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Research Article 研究报告

Transcriptome profiling of *Escherichia coli* responding to tellurite

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Abstract: Tellurite as a strong antimicrobial agent is highly toxic to a variety of microorganisms, while its toxicity mechanism remains indistinct. **[Objective]** The main goal of this work is to uncover the global changes of cell metabolism under tellurite stress and reveal the toxicity mechanism of tellurite. **[Methods]** The transcriptomes of *Escherichia coli* MG1655 exposed to tellurite stress and under normal conditions were compared to reveal the differentially transcribed genes. **[Results]** After being treated with 10 μg/mL tellurite for 1 h, the cells exhibited an obvious adaptive response with many metabolic processes influenced. The transcription levels of the genes involved in ribosome metabolism and flagellar assembly changed significantly, implying the two pathways were affected by tellurite. The genes encoding the transcriptional factors and small RNAs and those functioning in the cell motility, metal ion metabolism, and membrane function also showed varied transcription levels, which might participate in the metabolism regulation and damage repair to resist the toxicity of tellurite. **[Conclusion]** This work can facilitate the study of the toxicity mechanism and promote the clinical application of tellurite.

Keywords: tellurite; comparative transcriptome; differentially transcribed genes; ribosome metabolism; flagellar assembly

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基于转录组分析大肠杆菌响应亚碲酸盐的机制

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摘　要：亚碲酸盐对绝大多数微生物有高毒性,可用作抗菌剂；但其具体毒性机制仍不清楚。【目的】理解亚碲酸盐的毒性机制，揭示亚碲酸盐处理导致的代谢变化。【方法】本研究通过比较转录组分析与挖掘差异转录基因，探讨了大肠杆菌响应亚碲酸盐胁迫的机制。【结果】*Escherichia coli* MG1655 在 10 μg/mL 亚碲酸盐处理 1 h 后，比较和分析了亚碲酸盐处理组与对照组的转录水平差异，发现细胞呈现一种明显的适应性变化，许多参与重要代谢途径的基因转录水平改变。其中，与核糖体代谢和鞭毛组装相关基因的转录水平发生显著变化，表明这两条途径很可能是亚碲酸盐作用的主要途径。与细胞能动性、金属离子代谢、细胞膜功能相关的基因的转录水平也发生了明显变化，可能是由于其参与了抵抗亚碲酸盐毒性的细胞代谢调节和损伤修复。【结论】本项工作有助于推动亚碲酸盐毒性机理的研究，促进亚碲酸盐的临床应用。

关键词：亚碲酸盐；比较转录组；差异转录基因；核糖体代谢；鞭毛组装

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[1]. 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 concentration of up to 1 μg/mL[1]. Therefore, it has been used as the antimicrobial agent for a long period before the application of antibiotics. Now the constant emerging of antibiotic resistance related pathogens has become one of the most concerned health problems[2]. Tellurite in sub-lethal concentration has been found 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[3]. It means that understanding the toxicity mechanisms of tellurite to bacteria may shed a light on the current dilemma of antibiotic resistance.

Previous research indicated that tellurite is toxic to cells as it can cause severe oxidation stress[4–5]. After entering the cell through phosphate transport protein PitA, tellurite can be reduced into the elemental tellurium, causing the oxidation of intracellular thiols and glutathiones, producing reactive oxygens and free radicals (O_2^-)[4–6]. The balance of the intracellular redox

state is disrupted and the produced superoxides can act on the metabolic enzymes containing the iron-sulfur center cluster, resulting in the loss of Fe^{2+}, the inactivation of enzymes and the damage of central metabolism[7]. Meanwhile, the produced superoxide and the released Fe^{2+} can also act on nucleic acids and membrane lipids, generating the lipid peroxides and affecting the cell processes[8]. Proteomics analysis showed that under the tellurite stress, the expression of antioxidase, superoxide dismutase, catalase, and oxidoreductase in cells enhanced significantly[9]. Tellurite can also inhibit the heme synthesis under aerobic and anaerobic conditions and produce the toxic hydroxyl radicals (OH), damaging the biomolecules and causing cell death[10]. However, there are still some contradictions in these proposed hypotheses. The molecular mechanisms of tellurite toxicity are still not very clear. Although the excessive reactive oxygen is considered as the main toxicant, tellurite is still toxic to cells in anaerobic conditions[11]. Selenite can also cause a higher level of reactive oxygen in the cells; however, its toxicity is much less than that of tellurite[12]. Moreover, *Deinococcus radiodurans* is strongly resistant to reactive oxygen, sensitive to tellurite[13].

