Genome-wide screening and characterization of genes involved in response to high dose of ciprofloxacin in Escherichia coli

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Abstract

Background

Antibiotic resistance is an urgent threat to public health. Prior to the evolution of antibiotic resistance, bacteria frequently undergo response and tend to develop a state of adaption to the antibiotic. Ciprofloxacin is a broad-spectrum antibiotic by damaging DNA. With the widespread clinical application, the resistance of bacteria to ciprofloxacin continues to increase. This study aimed to investigate the transcriptome changes under the action of high concentration of ciprofloxacin in *Escherichia coli*.

Results

We identified 773 higher expressed differentially expressed genes (DEGs) and 645 lower expressed DEGs in ciprofloxacin treated cells. Enriched biological pathways reflected the up-regulation of biological process such as DNA damage and repair system, toxin/antitoxin systems, formaldehyde detoxification system, peptide biosynthetic process and cellular protein metabolic process. With KEGG pathway analysis, higher expressed DEGs of *kdsA* and *waa* operon were associated with “LPS biosynthesis”. *rfbABC* operon was related to “streptomycin biosynthesis” and “polyketide sugar unit biosynthesis ”. Lower expressed DEGs of *thrABC* and *fliL* operons were associated with “flagellum-dependent cell motility” and “bacterial-type flagellum” in GO terms, and enriched into “biosynthesis of amino acids” and “flagellar assembly” in KEGG pathways. After treatment of ciprofloxacin, bacterial lipopolysacchride (LPS) release was increased by two times, and the mRNA expression level of LPS synthesis genes, *waaB, waaP* and *waaG* were elevated ($P < 0.05$).

Conclusions

Characterization of the gene clusters by RNA-seq showed high dose of ciprofloxacin not only lead to damage of bacterial macromolecules and components, but also induce protective response against antibiotic action by up-regulating the SOS system, toxin/antitoxin system and formaldehyde detoxification system. Moreover, genes related to biosynthesis of LPS were also higher expressed by the treatment indicating that ciprofloxacin can enhance the production of endotoxin on the level of transcription. These results demonstrated that transient exposure of high dose ciprofloxacin is double edged. Cautions should be taken when administering the high dose antibiotic treatment for infectious diseases.

Background

Ciprofloxacin is among the most commonly prescribed class of fluoroquinolones that is widely used in the therapy of mild-to-moderate urinary and respiratory tract infections caused by *Enterobacteriaceae*. However, growing resistance to these agents leads to the decline of their clinical efficacy. Prior to the

evolution of antibiotic resistance, bacteria frequently undergo response and tend to develop a state of adaption to the antibiotic.

Antibiotic exposure has been shown to promote the emergence of resistant bacteria [1]. There are many environments or human tissues during therapy where bacteria are exposed to low concentrations of antibiotic (less than minimum inhibitory concentration, MIC). Many studies have shown the profound transcriptomic changes in the bacterial cells and their potential contributions to the evolution of resistance when exposed to low levels of ciprofloxacin [2, 3]. In the therapeutic use of ciprofloxacin, levels of ciprofloxacin in tissues or serum can be reach or exceed the MIC. Exposed to ciprofloxacin at concentrations above the MIC for a prolonged time will kill the susceptible bacteria and select the resistant mutation. However, less is known about the transcriptional influence of a transient, high level (> MIC) exposure of ciprofloxacin in bacteria. This study investigated the transcriptome changes under the action of high concentration ciprofloxacin in *Escherichia coli.*

Results

Bacteria growth

The growth of MG1655 before and after ciprofloxacin treatment were shown in Fig. 1. Bacterial strains were growth from cell concentration of 7.53 Log_{10}CFU/mL to 8.00 Log_{10}CFU/mL within 60 minutes under normal growth conditions. After treatment with 2 μg/mL ciprofloxacin, bacterial strains cell concentration of 3.66 Log_{10}CFU/mL at 30 minutes and 3.24 Log_{10}CFU/mL at 60 minutes.

Overview of sequencing data

The quality of sequencing data from ciprofloxacin treated MG1655 and the controls were summarized and showed in Table 1. A total of 68,032,024 raw reads, corresponded to 63,641,782 clean reads and 7.97 G bases were got, with Q_{20} value > 95%, Q_{30} value > 90%, and GC content ranged from 51.76–52.43%. High mapping rate (> 70%) was detected after BLASTx alignment to reference data in NCBI (Table 2). Also, almost all of the reads were mapped to CDS genome regions (> 98%, **Figure S1**). All the rest reads were unannotated reads (≤ 2.2%) because of the incomplete annotation, non-coding RNA, or background noise. With Pearson correlation analysis, we showed there were high repeatabilities and minor variations between samples (Fig. 2).

Table 1
Summary of sequencing data quality.

Sample	Raw reads	Clean reads	Clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
Con 1	11218210	10962456	1.37G	0.03	96.40	92.38	52.01
Con 2	11667262	10682158	1.34G	0.02	96.08	91.35	52.41
Con 3	11203348	10374598	1.30G	0.02	96.55	92.19	51.96
Cip 1	10744572	10476204	1.31G	0.03	96.28	92.18	51.79
Cip 2	11601186	10454834	1.31G	0.02	95.66	90.64	52.25
Cip 3	11597446	10691532	1.34G	0.02	96.55	92.18	52.43
Q20, Q30: percentage of bases with Phred value > 20, 30, resepectively.							

Table 2
Summary of clean reads mapped to reference genome.

Sample name	Con 1	Con 2	Con 3	Cip 1	Cip 2	Cip 3
Total reads	10962456	10682158	10374598	10476204	10454834	10691532
Total mapped	10912603 (99.55%)	8092348 (75.76%)	10313500 (99.41%)	10410681 (99.37%)	9959922 (95.27%)	10480412 (98.03%)
Multiple mapped	179843 (1.64%)	280058 (2.62%)	309376 (2.98%)	248721 (2.37%)	469673 (4.49%)	407095 (3.81%)
Uniquely mapped	10732760 (97.9%)	7812290 (73.13%)	10004124 (96.43%)	10161960 (97%)	9490249 (90.77%)	10073317 (94.22%)
Read-1	5366198 (48.95%)	3912311 (36.62%)	5007936 (48.27%)	5081448 (48.5%)	4754094 (45.47%)	5043002 (47.17%)
Read-2	5366562 (48.95%)	3899979 (36.51%)	4996188 (48.16%)	5080512 (48.5%)	4736155 (45.3%)	5030315 (47.05%)
Reads map to '+'	5366149 (48.95%)	3906099 (36.57%)	5002349 (48.22%)	5080583 (48.5%)	4745896 (45.39%)	5036828 (47.11%)
Reads map to '-'	5366611 (48.95%)	3906191 (36.57%)	5001775 (48.21%)	5081377 (48.5%)	4744353 (45.38%)	5036489 (47.11%)

Read-1 and − 2: the forward and reverse sequence of pair-end sequencing; '+' and '-': alignment to the + strand and − strand of the reference genome.

