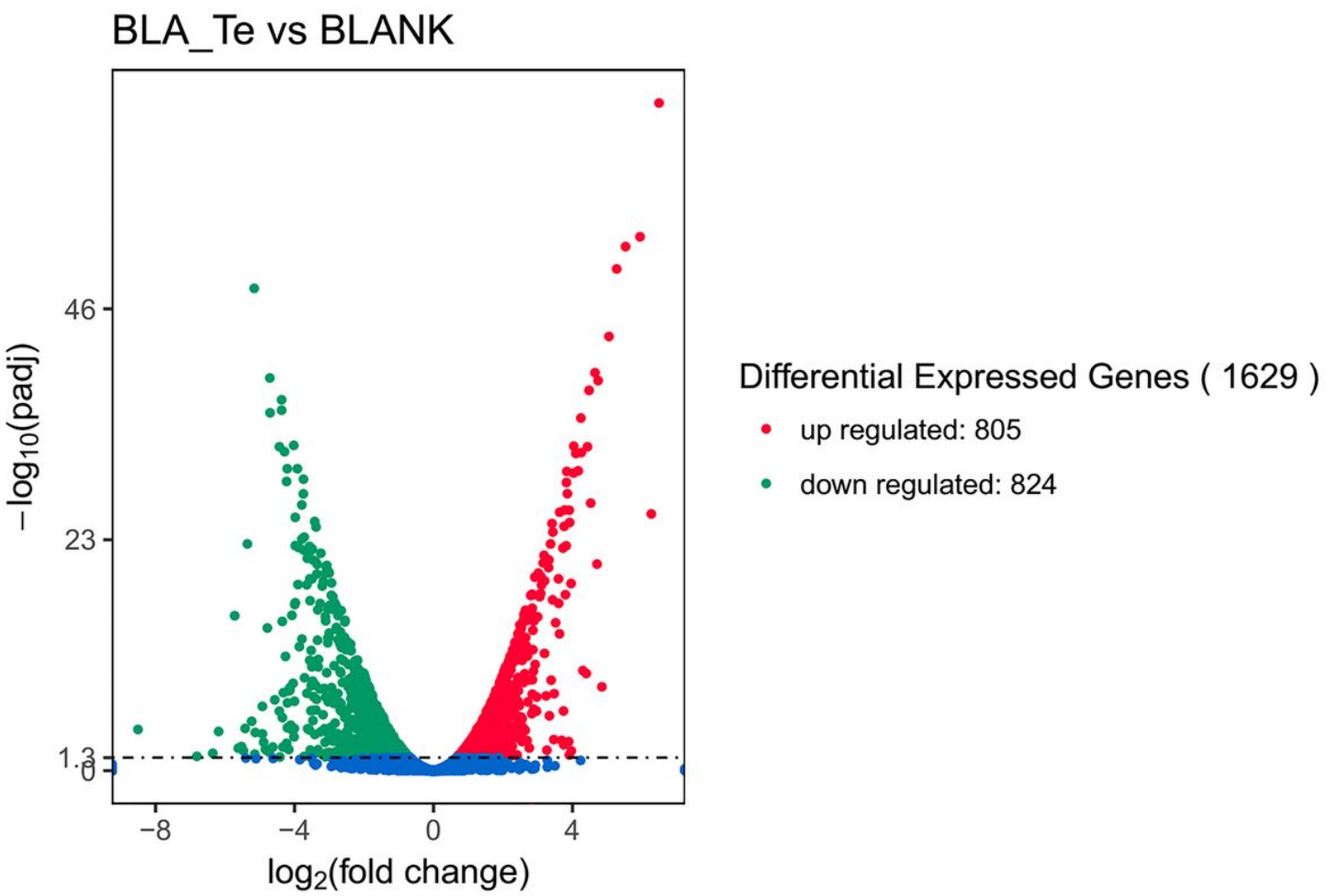

Figure 1

The volcano map of the differentially transcribed genes. BLA_Te was the tellurite treated groups and BLANK was the control group. The red dots represented the significantly up-regulated genes, the green dots represented the significantly down-regulated genes, and the blue dots were the representation of the genes with indistinctive transcription change. The X-coordinate was the transcription change folds in different samples, and the Y-coordinate was the statistically significant difference in the amount of gene transcription.