Up to now, understanding the toxicity mechanisms of tellurite to microorganisms mainly focuses on the oxidative stress and other side-effects, similar to the effect of other chemical oxidizing agents like hydrogen peroxide. To excavate the potential toxicity of tellurite on cell metabolism, comparative transcriptome analysis has been performed in this study. Comparative transcriptome analysis can find the differentially transcribed genes under different conditions and study the metabolic variations in microorganisms in different stresses[14]. After the cells were treated with tellurite of 0.5 μg/mL for 15 min, in the minimal inhibition concentration (MIC) of *E. coli* for short time, cells showed an obvious decrease in the oxygen consumption and converted into the anaerobic breathing[15]. In this work, the comparative transcriptome analysis of the genes in *E. coli* MG1655 treated with tellurite of 10 μg/mL 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 hypotheses 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.

1 Materials and methods

1.1 Strains and materials

The *E. coli* MG1655 strain (F^- λ^- *ilvG*$^-$ *rfb*$^-$ *rph*$^-$) 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 M9 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 mmol/L, $CaCl_2$ 100 μmol/L, $FeSO_4$ 1 μmol/L, $CuSO_4$ 1 μmol/L, 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. All other reagents of analytical reagent grade purchased were from Sigma-Aldrich. The oligonucleotides used for reverse transcription and quantitative PCR (RT-qPCR) were synthesized at Sangon Ltd.

1.2 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 °C for 200 r/min. 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 °C for 200 r/min 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 °C. The experimental groups were marked as BLA_Te_1/BLA_Te_2 and the control groups 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.

1.3 Analysis of the differentially transcribed genes

The transcription levels of the genes in *E. coli* MG1655 cells under the tellurite stress were detected and compared with those of the cells cultured in the glucose condition (the control group). 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 gene transcription was padj<0.05. After screening the differentially transcribed genes, the effects of tellurite on gene function in *E. coli* are expounded with GO (gene ontology, http://www.geneontology.org/) database for the enrichment analysis. 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 analysis (kyoto encyclopedia of genes and genomes, https://www.genome.jp/kegg/).

1.4 RNA extraction and RT-qPCR

The cells of *E. coli* MG1655 were cultured as above and the total RNA was extracted; the RNA yield was determined using a Nanodrop UV spectrometer (Thermo Scientific). 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 the gene-specific primers (Table S1). The cycling conditions were 95 °C for 3 min, and 40 cycles of 95 °C for 10 s, 55 °C for 20 s and 72 °C for 20 s. A final melting analysis was obtained by slow heating with 10 s increments of 0.5 °C from 54 °C to 95 °C. The threshold cycle (Cq) value of each sample was determined and the relative fold changes in mRNA quantities were calculated using the DDCt method[16]. Three independent experiments were performed for each sample and the average values with the standard errors were calculated.

1.5 Quality analysis of the transcriptome sequencing data

The data quality of the transcriptome sequencing was the basis of subsequent effective analysis. After the high-throughput sequencing, the numbers 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 numbers of clean reads were 14 634 334, 17 166 478, 21 053 516, and 18 834 606, respectively. The clean bases were 2.20 G, 2.57 G, 3.16 G, 2.83 G, respectively; the Q20 percent (%) reached 98.22, 97.90, 97.89, 98.05, and the Q30 percent (%) reached 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 were of high quality and could fit the requirement of the subsequent bioinformatics analysis. In addition, the reference sequences comparison showed that the sequence numbers 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 reached 78.39, 80.79, 58.81, 80.79, respectively. Besides, the percentage of the multiple positioning sequences to the total sequences was 3.74, 4.47, 2.61, 2.8. These results demonstrated that the reference genome was appropriate and no contamination happened 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 coefficients 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 that the transcription abundances were 42.43%, 40.00%, 34.88% and 33.39%, respectively, when the four samples were at FPKM>60. It meant that the transcription abundance and the transcription levels of these samples were high.

2 Results and analysis

2.1 Summary of the differentially transcribed genes

Comparing the transcriptome data of the tellurite-treated groups and the glucose-cultured groups, total of 1 629 genes in *E. coli* MG1655 cells were found to be of significant change in their transcription levels under the tellurite stress (the transcriptome data can be obtained in: https://www.ncbi.nlm.nih.gov/sra/PRJNA741877). The transcription levels of 805 genes were significantly up-regulated (P<0.05), and among them, the transcription levels of 633 genes were increased strikingly (P<0.01). On the other hand, the transcription levels 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 (Figure 1). Little difference was observed in the number of the up-regulated genes and the down-regulated genes in these *E. coli* MG1655 cells between the treatment of 0.5 μg/mL tellurite for 15 min and the treatment of 10 μg/mL for 1 h. It implied that the treatment of tellurite in high concentration for long time actually affected the transcription of certain genes, not the inhibited the transcription of indiscriminate genes. And *E. coli* cells would adopt an adaptive reaction to repair the damage and maintain cell survival by modifying certain metabolic pathways.

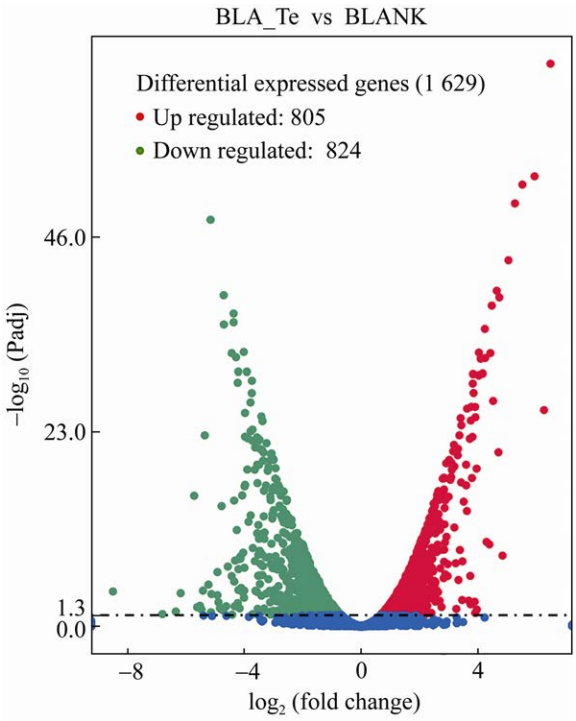

Figure 1 The volcano map of the differentially transcribed genes. BLA_Te was the tellurite treated group and BLANK was the control group. The red dots represented the significantly up-regulated genes, the green dots represented the significantly down-regulated genes, and the blue dots were the representation of the genes with indistinctive change in transcription. The X-coordinate represented the transcription change folds in different samples, and the Y-coordinate represented the statistically significant difference in the amount of gene transcription.

2.2 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 1 629 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 1 940 GO terms, and 46 GO terms showed the

significant enrichment (P<0.05). As to the down-regulated genes, 1 805 GO terms were enriched and 50 GO terms of them showed the significant enrichment (P<0.05).

For the 46 enriched GO terms of the up-regulated genes, 24 GO terms of them 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 differentially transcribed genes with the largest number were enriched into the molecular function (557 genes), followed by 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 cell (144 genes) and cell parts (144 genes) (Table S3). These results demonstrated that the genes related to molecular function, cell process and the organic substrate metabolism were significantly up-regulated after the treatment of tellurite in high concentration for long time, implying cells tried to resist the tellurite toxicity via strengthening the molecular syntheses and the central metabolic pathways.

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 differentially transcribed genes with the largest number 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 the oxidoreductase activity, the oxidation reduction process and the cofactors binding process in bacterial cells were inhibited significantly with the treatment of tellurite in high concentration for long time.

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 (Figure 2). Results indicated that most of the genes significantly changed in their transcription 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 the up-regulated genes was higher than that of the down-regulated genes. It meant that the effect of tellurite toxicity mainly functioned in 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 effects of tellurite.

2.3 The KEGG functional enrichment analysis of the differentially transcribed genes

KEGG functional enrichment analysis can systematically predict the metabolic pathways that the target genes involved in. The KEGG analysis of the 1 629 differentially transcribed genes of *E. coli* MG1655 cells under tellurite treatment was performed to explore the potential metabolic pathways affected by the tellurite stress. Results indicated that the up-regulated genes were enriched into 77 metabolic pathways, and down-regulated genes were enriched into 82 metabolic pathways. The top 20 enrichment pathways of the up-regulated genes and the down-regulated genes were shown (Figure 3).

The top 5 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

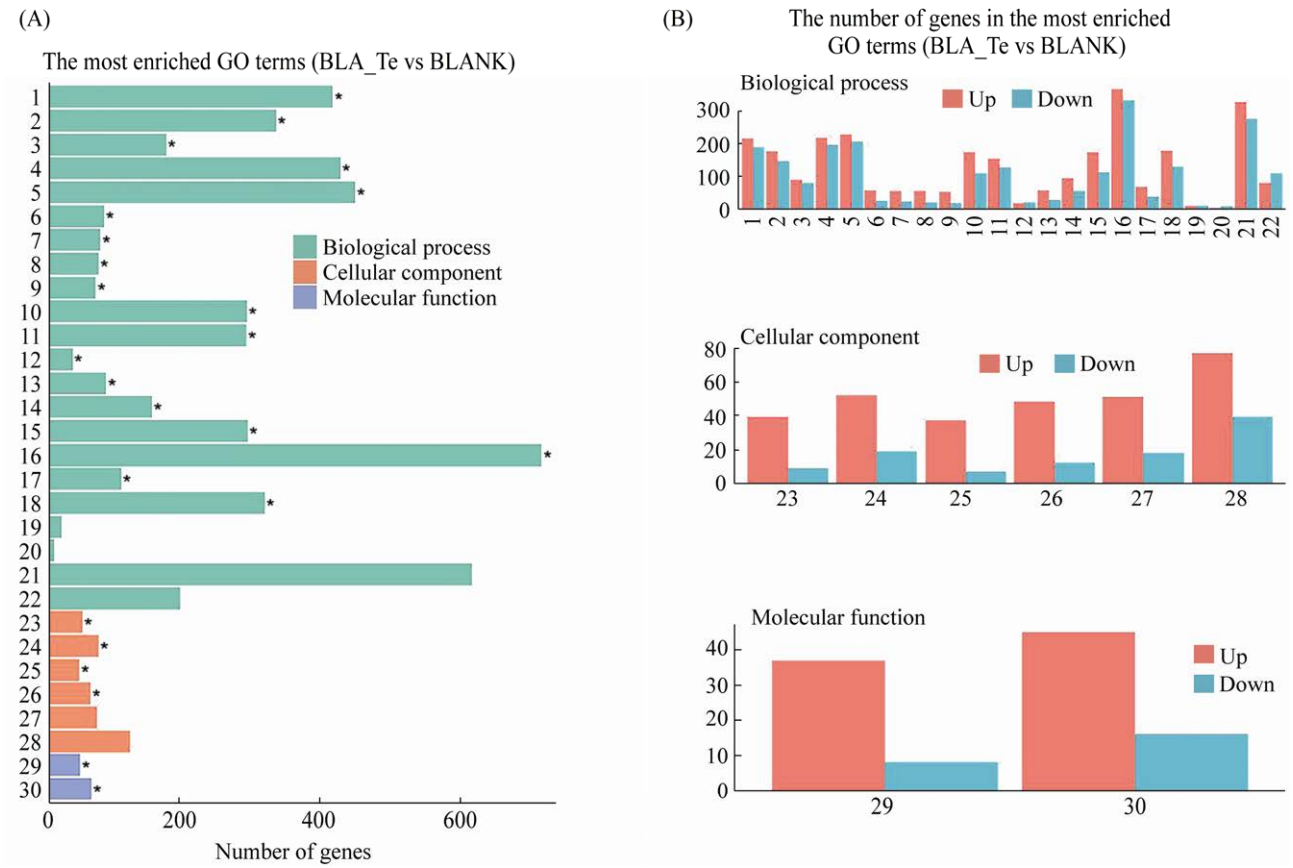

Figure 2 The enrichment of GO terms of the differentially transcribed genes. BLA_Te was the tellurite treated group and BLANK was the control group. A: the enriched GO terms of the differentially transcribed genes in biological process, molecular function and cellular component; B: the enriched GO terms 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.

arginine and proline metabolism. Among them, the ribosome pathway was strikingly enriched (Q value of 5.17E-06). There were 78 annotated genes involved in this pathway, and the transcription of 44 genes was significantly 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 3.386 1, 2.915 6, 2.709, 2.606 8, 2.604 8,

respectively, implying these genes and this pathway were activated significantly under the tellurite stress (Figure 3A). On the contrary, the top 5 enriched metabolic pathways of the down-regulated genes were the 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 the most significantly enriched with the Q value of 0.048 239 876; 23 genes of the 36 annotated genes in this pathway showed differential transcription and the rich factor was 0.64. The genes in the flagellar assembly metabolic pathway with the highest fold change were *flhC*, *flhD*, *flgH*, *fliS*, and *fliE*, and the down-regulated fold reached –3.488 0, –3.337 6, –3.201 8, –3.109 7, and –3.064 0, respectively, implying these genes and this pathway were inhibited significantly under the tellurite stress (Figure 3B). All these results demonstrated that the basic cellular biomolecular metabolic pathways and energy metabolism including amino acids, nucleotides, polysaccharides, lipids, vitamins, and etc., were severely affected by the treatment of tellurite in high concentration for long period. The great influence on the function of ribosome and the assembly of flagella indicated the cells were in an adaptive state to strengthen the ribosome activity and reduce the cell motility to ensure enough energy for the maintaining of core metabolism.

2.4 The RT-qPCR verification of the differentially transcribed genes

The transcription levels of several genes in *E. coli* MG1655 treated with tellurite were analyzed by RT-qPCR to verify the transcriptome results. According to their potential metabolic pathways, the target genes were the genes probably responding to the tellurite stress, 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 mechanisms of cells to tellurite stress, the gene *tehB* annotated as the tellurite resistance-related genes, was also selected. RT-qPCR results showed that the transcription levels 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 were observed in the transcription of gene *sraB* and *rygD* (Figure 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 changes of most of the functional genes were both detectable and in the similar trend, besides some differences in the transcription levels of some small RNAs (Table 1).

3 Discussion

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

Ribosomes are important places where mRNAs are translated into amino acids, producing proteins to maintain cellular structure and cell survival[17]. After the tellurite treatment, the ribosome metabolism pathway was significantly enriched; 44 genes were up-regulated and 2 genes were down-regulated (Figure 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, respectively[18–22]. Ribosome protein L4 can not only regulate the synthesis of its own protein and inhibit the translation of other ribosome proteins, but also can regulate the RNase E activity to enhance the expression levels of stress response proteins and sustain normal cellular metabolisms in adverse conditions[23–25]. The ribosome protein S16 participates in the assembly of 30S ribosome, and possesses the endonuclease activity dependent on