Transcript annotation

With the annotation to Nr database, we identified transcripts including DEGs, sRNA, and novel transcripts. A total of 51 novel transcripts (evalue ≤ 1e-5) were identified using assembly of sequenceing data against reference genome in Nr database. Also, 33 sRNAs were identified with the length ranged from 50 bp to 175 bp (average length of 78.45 bp, **Figure S2A**). Length of 3'-UTR (average length of 44.34 bp) and 5'-UTR (average length of 45.08 bp) of most of these transcripts was less than 100 bp (**Figure S2B and C**). Among those transcripts, a total of 647 lower expressed and 797 higher expressed differentially expressed transcripts (DETs) were identified (Fig. 3). Among these DETs, a total of 97 SNPs and 47 InDels were detected (Table 3). Also, 40 SNPs and 30 InDels were located at DEGs, the others were located at sRNAs and novel transcripts. With transformed $\log_{10}$(FPKM + 1) values, hierarchical clustering analysis of all DETs were performed and 6 distinct clusters were got (Fig. 4). Among those 797 higher expressed transcripts, 6 sRNAs, 18 novel transcripts, and 773 DEGs were included (including *lexA*, *recA*, *recX*, *tisB*, *gyrA*, *gyrB*, *stpA*, and *dnaN*), and the lower expressed transcripts were consisted of 2 novel transcripts and 645 DEGs (including *thrA-C*, *flgA-B*, and *fliF-L*; **Table S1**). These sRNAs targeted to thousands of genes (data not shown). Operon analysis showed there were 828 operons, and most of the operons were consisted of 2–5 tandem genes (91.06%, **Table S2**). Percentage of operons consisting of 10–15 tandem genes was 1.33%. This was in accordance with the promoter predication result that 1–20 promoter sequences with high scores were identified in one transcript (**Table S3**).

Table 3
Summary and distribution of SNP and InDel in differentially expressed transcripts.

Name	Con 1	Con 2	Con 3	Cip 1	Cip 2	Cip 3	Total
SNPs	38	32	31	51	43	41	97
InDels	32	39	27	33	39	33	47

GO and KEGG pathway enrichment analysis

In order to draw the functional annotation and pathways associated with these DEGs, we performed the GO and KEGG pathway enrichment analysis of DEGs induced by ciprofloxacin treatment.

Results showed higher expressed DEGs encoding "putative fimbrial-like adhesin protein" (e.g. *ybgD*, *yraH*, *yehA*, *yfcV* and *csgAB* operon) were enriched into pilus, cell part, cell adhesion and so on. Higher expressed DEGs encoding 50S/30S ribosomal subunit protein (e.g. *frmRAB*, *S10*, and spc ribosomal protein operons) and DEGs encoding regulator protein that represses *frmRAB* operon which encodes a formaldehyde detoxification system. DNA recombination and repair protein (*recA*) were associated with GO terms related to ribosome complex, cellular amide metabolic process, etc. Toxic membrane persister formation peptide gene *(tisB)* was also higher expressed, which is regulated by *lexA* and participate in toxin/antitoxin systems. Some DEGs were associated with DNA repair or replication pathways, e.g. *dnaN* and *holD* (**Table S4 and** Fig. 5A). Lower expressed DEGs encoding proteins related to flagellum

development (e.g. *flgG*, *flgB*, and *fliL* operons), including GO terms of cilium or flagellum-dependent cell motility, bacterial-type flagellum, and bacterial-type flagellum basal body (**Table S4 and** Fig. 5B).

With KEGG pathway analysis, the higher expressed DEGs and lower expressed DEGs were associated with 2 significant pathways, respectively (adjusted *p*-Value < 0.05, **Table S5 and** Fig. 6). However, these terms were too few to comprehensively understand the mechanisms related to ciprofloxacin responses in MG1655. So we selected the top 20 KEGG pathways to analyze the related mechanisms expendably. Accordingly, we found the higher expressed DEGs (e.g. *S10*, *spc, recR, ruvABC, waa, rfbABC*, and *frmRAB* operon) were enriched into biosynthesis of secondary metabolites, including pathways of ribosome, methane metabolism, thiamine metabolism, and so on (**Table S5 and** Fig. 6A). *waa* operon (includes 10 genes) was associated with LPS biosynthesis. *rfbABC* operon (encodes 3 polypeptides *rfbA*, *rfbB*, and *rfbC*) was related to streptomycin biosynthesis and polyketide sugar unit biosynthesis. Most of lower expressed DEGs (e.g. *thrABC*, *flgG*, *flgB*, and *fliL* operons) were enriched into pathways related to amino acids metabolism and flagellum development, including pathways of biosynthesis of amino acids, flagellar assembly, arginine and proline metabolism, etc. The nar operon (encodes 4 polypeptides NarG, NarH, NarJ and Narl), and *gltBD* operons (encodes *gltB* and *gltD*) were related to nitrogen metabolism (**Table S5 and** Fig. 6B).

Real-time PCR verification

The expression level of *recA* was elevated from 9.13 ± 2.20 to 62.25 ± 8.46 after ciprofloxacin treatment ($P < 0.001$). The mRNA expression level of LPS synthesis genes, *waaB, waaP* and *waaG* were also elevated after ciprofloxacin treatment. After 20 minutes of treatment with ciprofloxacin, the level of *waaB* increased from 0.76 ± 0.24 to 2.24 ± 0.60 ($P < 0.05$), *waaP* increased from 0.81 ± 0.12 to 2.34 ± 0.46 ($P < 0.01$) and *waaG* increased from 0.91 ± 0.18 to 2.16 ± 0.45 ($P < 0.05$) (Fig. 7).

Determination of LPS release after ciprofloxacin treatment

Based on the analysis of GO and KEGG database, LPS biosynthesis and *waa* operon were found to be higher expressed. So the release of LPS in the supernatant of MG1655 were determined after exposure to ciprofloxacin for a 20 min. It is shown that, bacterial LPS release was increased after treatment of ciprofloxacin ($P < 0.01$) (Fig. 8).

Discussion

The increasing ability of ciprofloxacin-resistance or tolerance may lead to high clinical failure rate of bacterial infections and increasing prevalence of infections by antibiotic-resistant bacteria [4, 5]. The canonical mechanisms of ciprofloxacin-resistance include genetic mutations of *gyrA* and parC or horizontal dissemination of antibiotic resistance genes which protects or modifies the targets the fluoroquinolone [6]. Many studies have shown that transcriptional response in bacterial cells induced by subinhibitory concentration of antibiotics contributes the formation of bacterial resistance [7, 8]. However,

less is known about the influence of a transient, high level exposure of ciprofloxacin in bacterial transcriptome. High dose therapy of antibacterial agent has been the commonly used regime when treating infections. The concentration of 2 μg/mL is approximate to the maximum concentration in tissue [9]. For *E. coli* MG1655 used in this study, this concentration is far exceeding the MIC of ciprofloxacin to the strain.

High concentration of antibiotics caused cellular damage by DNA damage and decrease of motive force.

Ciprofloxacin induces DNA damage such as breaking double-stranded DNA and forking stalled replication, which trigger genetic exchanges [10]. In the present study, we identified some genes related to DNA damage, including *gyrA*, *gyrB*, *stpA*, *ygbT*, *parE*, and *dnaN*, which were elevated in ciprofloxacin treated cells. Among these genes, *stpA* encodes a H-NS-like protein, which constrains DNA supercoils and influences DNA topology [11]. The *gyrA* gene contributes to DNA relaxation during DNA replication and mediates breakage and reunion of DNA strand [12]. The elevated expression of these genes suggested that high dose of ciprofloxacin had induced DNA damage in *E. coli* cells.