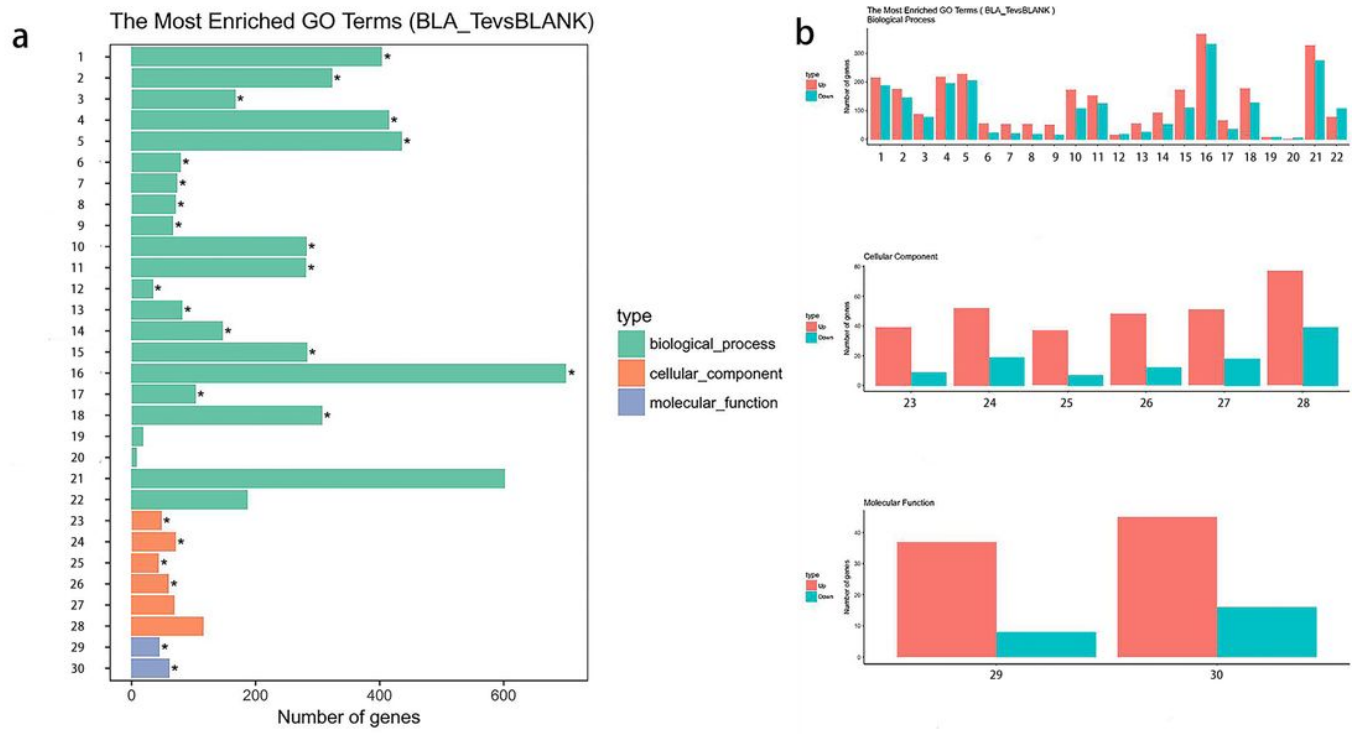

Figure 2

The enrichment of GO terms of the differentially transcribed genes. BLA_Te was the tellurite treated groups and BLANK was the control group. (a) the enriched GO terms of the differentially transcribed genes in biological process, molecular function and cellular component. (b) the enriched GO terms of the up-regulated genes and the down-regulated genes in biological process, molecular function and cellular component. The different GO terms were numbered and the GO terms with significant enrichment were marked with star. 1, cellular biosynthetic process; 2, cellular nitrogen compound biosynthetic process; 3. organonitrogen compound biosynthetic process; 4. organic substance biosynthetic process; 5. biosynthetic process; 6. amide biosynthetic process; 7. peptide metabolic process; 8. peptide biosynthetic process; 9. translation; 10. cellular macromolecule biosynthetic process; 11. organonitrogen compound metabolic process; 12. generation of precursor metabolic process; 13, cellular amide metabolic process; 14. protein metabolic process; 15. macromolecule biosynthetic process; 16. cellular process; 17. cellular protein metabolic process; 18. gene expression; 19. aspartate family amino acid metabolic process; 20. response to oxidative stress; 21. cellular metabolic process; 22. oxidation-reduction process; 23. ribonucleoprotein complex; 24. non-membrane-bounded organelle; 25. ribosome; 26. intracellular non-membrane-bounded organelle; 27. cytoplasmic part; 28. organelle; 29. structural constituent of ribosome; 30. structural molecule activity.