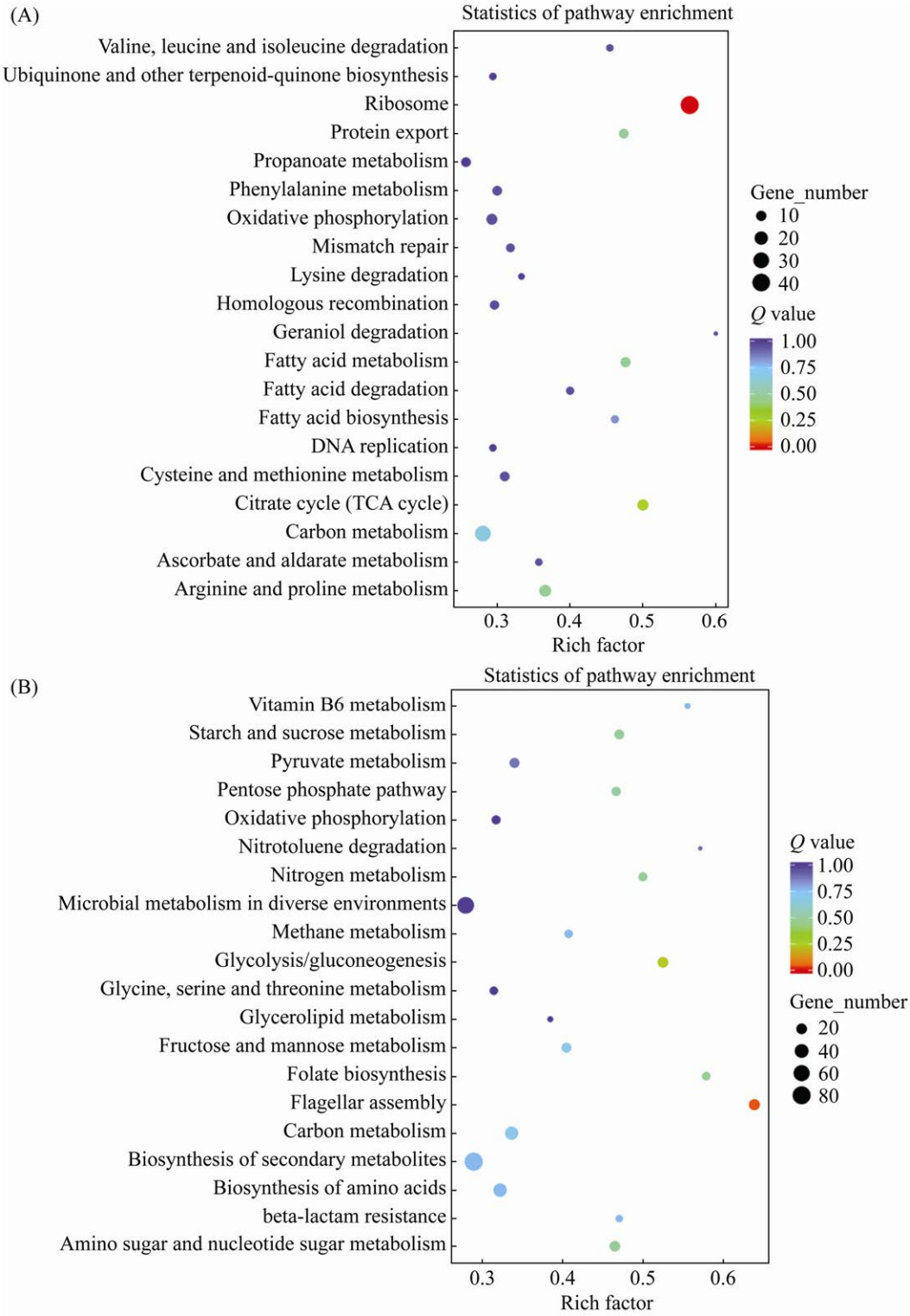

Figure 3 Scatter diagram of the KEGG analysis of the differentially transcribed genes. A: the top 20 enriched KEGG pathways of the up-regulated genes; B: the top 20 enriched KEGG pathways of the down-regulated genes. X-coordinate represented the rich factor, which was the ratio of the number of differentially transcribed genes to the number of annotated genes in one pathway. Y-coordinate indicated the pathways.

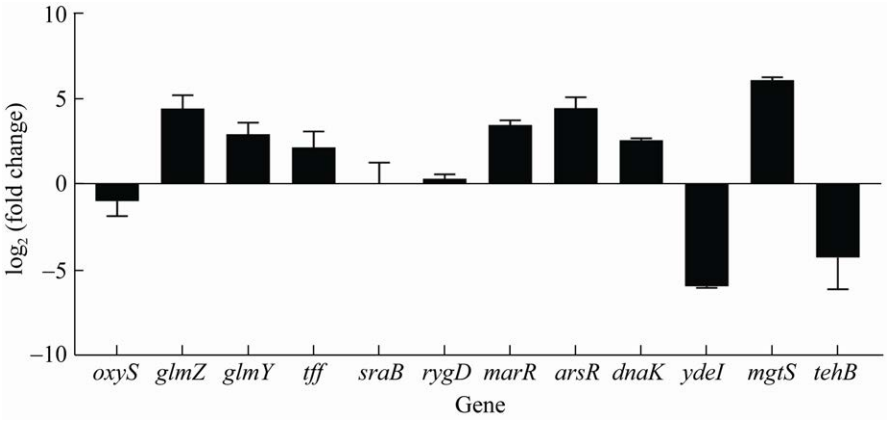

Figure 4 The transcription detection of target genes with RT-qPCR. The *E. coli* MG 1655 cells were cultivated in fresh M9 medium or M9 medium containing 10 μg/mL of tellurite and cultured for 1 h at 37 °C. The total RNA was extracted and the transcription levels of the target genes were detected by RT-qPCR. X-coordinate represented the target genes, Y-coordinate represented the fold change levels of the target genes.