In addition, high concentration of antibiotics can inhibit the motive force of bacteria. Among those DEGs in ciprofloxacin treated *E. coli*, gene clusters that were associated with curli or flagellum biogenesis and metabolisms were lower expressed (**Table S2**). Decreased expression of motility genes has been viewed as a generalized means of self-protection through energy conservation under particularly harmful conditions [13, 14]. The t*hrABC* operon or threonine operon consists of 4 threonine biosynthesis genes and is mediated by threonine and isoleucine levels in cytoplasm, which are essential for cell growth [15]. The *fliL* operon is consisted of 7 adjacent genes encode *fliL, fliM, fliN, fliO, fliP, fliQ, and fliR*, which is required for flagellar biogenesis and normal cell division [16]. The down-regulation of *thrABC* and *fliL* operons identified in ciprofloxacin treated *E. coli* suggests that ciprofloxacin treatment caused cellular damage and destroyed normal development of *E. coli* cells.

SOS system, toxin/antitoxin system and formaldehyde detoxification system activation, and their significance in drug resistance.

Many studies have demonstrated that subinhibitory concentrations of quinolones activate SOS pathway by DNA damage. The SOS-response in bacteria to antibiotic agents induces ciprofloxacin-resistance and promotes horizontal dissemination of antibiotic resistance genes, which are considered to induce the bacterial adaptive ability and evolution of bacterial resistance to antibiotics [17, 18]. SOS pathway includes the transcription of stress response genes, such as SOS regulon (*recA* and *lexA*), and the genes encode DNA repair proteins and polymerases [19]. Our current analysis identified the up-regulation of SOS-response genes including *recA* gene and its regulator *recX*, *lexA*.

Dörr *et al* demonstrated that SOS response-induced *tisB* "toxin", DNA damage inducible toxin, controlled the production of multidrug tolerant cells [20]. Besides, *tisB* toxin induction from SOS regulon could decrease bacterial growth rate and cause multidrug resistance [21]. In this study, *lexA* regulated genes *tisB* (toxic membrane persister formation peptide), *ybfE* (CopB family), and *ydjM* were higher expressed

after ciprofloxacin treatment. Toxin/antitoxin systems were also higher expressed upon ciprofloxacin exposure. High dose ciprofloxacin induced rapid increase of the *tisB* toxin within 20 min during exponential phase, indicating that bacterial cells are capable to response to the fluoroquinolone stress promptly that they tend to form persister cells before completing one cycle of binary fission.

The *frmRAB* operon and *rfb* clusters were higher expressed after ciprofloxacin treatment by RNA-seq analysis. The *frmRAB* operon encodes a formaldehyde detoxification system in *E. coli* and is responsible for glutathione (GSH) -mediated formaldehyde detoxification [22, 23]. GSH-mediated detoxification system prevents the deleterious effects of formaldehyde accumulation in *E. coli* [24]. We speculate that the cellular GSH induced by ciprofloxacin treatment triggered the formaldehyde degradation pathway and consequently increased the expression of the *frmRAB* operon in *E. coli. RfbABC* operon is essential for synthesis of deoxysugar and O-antigen, and multicellular development [25]. These demonstrated that ciprofloxacin treated *E. coli* cells produced a cellular protection system including enhanced formaldehyde detoxification and auto-repair mechanisms. These were in accordance with the activated SOS response and the ciprofloxacin tolerance or resistance in *E. coli* cells. In our study, the DEGs in *rfbABC* operon was also identified to be enriched into “streptomycin biosynthesis” pathway (**Table S5**).

Significance of increased LPS synthesis induced by high concentration of antibiotics.

Many studies have reported that subinhibitory concentration of quinolones can increase the LPS released by the bactericidal lysis of bacteria [26, 27]. RNA-seq analysis in this study revealed that genes related the biosynthesis of LPS were higher expressed, such as *rfb* gene cluster, *waa* operon and *kdsA*. Further qRT-PCR and LPS assay confirmed that both the LPS release of bacteria and the expression of LPS synthesis related genes increased under the action of ciprofloxacin at bactericidal concentration. These results suggest that high-dose fluroquinolone not only induces bacterial canonical stress response enabling bacterial populations to survive high concentration of antibiotic but also increases LPS production at the genetic level by increasing gene expression. LPS is a major constituent of the outer membrane. Over production of LPS is responsible for sepsis, because it stimulates monocytes and macrophages to produce large amounts of proinflammatory mediators, such as tumor necrosis factor alpha and interleukins [28]. It is known that LPS can trigger inflammatory responses against pathogens in human body, even at very low doses (1 ng/kg body mass) [29]. Therefore, it is very important to reduce the toxicity of bacterial endotoxin while using ciprofloxacin for antimicrobial treatment. In addition to the application of endotoxin scavengers such as polymyxin B to neutralize the endotoxin produced, agents designed to inhibit the expression of endotoxin genes are potential to promote the therapeutic efficiency and reduce the risk of endotoxemia, which is of great clinical importance.

Conclusions

We confirmed by RNA sequencing that high dose of ciprofloxacin to the exponential phase of *E. coli* induce the DNA damage, decrease of biosynthesis of amino acids and flagella assembly within 20 min exposure. In addition to the bacterial damaging effects, high concentration of ciprofloxacin can up-

regulate the transcriptional level of SOS system and toxin-antitoxin system, which are involved in the formation of persistent and resistant bacteria. While helping bacteria acquire adaptive resistance, high dose of ciprofloxacin may also produce bacteria with high transcriptional expression of LPS, which deserves to be vigilant during the antibiotic therapy. These results demonstrated that transient exposure of high dose ciprofloxacin is double edged. Cautions should be taken when administering the high dose antibiotic treatment for infectious disease.

Methods

Bacterial strain and growth conditions

E. coli K12 strain MG1655 was grown in 250 mL flasks with Luria-Bertani (LB) medium in a shaking incubator at 37 ℃ and 250 rpm overnight. For ciprofloxacin treatment, the overnight cultures were sub-cultured to early exponential phase and treated with 2 μg/mL ciprofloxacin (Sigma, USA) for another 20 min. Bacterial abundances are expressed as colony forming unit (CFU) per volume of fluid medium. The number of colony forming units (CFU) is multiplied by the dilution factor and expressed in CFU/mL.

RNA isolation, cDNA library construction and Illumina sequencing

Total RNA isolation from *E. coli* cells was performed using the Qiagen RNA protect bacterial reagent (Qiagen, Hilden, Germany) and purification was conducted using Qiagen RNeasy Plus Mini Kit (Qiagen) following the manufacturer's instruction. After assessment of RNA purity and integrity, RNA samples were treated using Epicentre Ribo-zero rRNA Removal Kit (Epicentre, Madison, WI, USA), followed by RNA fragmentation, synthesis of the first and second strands DNA, and appendence of sequencing adapter. Then the selected fragments by Agencourt AMPure XP Beads (Illumina Inc, San Diego, CA, USA) were amplified, followed by library purification, enrichment and quality determination. Accordingly, 6 cDNA libraries (3 from ciprofloxacin-treated *E. coli*, and 3 from non-ciprofloxacin-treated *E. coli*) were constructed and subjected to Illumina Hiseq 2500 sequencing platform. Genome accession numbers was NC_000913.3.