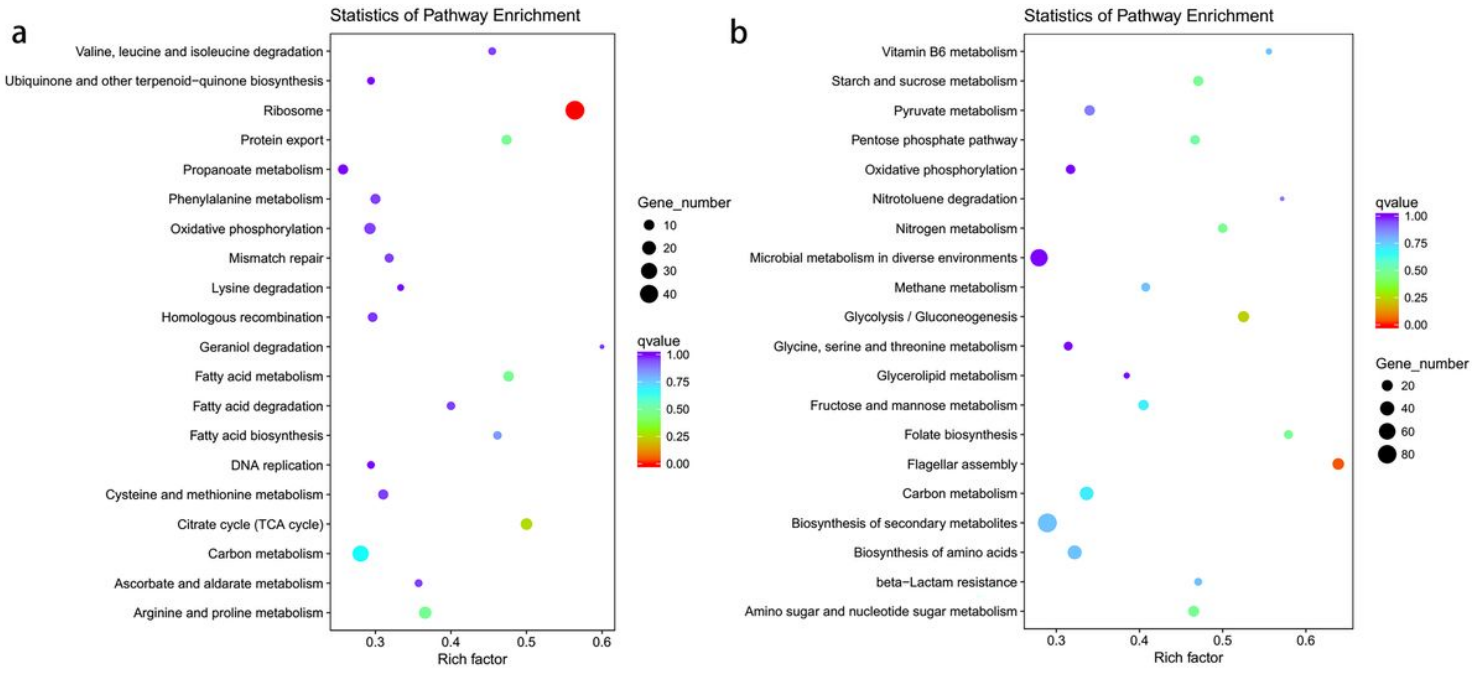

Figure 3

Scatter diagram of the KEGG analysis of the differentially transcribed genes. (a) the top 20 enriched KEGG pathways of the up-regulated genes. (b) the top 20 enriched KEGG pathways of the down-regulated genes. X-coordinate was the rich factor, which was the ratio of the number of differentially transcribed genes to the number of annotated genes in one pathway. Y-coordinate was the name of the pathways.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx