Research Article

Genome-Wide Transcriptional Profiling of the Response of Staphylococcus aureus to Cryptotanshinone

Haihua Feng,¹ Hua Xiang,¹ Jiyu Zhang,² Guowen Liu,¹ Na Guo,¹ Xuelin Wang,¹ Xiuping Wu,¹ Xuming Deng,¹ and Lu Yu¹

¹Key Laboratory of Zoonosis, Institute of Zoonosis, College of Animal Science and Veterinary Medicine, Jilin University, Ministry of Education, Changchun 130062, China
²Lanzhou Institute of Animal Science and Veterinary Pharmaceutics Science, Chinese Academy of Agricultural Sciences, Lanzhou 730050, China

Correspondence should be addressed to Lu Yu, yulu225@126.com

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Staphylococcus aureus (S. aureus) strains with multiple antibiotic resistances are increasingly widespread, and new agents are required for the treatment of S. aureus. Cryptotanshinone (CT), a major tanshinone of medicinal plant Salvia miltiorrhiza Bunge, demonstrated effective in vitro antibacterial activity against all 21 S. aureus strains tested in this experiment. Affymetrix GeneChips were utilized to determine the global transcriptional response of S. aureus ATCC 25923 to treatment with subinhibitory concentrations of CT. Transcriptome profiling indicated that the antibacterial action of CT may be associated with its action as active oxygen radical generator; S. aureus undergoes an oxygen-limiting state upon exposure to CT.

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1. Introduction

Staphylococcus aureus (S. aureus) is one of the most important pathogens in both hospitals and the community and can cause numerous syndromes in humans, such as endocarditis, osteomyelitis, and septicemia [1, 2]. Greater than 60% of S. aureus isolates are now resistant to methicillin (oxacillin), and some strains have developed resistance to more than 20 different antimicrobial agents [3]; new agents are therefore needed for the treatment of S. aureus.

For over 100 years, chemical compounds isolated from medicinal plants have served as the models for many clinically proven drugs and are now being reassessed as antimicrobial agents [4]. Cryptotanshinone (CT) is a major tanshinone of Dan Shen, the root of Salvia miltiorrhiza Bunge (Labiatae) [5]. CT exhibits antimicrobial activity against a broad range of Gram-positive, including S. aureus, and Gram-negative bacteria as well as other microorganisms [6, 7].

Although CT exhibited fairly high levels of activity against S. aureus, there have been no reports related to the inhibitory mechanisms of CT against S. aureus. Transcriptional profilings generated with Affymetrix GeneChips have been used to identify genes of S. aureus that were induced in response to the antibiotics vancomycin, oxacillin, d-cycloserine, or bacitracin [8, 9]. Transcriptional profiles of microorganisms treated with an inhibitor could provide valuable information for both pathway characterization, and for determination of the mechanism of inhibition [10, 11].

In this report, to investigate the antimicrobial activity and the possible action mechanisms of CT against S. aureus, we tested the minimum inhibitory concentrations (MICs) of CT against 21 S. aureus strains, as well as utilizing Affymetrix GeneChip analysis to identify differentially expressed genes for S. aureus treated with subinhibitory concentrations of CT.

2. Materials and Methods

2.1. Bacterial Strains and Materials. The S. aureus strains used in this study were comprised of 20 clinical isolates from the First Hospital of Jilin University, which have different antibiograms against 12 antimicrobial agents (Table 1), and the standard strain ATCC 25923 obtained...
from China Medical Culture Collection Center (CMCC). Mueller-Hinton broth II (MHB II) and Mueller-Hinton agar (MHA) were purchased from BD (Biosciences, Inc., Sparks, Md). CT was purchased from the China Medical Culture Collection Centre (CMCC), and stock solutions of varying concentrations were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.2. Antibiotic Susceptibility Testing. The minimum inhibitory concentrations (MICs) of CT against the 21 S. aureus strains were determined in triplicate by broth microdilution or broth macrodilution using twofold serial dilutions in MHB II, according to CLSI/NCCLS M100-S15. The MICs were defined as the lowest concentration at which no visible growth was observed. The minimum concentration of CT that inhibited 90% of the isolates tested was defined as the MIC$_{90}$.

2.3. Synergistic Study. A standard checkerboard assay was performed to assess the interaction of combination (CT and TMP/SXT) against S. aureus strain ATCC 25923 by a well-established method [12]. The fractional inhibitory concentration (FIC) index was calculated by a previously described method [12]. An FIC index (FICI) between two compounds less than or equal to 0.5 is considered synergism, an FIC index between 0.5 and 2 is considered indifference, and a FICI equal to or more than 2 is considered antagonism.

2.4. Growth Curves. S. aureus strain ATCC 25923 was grown to an optical density of 0.3 at 600 nm in MHB II, and 100 mL aliquots were then distributed into five 500 mL Erlenmeyer flasks. CT (dissolved in DMSO) was added to 100 mL aliquots were then distributed into five 500 mL to an optical density of 0.3 at 600 nm in MHB II, and strain ATCC 25923 was grown.