Data processing and annotation

Clean reads were obtained by filtering out low-quality, adaptor-polluted and high content of unknown base reads from raw reads. Evaluation of sequencing datas quality were assessed by base error rate (%), phred score (Q_{phred}, %), and GC content (%). The clean reads were aligned to the assembled reference to NCBI Nr database (Non-redundant protein) by Bowtie 2 (version, 2.2.3) with mismatch of 2 [30]. Searches were conducted by the BLASTx [31] program with an e-value cutoff of 1e-5. The resulted transcripts were employed for analysis of novel transcripts (Rockhopper, v1.2.1 [32]), gene structure identification (Rockhopper, v1.2.1[32]), single nucleotide polymorphisms (SNPs) and insertion-deletion (Indel) detection (GATK2, v3.2 [33]), and identification of differentially expressed genes (DEGs; with DEGseq v1.12.0 [34], DEseq v1.10.1 [35], and edgeR v3.0.8 [36]). In addition, the operon and promoter prediction (Rockhopper,

v1.2.1 [32]), 5'- (RBSfinder, v1.0 [37]) and 3'-UTR distribution (Trans-TermHP, v2.0.9 [38]), small RNA (sRNA) analysis (RNAfold, v2.0 [39]) and predication of sRNA targets were performed (IntaRNA, v1.2.5 [40]).

DEG identification, GO and KEGG pathway enrichment and statistical analysis

The DEGseq R package v1.12.0 [34], DEseq v1.10.1 [35], and edgeR v3.0.8 [36] software was used for identification of DEGs induced by ciprofloxacin by pairwise comparison. Gene expression level was measured in fragments per kilobase of exon per million reads mapped (FPKM). The threshold values of significant DEGs were set at $|\log FC| \geq 1$, FDR (false discovery rate) ≤ 0.05, and adjusted p value ≤ 0.05. Hierarchical clustering analysis of DEGs was performed with $\log_{10}(FPKM + 1)$. GO (Gene Ontology, http://www.geneontology.org/, GOseq [41]) term enrichment and KEGG (Kyoto Encyclopedia of Genes and Genomes, KOBAS [42]) pathway analysis of DEGs was performed. GO and KEGG pathway terms with adjust *p* value < 0.05 were considered as significant.

Quantitative Reverse Transcription PCR (qRT-PCR)

To detect the effect of ciprofloxacin on transcription of selected genes associated with motility, 1000-fold diluted overnight cultures of *E. coli* MG1655 was grown to early exponential phase. Ciprofloxacin (2 μg/mL) was added to the bacterial culture and incubated at room temperature for 30 min. Bacterial pellets were harvested by centrifugation and resuspended in PBS containing 100 mg/L lysozymes (Sigma-Aldrich) and incubated for 10 min at room temperature. Total RNA was extracted using TRIzol reagent (Invitrogen), digested with Turbo DNase-free kit (Ambion, Austin, TX) to remove contaminating DNA, and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). cDNA was synthesized with 100 ng of total RNA by using the M-MLV reverse transcriptase (Promega, Madison, WI, USA) and random hexamers (Takara).

Expression of genes associated with lipopolysaccharide (LPS) biosynthesis (Table S1) was quantified using a two-step qRT-PCR analysis. Quantitative reverse transcription-PCR (qPCR) was carried out with SYBR (TaKaRa), in a 20 μL volume using a CFX96 qPCR detection system (Bio-Rad, Hercules, CA, USA) under the following conditions: 50 ℃ for 2 min, initial denaturation at 95 ℃ for 10 min, and 45 cycles of 15 s at 95 ℃ and 1 min at 60 ℃. Results were analyzed with SDS software (v. 2.2 Applied Biosystems). Data were normalized to the endogenous reference gene (*mdh*) and analyzed by the threshold cycle method ($2^{-\Delta CT}$). This experiment was repeated three times with independently isolated RNA samples. The primers used in qPCR were shown in Table 4.

Table 4
Primers used in this work.

Gene name	Primer	Primer sequence (5'-3')	Comment
mdh	Forward	TGACCAAACGCATCCAGAAC	internal reference
	Reverse	GCACGAACCAGAGACAGACC	
recA	Forward	GGAAATCTGTGACGCCCTGG	DNA
	Reverse	AGATCAGCAGCGTGTTGGA	
waaB	Forward	GTGATCTCCTGCCTGTTTGC	DNA
	Reverse	TGATAGTCGGCACAGGTGAT	
waaP	Forward	AACCCGCCTGATATACGTGT	DNA
	Reverse	GCGGTGATTTATCCCTGCAG	
waaG	Forward	AATCGCCGATTTCCAGAAGC	DNA
	Reverse	GGCTGTTTGGGATTTGCTCA	
kdsA	Forward	TCGGCTATGACAACCTGGTT	DNA
	Reverse	GGGTCACGTCGAAAATCACC	

Statistical analysis

The statistical difference in gene expression of qRT-PCR results was analyzed by Student's t-test and false discovery rate (FDR) was also calculated to correct the *p*-value. It was considered to be statistically significant when p-value < 0.05 or q-value < 0.05.

Quantitation of LPS

Endotoxin contents in the supernatant of bacteria with or without ciprofloxacin treatment were detected by Limulus amebocyte lysate (LAL) assay kit (Themo Scientific) according to manufacturer's instruction. Briefly, bacteria were microcentrifuged. Supernatant was harvest and added in triplicate to equal volumn of LAL in a pyrogen-free microtiter plate. The mixture was incubated at 37 ℃ for 10 min, and 100 μL of chromogenic substrate solution was added and color development was terminated by addition of 20% acetic acid. The optical density was measured at 410 nm.

Abbreviations

DEGs: differentially expressed genes; LPS:lipopolysacchride; MIC:minimum inhibitory concentration

Declarations

Availability of data and materials

We promise that all the data supporting the set of our conclusions are included in this article and its additional files. The complete RNA sequencing data generated and analyzed in this study have been submitted to the Genome Expression Omnibus (GEO) database, and the accession number is GSE152445 (https://www.ncbi.nlm.nih.gov/geo/query/ acc.cgi?acc = GSE152445).

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

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Authors' contributions

YMF, FMZ and XQZ designed the study. Experiments were performed by RS, XH, QZ, PDJ and WLZ. Data analysis were performed by XYL and RS. YMF, RS, CMZ and XQZ wrote the manuscript. All authors read and approved the final manuscript.

