S. epidermidis is an opportunistic pathogen; however, it is capable of storing and transmitting drug-resistant genes (Bloemendaal et al., 2010; Otto, 2013). The ability of S. epidermidis to form biofilms in vivo allows it to be highly resistant to chemotherapeutics and can ultimately lead to chronic disease (Cerca et al., 2005). Some Chinese medicinal herbal products have been found to prevent biofilm formation in bacteria (Guan et al., 2013; Wang et al., 2015; Yang et al., 2016). Total alkaloids of S. alopecuroides (TASA) are an alkaloid mixture that has demonstrated a better inhibitory effect on the late stage of S. epidermidis biofilm thickening than ciprofloxacin (CIP), and erythromycin (ERY) (Li et al., 2016).

In order to investigate the inhibitory mechanism of TASA, the global transcriptome changes of biofilm-forming S. epidermidis response to total alkaloids of Sophora alopecuroides was observed. Bioinformatic analyses were further used to compare the differential gene expression between control and the treated samples. It was found that 282 genes were differentially expressed, with 92 up-regulated and 190 down-regulated. These involved down-regulation of the sulfur metabolism pathway. It was suggested that inhibitory effects on Staphylococcus epidermidis and its biofilm formation of the total alkaloids of S. alopecuroides was mainly due to the regulation of the sulfur metabolism pathways of S. epidermidis.

Key words: Staphylococcus epidermidis, sulfur metabolism, total alkaloids of Sophora alopecuroides, transcriptome

Global Transcriptome Changes of Biofilm-Forming Staphylococcus epidermidis Responding to Total Alkaloids of Sophora alopecuroides

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Abstract

Transcriptome changes of biofilm-forming Staphylococcus epidermidis response to total alkaloids of Sophora alopecuroides was observed. Bioinformatic analyses were further used to compare the differential gene expression between control and the treated samples. It was found that 282 genes were differentially expressed, with 92 up-regulated and 190 down-regulated. These involved down-regulation of the sulfur metabolism pathway. It was suggested that inhibitory effects on Staphylococcus epidermidis and its biofilm formation of the total alkaloids of S. alopecuroides was mainly due to the regulation of the sulfur metabolism pathways of S. epidermidis.

Key words: Staphylococcus epidermidis, sulfur metabolism, total alkaloids of Sophora alopecuroides, transcriptome
dataset was used as inputs into DESeq2 package (Love et al., 2014) to analysis the unigenes expression based on RPKM (reads per kilobase transcriptome per million mapped reads). A fold change of ≥ 2 and a minimum false discovery rate (FDR) of < 0.01 were accepted as indicators of the differentially expressed genes (DEGs) after Benjamini-Hochberg post hoc correction. The DEGs were BLASTX against the Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Group (COG) and Gene Ontology (GO) to identify their predicted biological function. In order to verify the RNA-Seq results, the expression levels of selected DEGs were quantified by RT-qPCR.

First, Nano 2000 (Thermo Scientific, Wilmington, USA) was used to measure the concentration of total mRNA and 500 ng of RNA was reverse-transcribed into cDNA with PrimeScript™ RT Master Mix (TaKaRa). RT-qPCR was performed with an iQ5 light cycler (Bio-Rad) by using SYBR® Premix EX Taq™ II (TaKaRa) in a 20 μl reaction volume, which consisted of 0.25 mM of each primer, 10 μl of SYBR Premix Ex Taq II and 1 μL of template cDNA. PCR conditions were as follows: 30 s at 95°C followed by 40 cycles of 5 s at 95°C and 30 s at 58°C. The reference gene was 16S rRNA, data were acquired through Bio-Rad and analyzed by using the 2−ΔΔCT method (Livak and Schmittgen, 2001). All experiments were performed in triplicate.