2.5. Treatment with CT. S. aureus strain ATCC 25923 was grown overnight at 200 rpm in a rotary shaker at 37°C in 10 mL of MHB II. Six 250-mL Erlenmeyer flasks, each containing 100 mL of MHB II, were inoculated with an overnight culture to an initial OD$_{600}$ of 0.05. The bacteria were then grown at 37°C at 200 rpm to an OD$_{600}$ of 0.3. Subsequently, 500 μL of a 12 800 μg·mL CT stock solution, prepared in dimethyl sulfoxide (DMSO), was added to three of the cultures (experimental cultures), yielding a final concentration of 1/2 × MIC (2 μg·mL). Hence, the final concentration of solvent in each CT treatment was 1% (vol/vol) DMSO, which did not alter the pH of the medium. The other three cultures lacking CT and supplemented with 1% (vol/vol) DMSO were used as the control. All bacterial suspensions (both CT treatment or control condition) were further incubated for 30 minutes at 37°C for RNA isolation.

2.6. RNA Isolation and cDNA Labeling. Bacterial cells were treated with RNA Protect bacterial reagent (QIAGEN, Inc., Valencia, Calif) to minimize RNA degradation immediately before harvesting. Cells were collected by centrifugation and stored at −80°C. RNA isolation and cDNA labeling were performed as previously described [13]. Three independent RNA preparations and cDNA labelings were performed on different days.

2.7. GeneChip Hybridization and Analysis. The GeneChip S. aureus genome array (antisense) was provided by CapitalBio Corporation (http://www.capitalbio.com/index.asp, Beijing, China), a service provider authorized by Affymetrix Inc. (Santa Clara, CA). This GeneChip includes N315, Mu50, NCTC 8325, and COL. The array contains probe sets to over 3 300 S. aureus ORFs and over 4 800 intergenic regions. GeneChip hybridization, washing, staining, and scanning were performed as previously described [13].

The images were processed with Microarray Analysis Suite 5.0 (Affymetrix). The raw data from the array scans were normalized by median-centering genes for each array, followed by log transformation. Expressed genes were identified using Affymetrix GeneChip Operating Software (GCOS, Ver.1.0), which utilizes statistical criteria to generate a “present” or “absent” call for genes represented by each probe set on the array. Additionally, genes with “absent” scores were filtered out of the dataset, and the remaining genes were analyzed. To identify genes that are differentially expressed in CT-treated samples compared to controls, the Significance Analysis of Microarrays (SAM) software (http://www-stat.stanford.edu/~tibs/SAM/index.html) was used. To select the differentially expressed genes, we used threshold values of ≥1.5- and ≤−1.5-fold change between three RH treatment samples and three control samples; the FDR significance level was <5%.

2.8. Quantitative Real-Time RT-PCR. Quantitative real-time reverse transcription (RT)-PCR was used to verify the microarray results. Aliquots of the RNA preparations from CT-treated and control samples used in the microarray experiments were also used for quantitative real-time RT-PCR follow-up studies. The cDNA was subjected to real-time PCR using the primer pairs listed in Table 2. Quantitative real-time PCR was performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), as previously described [13].

3. Results and Discussion

3.1. MICs of CT Against S. aureus Strains and Influence of CT on Growth Curves. In this experiment, the MICs of CT against 21 S. aureus strains ranged from 4 to 64 μg·mL (Table 1), and the MIC$_{90}$ was 16 μg·mL. The MIC value of S. aureus strain ATCC 25923 versus CT was 4 μg·mL. This result demonstrated that CT is a potentially effective antimicrobial against S. aureus.

We performed growth curve of S. aureus ATCC 25923 with CT (1, 2, 4, 8, and 16 μg·mL) added at the time
Table 1: Antibiograms of 21 Staphylococcus aureus strains used in this study.

| Strains    | Source       | Date of isolation (mo/yr) | Antibiotic resistances | V MIC (μg·mL) | CIP MIC (μg·mL) | OX MIC (μg·mL) | CT MIC (μg·mL) |
|------------|--------------|---------------------------|------------------------|---------------|----------------|---------------|---------------|
| SA003      | Blood        | 2/2003                    | P                      | 1             | 0.12           | 0.12          | 4             |
| SA006      | Skin         | 2/2003                    | M, P, CI, CM, E        | 1             | 64             | 128           | 8             |
| SA009      | Skin abscess | 3/2003                    | M, CI, CM, E           | 0.5           | 128            | 256           | 16            |
| SA017      | Skin abscess | 5/2003                    | P, T                   | 2             | 0.5            | 0.5           | 4             |
| SA018      | Broncheal swab| 6/2003                  | M, P, CI, CM, E, G    | 2             | 128            | 512           | 16            |
| SA025      | Sputum       | 10/2003                   | —                      | 0.25          | 0.25           | 0.12          | 4             |
| SA039      | Broncheal swab| 2/2004                  | M, P, CI, CM, E, G, RI, T, TMP/SXT | 1 | 512 | 512 | 64 |
| SA059      | Broncheal swab| 6/2004                  | M, P, CI, T, TMX/SXT  | 2             | 128            | 512           | 8             |
| SA079      | Sputum       | 9/2004                    | M, P, CI, CM, E, G, TMP/SXT | 4 | 128 | 256 | 32 |
| SA092      | Abscess      | 10/2004                   | M, P, E                | 1             | 16             | 128           | 16            |
| SA106      | Wound isolate| 1/2005                   | M, P, CI, CM, E, G, T, TMP/SXT | 0.5 | 256 | 512 | 16 |
| SA118      | Blood        | 1/2005                    | M, P, E, G            | 1             | 64             | 256           | 16            |
| SA121      | Wound isolate| 3/2005                   | M, P, CM, E, G        | 1             | 128            | 256           | 16            |
| SA142      | Abscess      | 5/2005                    | P, T                   | 1             | 1              | 0.5           | 8             |
| SA146      | Skin abscess | 5/2005                    | M, P, CM, E, T        | 2             | 64             | 128           | 16            |
| SA165      | Urine        | 6/2006                    | P, CI, CM, E, G, RI, T| 2             | 256            | 512           | 16            |
| SA173      | Broncheal swab| 8/2005                   | M, P, T                | 0.25          | 32             | 128           | 8             |
| SA179      | Blood        | 9/2005                    | M, CI, CM, E          | 1             | 128            | 512           | 16            |
| SA192      | Urine        | 10/2005                   | M, P, CI, CM, E, G    | 1             | 64             | 512           | 16            |
| SA203      | Wound isolate| 12/2005                  | M, P, CM, T           | 2             | 64             | 512           | 16            |
| ATCC25923  | CMCC         | —                         | —                      | 1             | 0.25           | 0.25          | 4             |

Abbreviations: M: methicillin; P: penicillin; CI: clindamycin; CM: chloramphenicol; E: erythromycin; G: gentamicin; RI: rifampin; T: tetracycline; TMP/SXT: trimethoprim-sulfamethoxazole; V: vancomycin; CIP: Ciprofloxacin; OX: Oxacillin.

point of OD600 = 0.3. Equivalent DMSO was added to the experimental cultures and control cultures. Bacterial cells samples were collected at certain time intervals in this experiment with a time course of 1440 minutes, and the growth curves of CT-treated and untreated strain were measured spectrophotometrically. With 1 and 2 μg·mL of CT and controls, we found the optical density increased steadily, while with 4 μg·mL, 8 μg·mL, and 16 μg·mL of CT, we did not observe increase in the optical density (Figure 1). This result showed that CT displays bacteriostatic action against S. aureus, which is consistent with previous reports [15, 16].

3.2. Gene Transcription Responses to CT Exposure. GeneChip analysis revealed that a substantial number of genes (185) were differentially regulated in response to CT. Of these, 100 genes exhibited a significant increase in transcript abundance, and 85 exhibited a significant decrease in transcript abundance. The microarray-related data were submitted to Gene Expression Omnibus (GEO) under the accession number: GSE13203. The distribution of CT-responsive genes and their biological roles are shown in Figure 2. A complete list of all genes differentially expressed by CT can be found in the supplemental material (See supplementary Table S1 in supplementary material available online at doi: 10.1155/2009/617509). Herein, our interest mainly focused on specific genes that may allow the organism to survive in the presence of CT.

3.3. Genes of the Sortase Enzyme and Iron-Regulated Surface Determinants (Isd) are Inhibited in Response to CT. In the present study, the sortase-encoding gene srtB (SA0982) was repressed 3-fold, and some surface protein-encoding genes of isdABDEFGH were also significantly inhibited in response to CT. The Isd cluster of S. aureus is composed of 10 genes (isdABCDEFGHI and srtB). Pathogens, such as S. aureus, require iron for survival and have evolved specialized proteins, such as Isd, to scavenge heme from their host [17]. Therefore, the staphylococcal Isd system can be viewed as a pathogenic strategy for scavenging heme iron during infection by tapping into the iron rich source of hemoproteins.
Table 2: Primers used in real-time RT-PCR with SYBR green probes.

| Primer     | N315 ORF(a) | Sequence                      |
|------------|-------------|-------------------------------|
| 16S rRNAfor(b) | SarRNA01   | CGTGCTACAATGGACAATACAAA       |
| 16S rRNArev(b) | SarRNA01   | ATCTACATGTCTGGTGCTTAC         |
| ribAfor     | SA1587      | GCTTACATTCTGCGTGCTTAC         |
| ribArev     | SA1587      | ACAATGTCTATGCACAGGCT          |
| isdAfor     | SA0977      | GCAACGGCGAAATCTGAAAG          |
| isdArev     | SA0977      | AAGGCAACTGTGCTAATAAAG         |
| modAfor     | SA2074      | TTACATGTGGTGATGAGGG           |
| modArev     | SA2074      | CATGCGCTTTATTCTTGGTCT         |
| seofor      | SA1648      | AGTCAAGTGTCAGCGCTATT          |
| seorev      | SA1648      | AGATATTCATCTTACAAAT           |
| SA1831for   | SA1831      | CGTGCTGTTATGCACTTTGAGT       |
| SA1831rev   | SA1831      | TATCTCTTTATTCTTGTGCG          |
| srtBfor     | SA0982      | TAAATCAAAGGACATTGTGGATGG      |
| srtBrev     | SA0982      | TACGTGATGTCTTGCTGCA           |
| glpTfor     | SA0325      | CGACTTGTGTCACAGCGGAAAA       |
| glpTrev     | SA0325      | CGCCCAATCAAGTGACAA           |
| oppFfor     | SA1989      | TCAGAAGGCTTTATTGTT            |
| oppFrev     | SA0198      | GAATGATTAGTGGTGGTTTA         |
| SA2135for   | SA2135      | AACATCAAGGCCGCAAATTG         |
| SA2135rev   | SA2135      | TAATTTCCACCGCCGAC          |
| msmXfor     | SA2060      | CATTGCGGCTAAAGCTACG          |
| msmXrev     | SA2060      | GACGCTTGCACGACGATAA          |
| opp-1Ffor   | SA2251      | TCATCAATTACGCCCTT            |
| opp-1Frev   | SA2251      | GCTTATAGTACGCGACT            |
| dpsfor      | SA1951      | TTACGGGATAGGAGGAAA            |
| dpsrev      | SA1951      | ATCACGGCAAGATTACC           |
| SA0302for   | SA0302      | GAATGGAAAAACAGAAAAAC         |
| SA0302rev   | SA0302      | GCAAACACATTAGCGCAAATAG       |

(a) ORF, open reading frame.
(b) refer to references [14].

Our results suggest that CT significantly affected the staphylococcal Isd system. In our study, the transcriptional level of srtCHI was not differentially modulated by a factor of ≥2.0 or ≤−2.0 upon exposure to CT.

3.4. Anaerobic Respiration and Fermentation, and Oxidative Stress Resistance Genes Regulated by CT. The expression of some genes involved in anaerobic respiration and fermentation was induced by CT during this study, including fdaB, pflB, pflA, nirB, nirD, narG, narH, and narI. The fdaB gene encodes fructose-bisphosphate aldolase. The transcription of the fdaB gene, which is involved in glycolysis, was upregulated, indicating enhanced glycolytic activity by CT. Similar upregulation results were obtained for fdaB under anaerobic conditions [19]. The pflB and pflA genes encode enzymes of the fermentation pathways, which are involved in acetate and ethanol formation [19]. Prior studies demonstrated that the pflA/pflB pair is involved in energy metabolism when E. coli grows under oxygen-limiting conditions [20]. NarG (respiratory nitrate reductase, alpha subunit), NarH (respiratory nitrate reductase, beta subunit), and NarI (respiratory nitrate reductase, gamma subunit) have been shown to be involved in nitrate respiration in E. coli [21]. NirB (nitrite reductase, large subunit) and NirD (nitrite reductase, small subunit) are essential and sufficient for NADH-dependent nitrite reduction [22]. Moreover, S. aureus is able to use nitrate or nitrite as alternative electron acceptors [19, 23]. Transcripts of the nar and nir operon were found to be present at elevated levels under anaerobic conditions [19]. Additionally, CT also induced the arcABCD genes, which are associated with energy metabolism. The S. aureus arginine deiminase (ADI) metabolic pathway genes are organized in an operon arcABDC [24]. The proteins encoded by arcABCD are responsible for the ADI pathway, which enables arginine-dependent anaerobic growth [25]. Oxygen deficiency promotes the autophosphorylation of ArcB that activates phosphorylation of ArcA and results in regulation of numerous operons that provide control of carbon catabolism and cellular redox status [26]. Consequently, our results described herein imply that CT may induce anaerobic respiration and fermentation in S. aureus.
Expression of some genes involved in oxidative stress resistance was affected by CT, including ahpC, ahpF, katA, and sodM. The sodM gene encodes superoxide dismutase, and katA encodes the catalase enzyme. KatA is the only major catalase in S. aureus, accumulates extracellularly, detoxifies H$_2$O$_2$, and was proposed to be a major virulence determinant in S. aureus [27, 28]. Our results showed upregulation of the katA gene and downregulation of the sodM gene, thus connecting CT to oxidative-stress response. Surprisingly, a similar transcriptional response also occurs when cells are subjected to 1 mM peracetic acid [29]. The ahpC gene encodes alkyl hydroperoxide reductase subunit C, and ahpF encodes alkyl hydroperoxide reductase subunit F. Our results also demonstrated an upregulation of the ahpC and ahpF genes. Alkyl hydroperoxide reductase subunit C (AhpC) protects cells against ONOO$^-$, which is generated within neutrophils and macrophages [30].

Recent report suggested that superoxide radical formation might be the cause of antibacterial activity of CT [7]. As mentioned above, our results showed clearly that genes fdaB, pflB, pflA, nirB, nirD, narG, narH, and narL involved in anaerobic respiration and fermentation were upregulated, and genes ahpC, ahpF, and katA involved in oxidative stress resistance were upregulated by CT. Chang et al. indicated that hydrogen peroxide, a reactive oxygen species, led to genes pflBA and arcBC increases in transcription levels; they suggested that S. aureus might undergo an oxygen-limiting state in response to hydrogen peroxide-driven oxidative stress [31]. Further, previous result showed that in E. coli pfl is significantly induced by shifting the culture condition from an aerobic to a microaerobic state [32]. Moreover, transcriptome and proteome analysis of Bacillus subtilis gene expression in response to superoxide and peroxide stress showed that genes katA and ahpCF were significantly induced [33]. Thus, our microarray result is consistent with previous observations which revealed that CT might act as superoxide radicals generator; Lee et al. proposed that this phenomenon benefited S. aureus by preventing further cytotoxicity arising from reactive oxygen species produced during oxygen respiration [7]. Lactoquinomycin A (LQMA), an antibiotic having a quinone moiety like CT, also generated superoxide radicals during reduction of the quinone moiety by quinone reductase and resulted in other active oxygens [7].

3.5. Antibiotics Resistance Genes Affected by CT. Genechip analysis showed that several antibiotic resistance genes were differentially regulated by CT exposure, including dfrA, drp35, cdsA, and pgsA. Among these, the transcription of dfrA was upregulated, whereas the transcription of drp35, pgsA (−2.0-fold), and cdsA was significantly downregulated by CT exposure. The dfrA gene encodes dihydrofolate reductase, which is responsible for trimethoprim resistance (Tp$r^+$) [34]. In present study, we found that CT has high MIC values in trimethoprim-sulphamethoxazole (TMP/SXT) susceptible and resistant strains. In order to test the interaction between CT and TMP/SXT, we conducted additional experiment to assay the vitro antimicrobial activity of CT against S. aureus strain ATCC 25923 in combination with TMP/SXT using checkerboard microdilution method. The result showed that there is an antagonism in combination of CT and TMP/SXT against S. aureus 25923, with FICI of 4. It is indicated that the increased expression of gene dfrA induced by CT might increase the resistance of the S. aureus to TMP/SXT. This result suggested that it is necessary to avoid simultaneous use of CT and TMP/SXT. The drp35 gene encodes Drp35, which possesses calcium-dependent lactonase activity and is a cytoplasmic protein induced by cell wall-affecting antibiotics or detergents [35]. Antibiotic susceptibility experiments using a drp35-defective strain and an overexpressing strain of S. aureus revealed that Drp35 is correlated with bacitracin resistance [36]. Two essential genes, cdsA and pgsA, encoding phosphatidate cytididytranferase and phosphatidylglycerophosphate synthase, respectively, are required for synthesis of phosphatidylglycerol phosphate, which might be converted into phosphoglycerol by a nonspecific phosphatase [37]. Conditional lethal alleles of genes involved in phospholipid biosynthesis in S. aureus, including pgsA and cdsA, have also been found to confer hypersusceptibility to macrolides [38].

3.6. Other Genes Differentially Regulated by CT. We found that 2 carotenoid pigment-associated genes, crtM and crtN, of S. aureus were upregulated in response to CT. Carotenoids are natural pigments with important biological activities [39–41]. The species description of S. aureus reflects the colony color (L. aureus: golden or orange) and distinguishes this species from Staphylococcus epidermidis (formerly Staphylococcus albus) [42]. Additionally, CT exposure also induced the ald gene that encodes aldehyde dehydrogenase, which is associated with biosynthesis of C30 carotenoids in S. aureus. The ald gene was located between crtN and crtNb in the same gene cluster [43]. Moreover, some putative transporter genes were differentially regulated by CT exposure. In our study, a large number of putative transporter genes (39) were differentially regulated upon exposure to CT; 12 of these genes were
**Figure 2**: CT-responsive genes grouped by functional classification. The differentially regulated genes were divided into 33 functional categories. The number of genes upregulated and downregulated for each functional group was represented.

**Table 3**: Real-time RT-PCR analysis of gene expression.

| N315 ORF | N315 gene | N315 description | Fold change ± SD*(a) |
|----------|-----------|------------------|---------------------|
|          |           |                  | RT-PCR               |
| SA1587   | ribA      | Riboflavin biosynthesis protein | −3.8 ± 1.9          |
| SA0977   | isdA      | Cell surface protein | −4.3 ± 2.3          |
| SA2074   | modA      | Probable molybdate-binding protein | −2.8 ± 0.9          |
| SA1648   | seo       | Enterotoxin SeO [Pathogenicity island SaPIn3] | −3.8 ± 1.2          |
| SA1831   |           | Hypothetical protein [Pathogenicity island SaPIn1] | −19.4 ± 6.1          |
| SA0982   | srtB      | NPQTN specific sortase B | −3.2 ± 0.8          |
| SA0325   | glpT      | Glycerol-3-phosphate transporter | −2.7 ± 0.3          |
| SA0198   | oppF      | Oligopeptide transport ATP-binding protein | −2.8 ± 0.5          |
| SA2135   |           | Hypothetical protein, similar to sodium/glutamate symporter | −2.8 ± 0.8          |
| SA0206   | msmX      | Multiple sugar-binding transport ATP-binding protein | −3.4 ± 1.0          |
| SA2251   | opp-1F    | Oligopeptide transporter putative ATPase domain | −2.6 ± 0.7          |
| SA1941   | dps       | General stress protein 20U | 10.5 ± 2.8          |
| SA0302   |           | Probable pyrimidine nucleoside transport protein | 18.4 ± 5.4          |

*(a)* + indicates reduction, and − indicates increase. Standard deviation were calculated based on three independent experiments.
upregulated, while 27 were downregulated. These genes included 26 ATP-dependent transporter genes (all involved in the ATP-binding cassette (ABC) superfamily) and 12 secondary transporter genes (3 genes are associated with the major facilitator superfamily (MFS)). In addition, a phosphotransferase system (PTS) gene, \textit{mtlA} (SA1962), was expressed at an increased level. Among the putative transporter genes regulated by CT, the \textit{semB} gene is a multidrug resistance (MDR) transporter, which was expressed at an increased level of 2.8-fold.

3.7. Validation of Microarray Data by Real-Time RT-PCR. Real-time quantitative RT-PCR was conducted to validate microarray data using the same RNA from the original microarray experiment, and 13 genes were selected for this analysis (i.e., \textit{ribA}, \textit{isDA}, \textit{modA}, \textit{seo}, \textit{SA1831}, \textit{srtB}, \textit{glpT}, \textit{oppF}, \textit{SA2135}, \textit{msmX}, \textit{opp-1F}, and \textit{dps}, and \textit{SA0302}). In general, there were positive correlations between microarray data and real-time RT-PCR data for all 13 genes (Table 3). However, expression of the genes \textit{SA1831} and \textit{SA0302} was observed at greater n-fold changes by real-time RT-PCR than with microarray analysis, indicating that real-time RT-PCR analysis may be more sensitive to changes than microarray analysis in transcript levels. For the remaining 11 genes (i.e., \textit{ribA}, \textit{isDA}, \textit{modA}, \textit{seo}, \textit{srtB}, \textit{glpT}, \textit{oppF}, \textit{SA2135}, \textit{msmX}, \textit{opp-1F}, and \textit{dps}), the levels of gene induction did not differ markedly between microarray data and real-time RT-PCR data.

4. Conclusion
In summary, the presented antibacterial activity and growth curve experiments demonstrated that CT is a bacteriostatic agent against \textit{S. aureus}. Transcriptional profiling revealed that the action mechanism of CT on \textit{S. aureus} is correlated to its action as active oxygen radical generator; \textit{S. aureus} might undergo an oxygen-limiting state upon exposure to CT. To our knowledge, this genome-wide transcriptomic approach revealed the first insights into the response of \textit{S. aureus} to CT challenge. Recognition of CT lays the groundwork for developing new agents owning good activity based on chemical structure modification of the lead compound.

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References
[1] I. M. Smith and A. B. Vickers, “Natural history of 338 treated and untreated patients with staphylococcal septicemia (1936–1955),” \textit{The Lancet}, vol. 1, no. 7138, pp. 1318–1322, 1960.
[2] G. L. Archer, “\textit{Staphylococcus aureus}: a well-armed pathogen,” \textit{Clinical Infectious Diseases}, vol. 26, no. 5, pp. 1179–1181, 1998.
[3] I. T. Paulsen, M. H. Brown, and R. A. Skurray, “Proton-dependent multidrug efflux systems,” \textit{Microbiological Reviews}, vol. 60, no. 4, pp. 575–608, 1996.
[4] G. B. Mahady, “Medicinal plants for the prevention and treatment of bacterial infections,” \textit{Current Pharmaceutical Design}, vol. 11, no. 19, pp. 2405–2427, 2005.
[5] H. C. Lin and W. L. Chang, “Diterpenoids from salvia miltiorrhiza,” \textit{Phytochemistry}, vol. 53, no. 8, pp. 951–953, 2000.
[6] G. Honda, Y. Kezuka, and M. Tabata, “Isolation of an antidermatophytic substance from the roots of \textit{Salvia miltiorrhiza},” \textit{Chemical & Pharmaceutical Bulletin}, vol. 36, no. 1, pp. 408–415, 1988.
[7] D.-S. Lee, S.-H. Lee, J.-G. Noh, and S.-D. Hong, “Antibacterial activities of cryptotanshinone and dihydrotanshinone I from a medicinal herb, \textit{Salvia miltiorrhiza} buhe,” \textit{Bioscience, Biotechnology and Biochemistry}, vol. 63, no. 12, pp. 2236–2239, 1999.
[8] F. McAleese, S. W. Wu, K. Sieradzki, et al., “Overexpression of genes of the cell wall stimulon in clinical isolates of \textit{Staphylococcus aureus} exhibiting vancomycin-intermediate-\textit{S. aureus}-type resistance to vancomycin,” \textit{Journal of Bacteriology}, vol. 188, no. 3, pp. 1120–1133, 2006.
[9] S. Utaida, P. M. Dunman, D. Macapagal, et al., “Genome-wide transcriptional profiling of the response of \textit{Staphylococcus aureus} aureus to cell-wall-active antibiotics reveals a cell-wall-stress stimulon,” \textit{Microbiology}, vol. 149, no. 10, pp. 2719–2732, 2003.
[10] H. Yin, Y. Zhao, Y. Zhang, et al., “Genome-wide analysis of the expression profile of \textit{Saccharomyces cerevisiae} in response to treatment with the plant isoflavone, wighteone, as a potential antifungal agent,” \textit{Biotecnology Letters}, vol. 28, no. 2, pp. 99–105, 2006.
[11] S. Draghichi, P. Khatri, R. P. Martins, G. C. Ostermeier, and S. A. Kravetz, “Global functional profiling of gene expression,” \textit{Genomics}, vol. 81, no. 2, pp. 98–104, 2003.
[12] Y. Ge, S. Difuntorum, S. Touami, et al., “In vitro antimicrobial activity of GSQ1530, a new heteroaromatic polycyclic compound,” \textit{Antimicrobial Agents and Chemotherapy}, vol. 46, no. 10, pp. 3168–3174, 2002.
[13] D. Wang, L. Yu, H. Xiang, et al., “Global transcriptional profiles of \textit{Staphylococcus aureus} treated with berberine chloride,” \textit{FEMS Microbiology Letters}, vol. 279, no. 2, pp. 217–225, 2008.
[14] D. Y. Wang, C. C. Yeh, J. H. Lee, C. F. Hung, and J. G. Chung, “Berberine inhibited arylamine N-acetyltransferase activity and gene expression and DNA adduct formation in human malignant astrocytoma (G9T/VGH) and brain glioblastoma multiforms (GBM 8401) cells,” \textit{Neurochemical Research}, vol. 27, no. 9, pp. 883–889, 2002.
[15] C. X. Liu and P. G. Xiao, “Recalling the research and development of new agents originating from chinese traditional medicines,” \textit{SAGE-The Genus Salvia}, vol. 2, no. 2, pp. 133–156, 2002.
[16] D. Baricic and T. Bartol, “The biological/pharmacological activity of the \textit{Salvia genus},” in \textit{SAGE-The Genus Salvia}, S. E. Kintzios, Ed., pp. 143–184, Harwood Academic Publishers, Amsterdam, The Netherlands, 2000.
[17] E. P. Skaar and O. Schneewind, “Iron-regulated surface determinants (Idp) of \textit{Staphylococcus aureus}: stealing iron from heme,” \textit{Microbes and Infection}, vol. 6, no. 4, pp. 390–397, 2004.
[18] S. K. Hazmanian, E. P. Skaar, A. H. Gaspar, et al., “Passage of heme-iron across the envelope of \textit{Staphylococcus aureus},” \textit{Science}, vol. 299, no. 5608, pp. 906–909, 2003.
J. Mostertz, J. Pané-Farré, C. Kohler, M. Hecker, and G. Homuth, “Anaerobic gene expression in Staphylococcus aureus,” *Journal of Bacteriology*, vol. 189, no. 11, pp. 4275–4289, 2007.

G. Sawers and G. Watson, “A glycy radical solution: oxygen-dependent interconversion of pyruvate formate-lyase,” *Molecular Microbiology*, vol. 29, no. 4, pp. 945–954, 1998.

F. Sargent, “Constructing the wonders of the bacterial world: biosynthesis of complex enzymes,” *Microbiology*, vol. 153, no. 3, pp. 633–651, 2007.

N. R. Harborne, L. Griiffiths, S. J. W. Busby, and J. A. Cole, “Transcriptional control, translation and function of the products of the five open reading frames of the *Escherichia coli* nir operon,” *Molecular Microbiology*, vol. 6, no. 19, pp. 2805–2813, 1992.

K. A. Burke and J. Lascelles, “Nitrate reductase system in *Staphylococcus aureus* wild type and mutants,” *Journal of Bacteriology*, vol. 123, no. 1, pp. 308–316, 1975.

J. Makhlin, T. Kofman, I. Borovok, et al., “*Staphylococcus aureus* ArcR controls expression of the arginine deiminase operon,” *Journal of Bacteriology*, vol. 189, no. 16, pp. 5976–5986, 2007.

A. Magnhouj, T. F. de Sousa Cabral, V. Stalon, and C. Vander Waven, “The arcABDC gene cluster, encoding the arginine deiminase pathway of *Bacillus licheniformis*, and its activation by the arginine repressor ArcR,” *Journal of Bacteriology*, vol. 180, no. 24, pp. 6468–6475, 1998.

S. Alexeeva, K. J. Hellingwerf, and M. I. Teixeira de Mattos, “Requirement of ArcA for redox regulation in *Escherichia coli* under microaerobic but not anaerobic or aerobic conditions,” *Journal of Bacteriology*, vol. 185, no. 1, pp. 204–209, 2003.

G. L. Mandell, “Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. In vitro and in vivo studies with emphasis on staphylococcal leukocyte interaction,” *Journal of Clinical Investigation*, vol. 55, no. 3, pp. 561–566, 1975.

K. Cosgrove, G. Coutts, L.-M. Jonsson, et al., “Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*,” *Journal of Bacteriology*, vol. 189, no. 3, pp. 1025–1035, 2007.

W. Chang, F. Toghrul, and W. E. Bentley, “Toxicogenomic response of *Staphylococcus aureus* to peracetic acid,” *Environmental Science and Technology*, vol. 40, no. 16, pp. 5124–5131, 2006.

L. Chen, Q.-W. Xie, and C. Nathan, “Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates,” *Molecular Cell*, vol. 1, no. 6, pp. 795–805, 1998.

W. Chang, D. A. Small, F. Toghrul, and W. E. Bentley, “Global transcriptome analysis of *Staphylococcus aureus* response to hydrogen peroxide,” *Journal of Bacteriology*, vol. 188, no. 4, pp. 1648–1659, 2006.

L. Peng and K. Shimizu, “Global metabolic regulation analysis for *Escherichia coli* K12 based on protein expression by 2-dimensional electrophoresis and enzyme activity measurement,” *Applied Microbiology and Biotechnology*, vol. 61, no. 2, pp. 163–178, 2003.

J. Mostertz, C. Scharf, M. Hecker, and G. Homuth, “Transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress,” *Microbiology*, vol. 150, no. 2, pp. 497–512, 2004.

H.-K. Young, R. A. Skurray, and S. G. B. Amyes, “Plasmid-mediated trimethoprim-resistance in *Staphylococcus aureus* Characterization of the first Gram-positive plasmid dihydrofolate reductase (type S1),” *Biochemical Journal*, vol. 243, no. 1, pp. 309–312, 1987.

H. Murakami, H. Matsumaru, M. Kanamori, H. Hayashi, and T. Ohta, “Cell wall-affecting antibiotics induce expression of a novel gene, *drp* 35, in *Staphylococcus aureus*,” *Biochemical and Biophysical Research Communications*, vol. 264, no. 2, pp. 348–351, 1999.

K. Morikawa, T. Hidaka, H. Murakami, H. Hayashi, and T. Ohta, “Staphylococcal Drp35 is the functional counterpart of the eukaryotic PONs,” *FEBS Microbiology Letters*, vol. 249, no. 1, pp. 185–190, 2005.

K. Kobayashi, S. D. Ehrlich, A. Albertini, et al., “Essentia *Bacillus subtilis* genes,” in *Proceedings of National Academy of Sciences of the United States of America*, vol. 100, no. 8, pp. 4678–4683, 2003.

P. K. Martin, T. Li, D. Sun, D. P. Biek, and M. B. Schmid, “Role in cell permeability of an essential two-component system in *Staphylococcus aureus*,” *Journal of Bacteriology*, vol. 181, no. 12, pp. 3666–3673, 1999.

J. D. Blount, N. B. Metcalfe, T. R. Birkhead, and P. F. Surai, “Carotenoid modulation of immune function and sexual attractiveness in zebra finches,” *Science*, vol. 300, no. 5616, pp. 125–127, 2003.

S. T. Mayne, “Beta-carotene, carotenoids, and disease prevention in humans,” *FASEB Journal*, vol. 10, no. 7, pp. 690–701, 1996.

D. Umeno and F. H. Arnold, “Evolution of a pathway to novel long-chain carotenoids,” *Journal of Bacteriology*, vol. 186, no. 5, pp. 1531–1536, 2004.

W. E. Kloos, K. H. Schleifer, and F. Gotz, “The genus staphylococcus,” in *The Prokaryotes*, B. Balows, H. G. Triiper, G. M. Dworkin, W. Harder, and K. H. Schleifer, Eds., pp. 1259–1420, Springer, New York, NY, USA, 1991.

L. Tao, A. Schenze, J. M. Odom, and Q. Cheng, “Novel carotenoid oxidase involved in biosynthesis of 4′-diapolycolepine dialdehyde,” *Applied and Environmental Microbiology*, vol. 71, no. 6, pp. 3294–3301, 2005.