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Figures

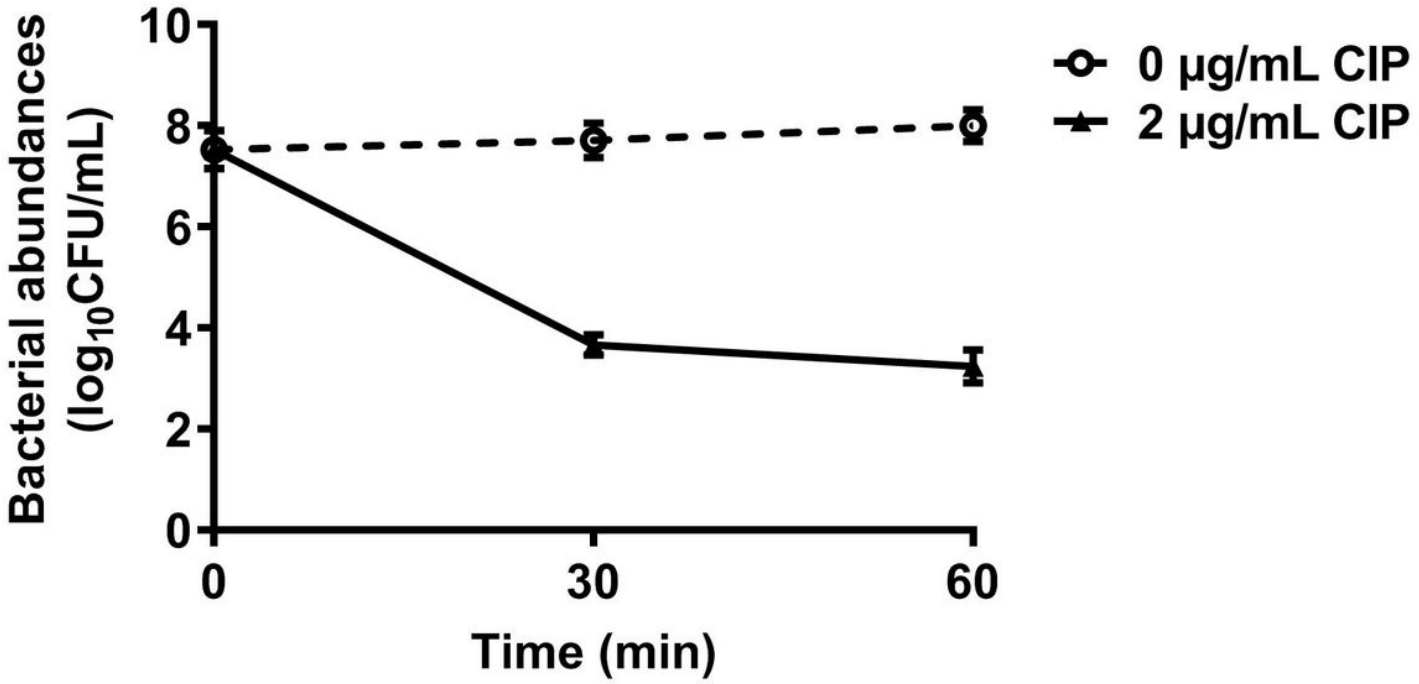

Figure 1

Survival curves of MG1655 before and after ciprofloxacin treatment.

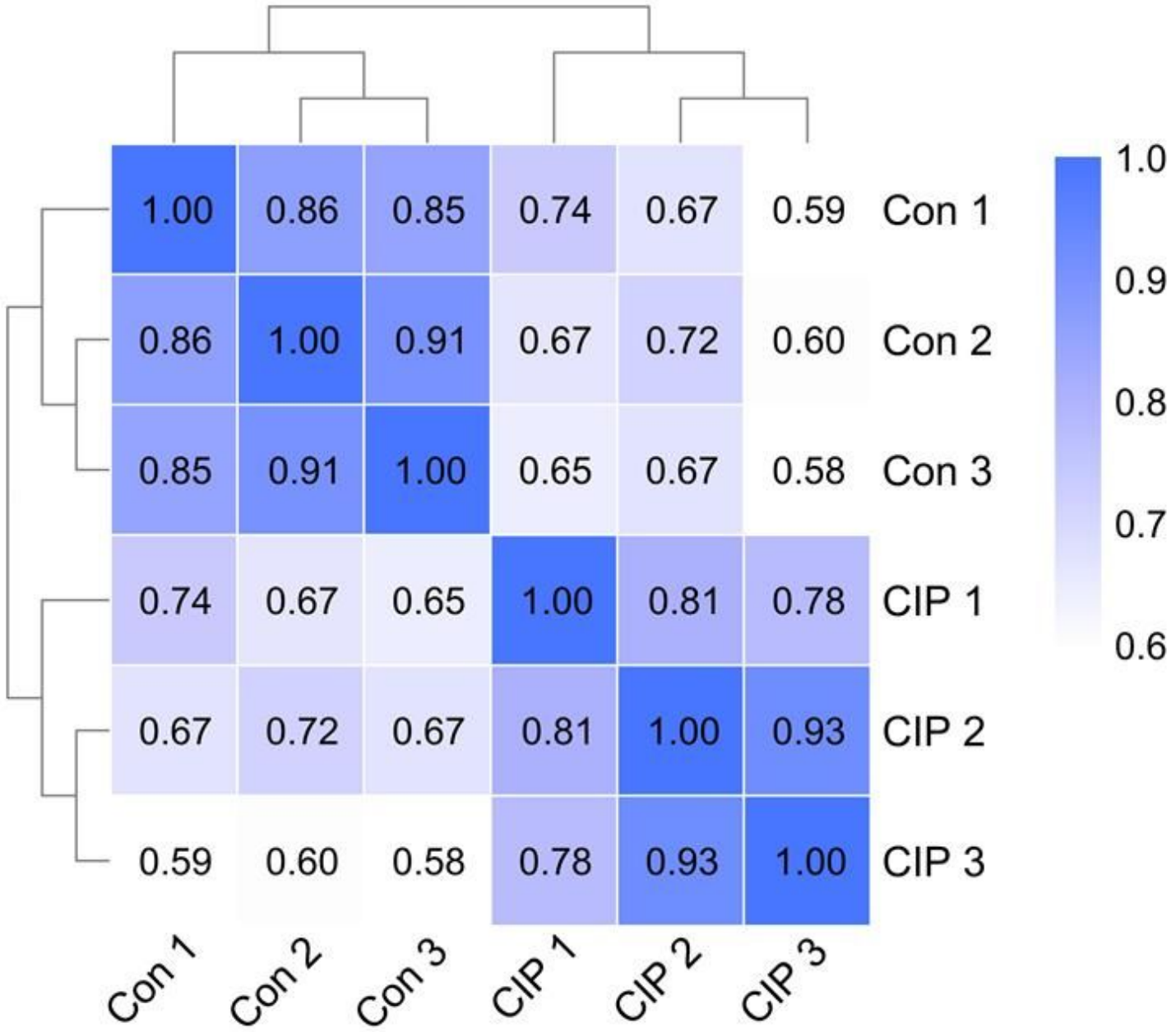

Figure 2

Analysis of Pearson correlation between samples. High correlation between samples under the same condition was identified (R2 > 0.8). Numeric values in boxes are squares of Pearson correlation coefficient (R2).