Table 1 The transcription levels of the target genes detected by transcriptome sequencing and RT-qPCR

Genes	Function	Transcriptome sequencing (log_2 fold change±*Padj*)	RT-qPCR (log_2 fold change±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±1.361 2E-30	3.480±0.274
arsR	Responsible for arsenic efflux	2.352 6±0.000 175 8	4.451±0.659
dnaK	Molecular chaperones, in response to heat shock and protein aggregation	2.485 7±3.11E-15	2.587±0.127
ydeI	Molecular chaperones, in response to oxidative stress	–3.377 8±0.018 72	–5.924±0.082
mgtS	Responsible for Mg^{2+} transport	4.706 3±2.64E-21	6.091±0.190
tehB	Responsible for tellurite resistance	–1.185 5±0.034 073	–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 means from the square of the mean (variance). –: no result.

Mg^{2+} and Mn^{2+}[21,26]. Ribosome protein L19 is responsible for assembly of 50S ribosome, connecting the large subunit to the small subunit[21,27]. With the treatment of tellurite in high concentration for long time, the ribosome metabolism pathway in cells was enhanced to promote the protein synthesis by restoring the assembly of ribosome subunits and improving the ribosome activity. Meanwhile, the intracellular RNase E and endonuclease activity were also increased to strengthen the stress responses and damage repair processes.

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

Flagellum is responsible for cell motility and chemotaxis, and its assembly is an energy

consuming process[28]. Under the nutritional deficient conditions, cells will lose flagella[29]. After the tellurite treatment, the flagella assembly pathway was significantly enriched; 23 genes were down-regulated and only 4 genes were up-regulated (Figure 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 an activator for the transcription activation of gene *fliA* and participated in the regulation of the flagella synthesis[30–31]. FlgH is the component of the L-ring located on the outer membrane layer, forming a molecular tube with the P-ring[32–33]. And FliS is the regulator of flagella synthesis and the component of flagella hook and substrate complex[34]. Therefore, after the treatment of tellurite in high concentration for long time, the energy generation processes in bacterial cells were inhibited. Cells tended to reduce the synthesis and assembly of flagella and preserve the cellular energy to maintain the core metabolism and conduct the damage repair.

3.3 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 were 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 structures of water-soluble proteins and promote synthesis of ATP through the synthesis of nicotinamide adenine dinucleotide and reduction of flavin adenine dinucleotide. The transcription levels 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[35]. 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[36]. In the lysine biosynthesis pathway, three genes were up-regulated, and five genes were down-regulated; the transcription of gene *dapD* was repressed most[37]. In arginine and proline metabolism pathway, the transcription levels of fifteen genes were enhanced and those of five genes were reduced; the putrescine metabolic gene, *puuD* was up-regulated significantly[38]. As to the amino sugar and nucleotide metabolism, twenty genes were decreased and gene *nanA* participating in the degradation of sialic acids changed significantly[39]. 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[40]. These results showed that the protein synthesis in *E. coli* was repressed and the protein degradation process was accelerated after the treatment of high-concentrated tellurite for long time. As the fundamental protein metabolism was affected, cells were hard to survive.

The glycolysis/gluconeogenesis pathway is the main route of sugar metabolism to maintain the cellular balance of sugar[41]. The transcription levels of most of the genes (21 genes) in this pathway were reduced, and gene *adhE* encoding the ethanol/acetaldehyde dehydrogenase was repressed significantly[42]. 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, sugars and even nucleic acids[43]. Thirteen genes in this pathway were 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 needed 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. It demonstrated that the transcription levels of the most genes related to energy metabolism and material metabolism changed significantly under the stress of tellurite, implying the genes and pathways in bacterial cells responding to tellurite were numerous and complex.

3.4 Tellurite treatment influenced the cellular oxidation-reduction balance

Previous work showed that tellurite was toxic to cells due to their oxidation, including the thiol consumption, the alteration of reduction potential and the production of reactive oxygen; the expression of reactive oxygen detoxifying enzymes in cells were also increased with the addition of tellurite[6,13,44–45]. 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 numbers of the up-regulated genes in the three pathways were 5, 5 and 9, and the numbers of the up-regulated genes were 3, 1 and 6, respectively. The UbiA (the transferase in the biosynthesis pathway of ubiquinone and terpenoid-quinone), the UlaF (the epimerase in ascorbic acid and aldehydic acid metabolism pathway) and the MetB (the lyase in cysteine and methionine metabolism pathway) showed the most significant changes in their transcription levels[46–48]. In addition, the transcription levels of SodB (the superoxide dismutase Fe-SOD) and SoxS (the transcription activator) were also increased significantly[49–50]. These results meant that the reactive oxygen was produced due to the redox unbalance under the stress of tellurite in high concentration, and cells needed to upregulated the genes responsible for energy metabolism and oxidation-reduction system to produce a large amount of antioxidant products for the damage repair.