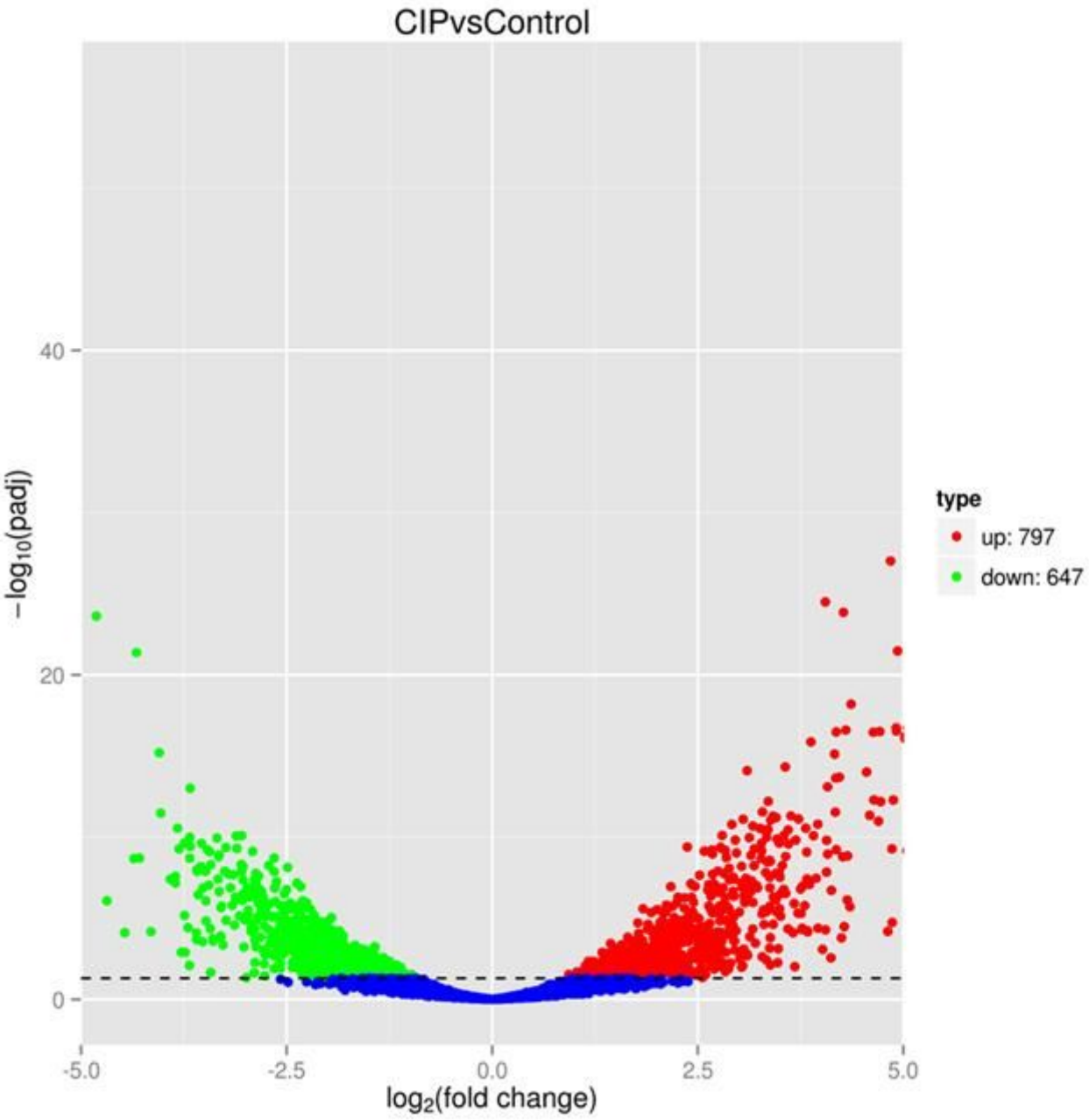

Figure 3

Volcanoplot of differentially expressed transcripts in ciprofloxacin treated E. coli vs. control. Red and green indicates the up- and lower expressed transcripts in Ciprofloxacin (Cip) treated E. coli vs. control with |logFC| ≥1 and adjusted p value ≤ 0.05. Blue indicates transcripts with |logFC| < 1 and/or adjusted p value > 0.05.

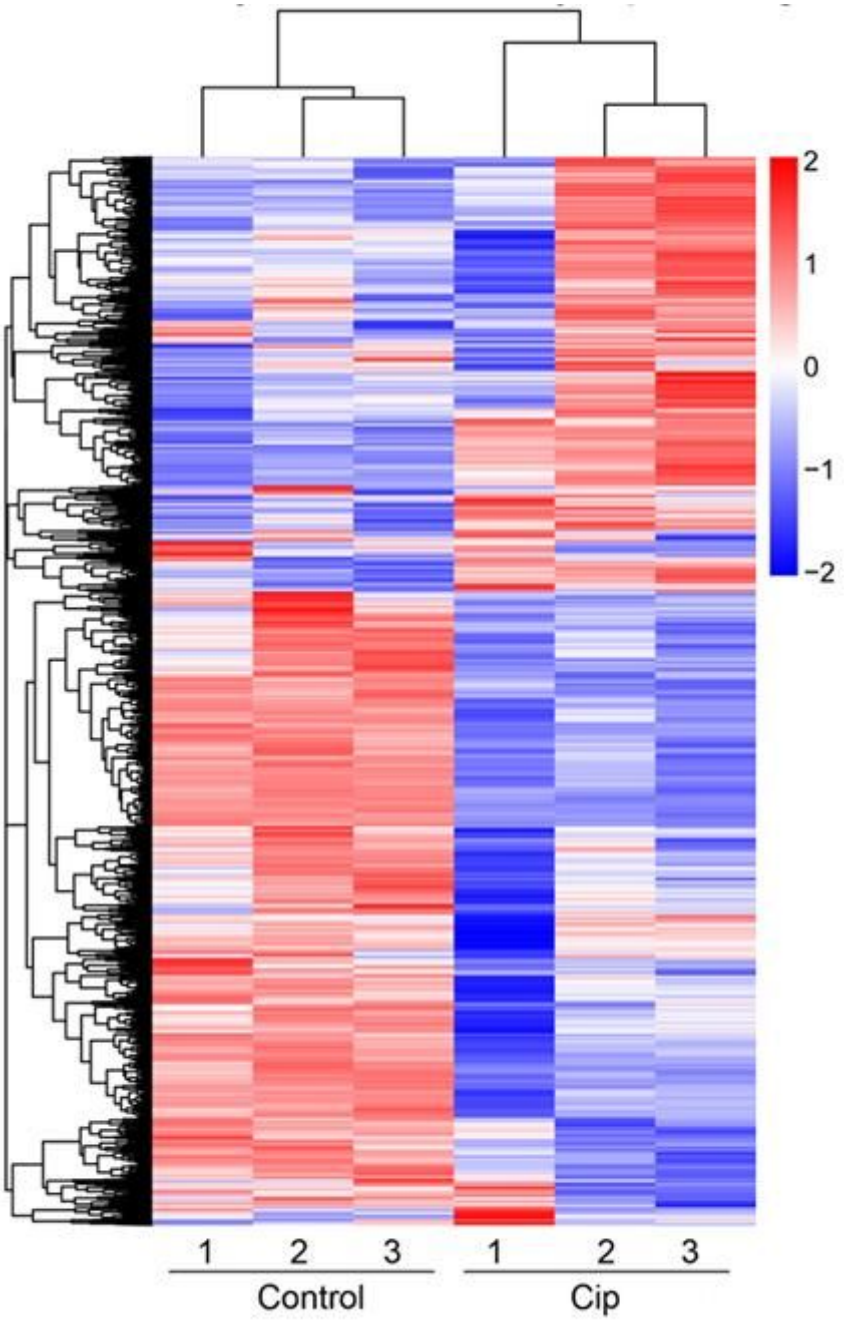

Figure 4

Clustering analysis for differentially expressed transcripts (DETs). Hierarchical clustering analysis was performed according to the gene expression levels measured in FPKM, log10-transformed FPKM+1 values. Red indicates higher expressed DETs and blue indicates lower expressed DETs in ciprofloxacin treated E. coli vs. control.

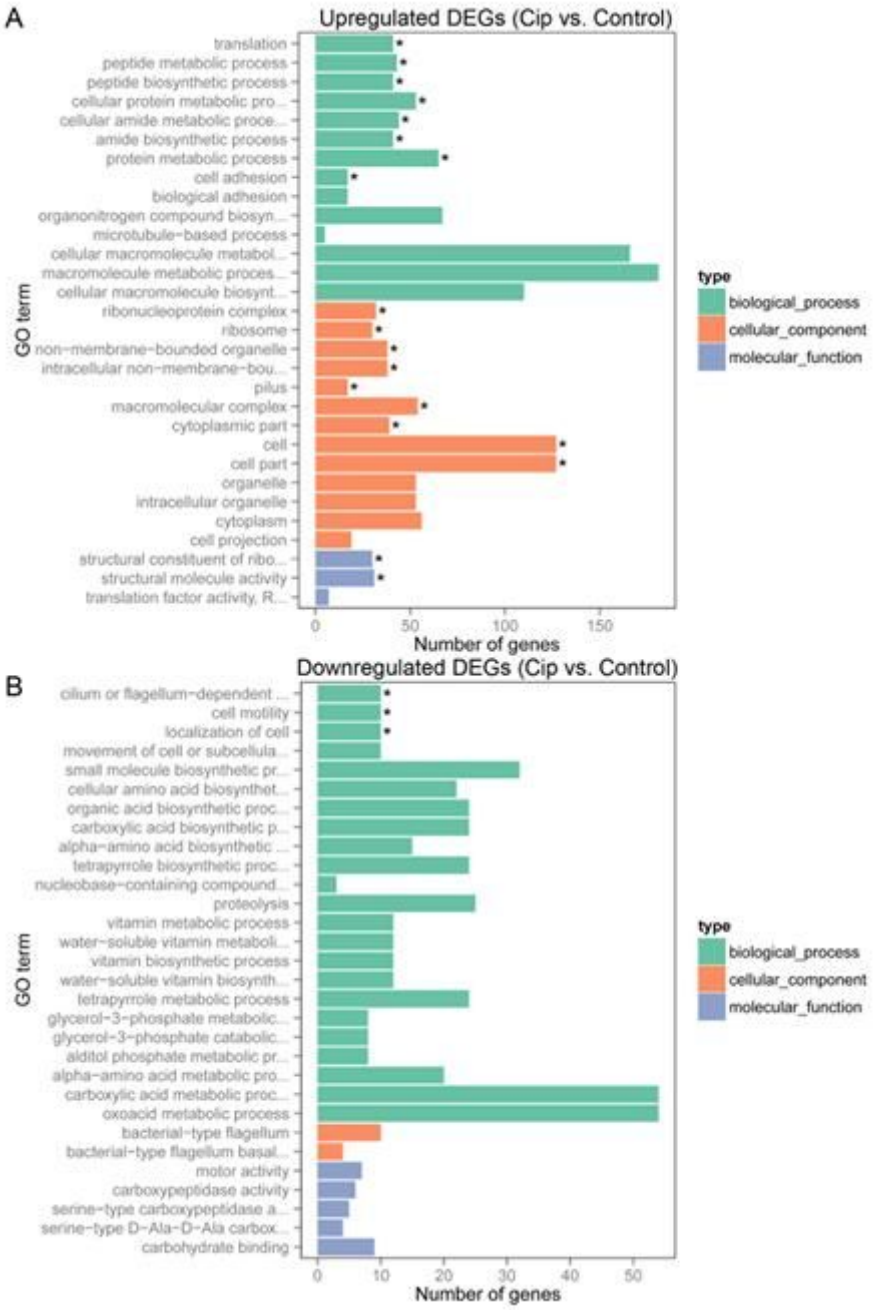

Figure 5

The top 30 enriched GO terms of differentially expressed genes (DEGs) in E. coli induced by ciprofloxacin. Color bars indicates adjusted p value of the enriched GO terms. Red to blue: p value of 0 to 1. The up- and lower expressed DEGs, respectively, were enriched in 18 and 3 significant GO terms. * indicates the significant terms with adjusted p value ≤ 0.05.

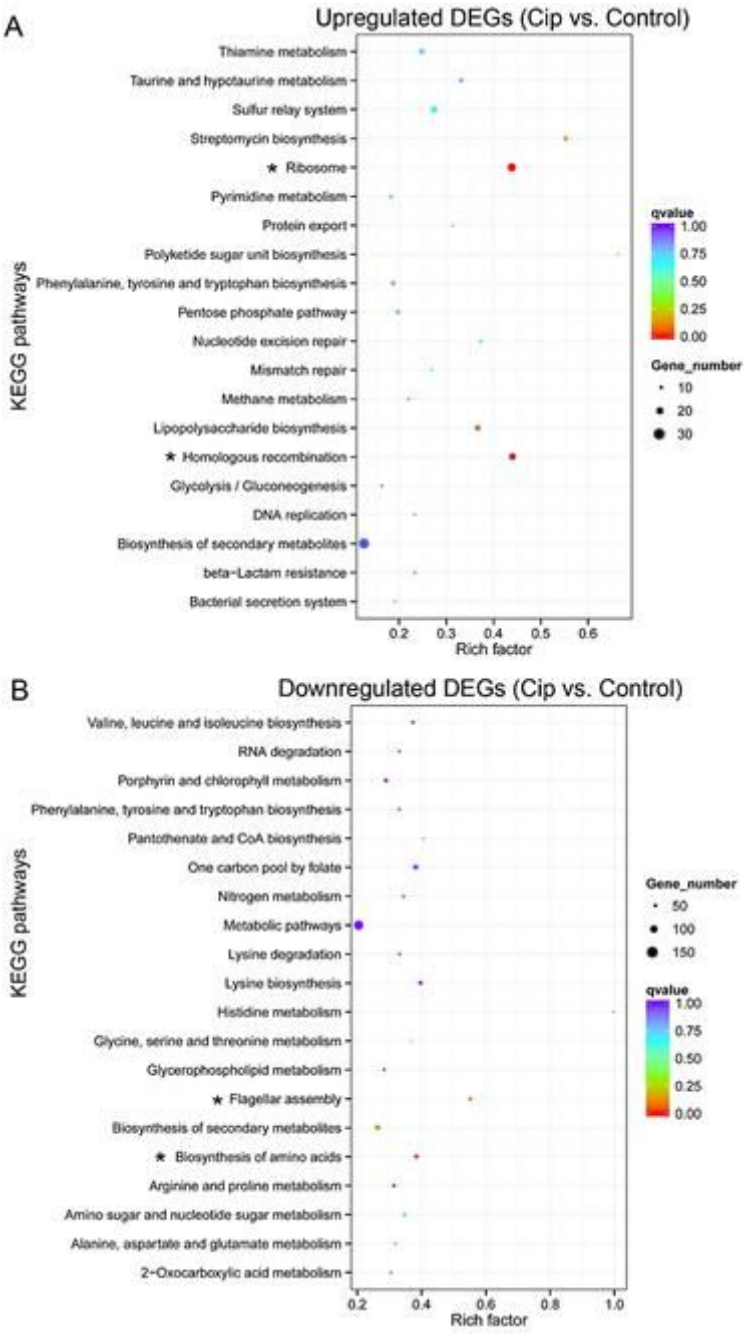

Figure 6

The top 20 enriched KEGG pathways of differentially expressed genes (DEGs) in E. coli induced by ciprofloxacin. Color bars indicates adjusted p value of the enriched pathways. Red to blue: p value of 0 to 1. The up- and lower expressed DEGs, respectively, were enriched in two significant pathways only. * indicates the significant pathways with adjusted p value ≤ 0.05.