3.5 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* cells also showed significant variation. The transcription levels of genes related to DNA replication, homologous recombination and mismatch repair pathways were changed significantly. DnaK protein acts as molecular partner to aid protein folding and maintain the protein function; its transcription was enhanced[51–52]. MarR, the transcriptional regulator with promoting the transcription of drug efflux protein MarRAB, was also up-regulated significantly, which may improve the cell resistance to tellurite[53–54]. 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[55]. The transcription levels of several sRNAs including *glmZ*, *glmY*, *tff* and *rygD* were up-regulated, while that of sRNA *oxyS* showed a significant decrease under the stress of tellurite. sRNA *oxyS* is a global regulator induced by oxidative stress; 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 tellurite in high concentration 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.

3.6 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[56]. 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[57]. The transcription level of gene *arsR* was increased significantly under the stress of tellurite, meaning that ArsR may participate 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[58–59]. It has been reported that tellurite resistance genes are 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 needed to pump out the tellurite and its reduced products, in a synergistic function against heavy metals which may be also achieved by increasing the efflux transportation of heavy metal ions. The tellurite anions 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 transcription levels of genes involved in their transportation were also changed under the stress of tellurite. Magnesium is vital for ribosome stability; ribosome can bind more than 170 magnesium ions and keep its stability[60]. The ribosome metabolism pathway was one of the most significantly up-regulated pathways in *E. coli* MG1655 after the treatment of tellurite. The intracellular concentration of magnesium ions was also important for the flagella motor power[61]. 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[62–63]. MgtS can combine with small RNA MgrR and modulate the PitA phosphate symporter, involved in the entrance of tellurite, to boost intracellular magnesium levels[64]. 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 a down-regulation trend[65]. These results indicated that the ribosome pathway was probably the main functioning pathway of tellurite toxicity. Tellurite might enter 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 to adapt the stressful environments and the genes for calcium transportation were also up-regulated with the treatment of tellurite[66]. 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 tellurite can also affect cell respiration and membrane potential, resulting to growth inhibition and cell death.

3.7 Tellurite affected some membrane proteins in *E. coli*

The transportation and reduction of tellurite are driven by some membrane proteins. Results showed that the transcription levels 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*[67]. 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 be related to the transport and detoxication of tellurite in *E. coli*.

In a word, to promote the toxicity mechanism study of tellurite, this work tried to analyze the global transcriptional changes of genes in *E. coli* MG1655 cells treated by tellurite in high concentration for long time through the transcriptome analysis. Some results of this work were consistent with those of previous transcriptomic and proteomics reports in which *E. coli* cells were treated with tellurite of low concentration for short period[9,15]. The transcription levels of genes related to basic metabolic pathways, oxidative stress and resistance pathways changed significantly, implying that the basic metabolisms for proteins, lipids, sugars, nucleic acids and other molecules were 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 enrichments after the treatment of tellurite, implying the ribosome metabolism and flagellar assembly pathways may be the main pathways affected by tellurite. However, genes responsible for DNA breaks in the oxidative stress of H_2O_2, encoding protein RecFJQOR and RuvA, failed to show significant enrichments[68–69]. It indicated that cells had been in an adaptive reaction and began to activate multiple pathways to deal with stress and repair damage through a specific mechanism under the treatment of tellurite in high concentration for long-time. It can also 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. Therefore, this work can preliminarily help to reveal the main and specific toxicity mechanism of tellurite and the molecular mechanism of cells resistance. And the difference of toxicity mechanisms between tellurite and other oxidizing agents like hydrogen peroxide, may further promote the potential appliance for drug-resistant pathogens.

Conflict of interest: all authors declare that they have no conflict of interest.

Ethical approval: this article does not contain any studies with human participants performed by any of the authors.

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补充材料

Table S1　The oligonucleotides for RT-qPCR detection
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Table S2　List of the genes with significantly differential transcription
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Table S3　The GO functional enrichment analysis of the up-regulated and the down-regulated genes
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