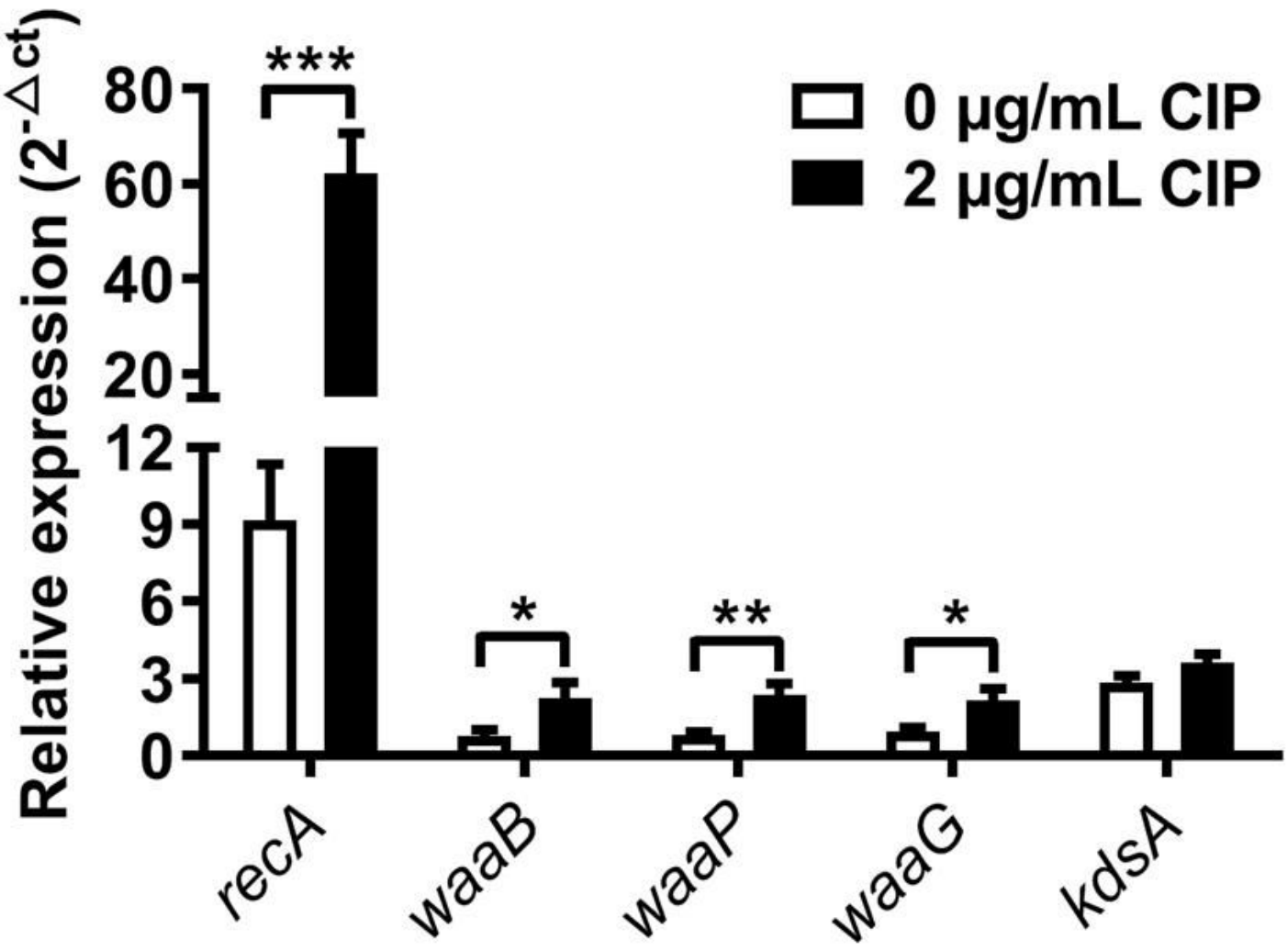

Figure 7

The expression levels of recA and LPS synthesis genes after high concentration ciprofloxacin treatment. After 20 minutes of treatment with ciprofloxacin, the levesl of recA, waaB, waaP and waaG (*P < 0.05,**P < 0.01,***P < 0.01).

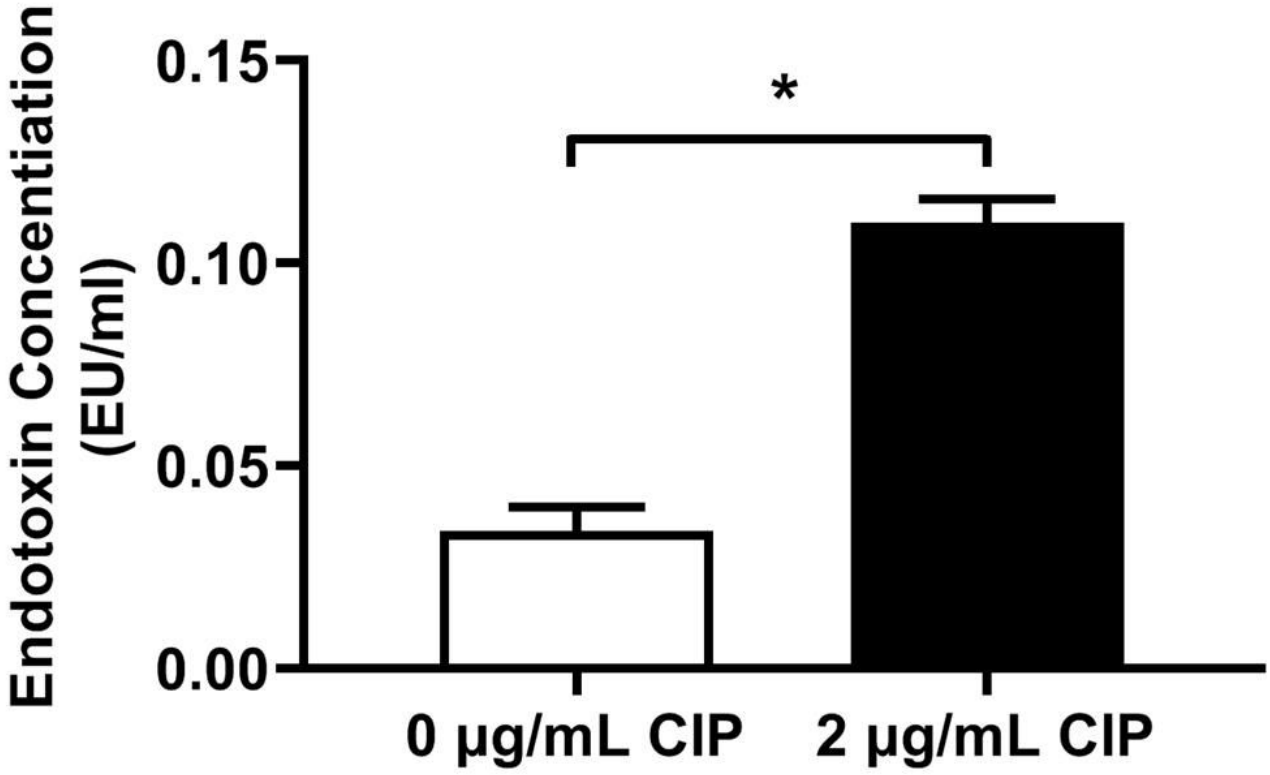

Figure 8

Endotoxin level released by E. coli after high concentration ciprofloxacin treatment. After 20 minutes of treatment with ciprofloxacin, the level of endotoxin in culture medium increased from 2.66 ± 0.22 to 3.82 ± 0.31. The difference was statistically significant (**P < 0.01).

Supplementary Files

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