282 unigenes were differentially expressed under the screening criteria (fold change ≥ 2 and FDR < 0.01). Within these DEGs, 92 unigenes were significantly up-regulated and 190 unigenes were significantly down-regulated. The DEGs were searched against the KEGG database to identify their biological pathways. The 20 greatest enriched pathways were listed in Fig. 1. Pathways (represented by symbols) in the upper left quadrant of Fig. 1 contain DEGs with more significant and reliable enrichment levels, therefore the sulfur metabolism pathway ranked first. In sulfur metabolism pathway, there are nine DEGs which were all down-regulated significantly, corresponding to the encoded enzymes included serine acetyltransferase (CysE, EC 2.3.1.30), phosphoadenosine phosphosulfate reductase (CysH, EC 1.8.1.2), sulfite reductase (CysL and CysJ, EC 1.8.4.8), cystathionine beta-lyase (Cbl, EC 2.5.1.48), cysteine synthase (CysK and CysM, EC 2.5.1.47), sulfate adenylyl transferase (Sat, EC 2.7.7.4) and adenyllysulfate kinase (CysC, EC 2.7.1.25) (Fig. 2). These enzymes directly affect the content of sulfur metabolites such as cysteine (Cys), methionine (Met), glutathione (GSH), etc. RT-qPCR results further demonstrated that all nine genes showed similar expression patterns to those of

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**Fig. 1.** KEGG pathway scatter diagram of differentially expressed genes. Each pattern represents a KEGG pathway with its name listed in the right. X-coordinate represents the enrichment factor showing the ratio of all genes annotated in the pathway, in contrast to the differentially expressed genes annotated in the same pathway. The smaller the enrichment factor, the enrichment level of the differentially expressed genes was more significant in this pathway. Y-coordinate represents log10(Q_value), the enrichment level of the differentially expressed genes was more significant and reliable in this pathway.
RNA-seq, confirming the RNA-seq based transcriptome datasets were accurate and robust (Fig. 3).

In this research, according to the analysis of transcriptome data, the sulfur metabolism pathway in *S. epidermidis* was greatly influenced by TASA. Sulfur metabolism is an important metabolic pathway in bacteria and its metabolites are involved in many physiological and biochemical processes in cells (Zeng et al., 2013). Cysteine (Cys), methionine (Met) and glutathione (GSH) are associated with bacterial activity and biofilm formation (Murillo et al., 2005; Gales et al., 2008; Soutourina et al., 2009; Li et al., 2013; Solis et al., 2016). A link between sulfate assimilation, Met biosynthesis and biofilm formation have been found in *Candida albicans* (Murillo et al., 2005). Deletion of the *ecm17* gene encoding the sulfite reductase beta subunit resulted in reduced adhesion and poor biofilm formation in *C. albicans* (Li et al., 2013). Additionally, deletion of the master regulator of Cys metabolism *cymR* in *S. aureus* also results in diminished biofilm formation (Soutourina et al., 2009). A recent research has provided further evidence of a role for sulfate assimilation and Cys/Met biosynthesis in *S. epidermidis* ATCC 35984 (RP62A) biofilm formation (Solis et al., 2016). According to the comparison of non-biofilm forming *S. epidermidis* ATCC 12228 and biofilm-forming ATCC 35984, Solis et al. (2016) showed that the sulfate assimilation and cysteine/methionine biosynthesis pathways in ATCC 35984 contained elevated levels (~25% increase) of methionine that were likely linked to biofilm formation. GSH, another important sulfur metabolite, which is associated with intracellular reactive oxygen species (ROS) and hydrogen sulfide (H₂S) also played an important role in biofilm formation (Gales et al., 2008; Klare et al., 2016; Ooi and Tan, 2016). In this study, TASA significantly influenced the sulfur metabolism by down-regulation of nine important genes in this pathway. The concentration of Cys, Met and GSH were directly affected, and most probably finally disrupted the biofilm formation of *S. epidermidis*. This result also validated the above-mentioned relationship between sulfur metabolism and biofilms.

In the pre-experiments, the relative expression of biofilm-related genes including ica, sigB, agr and fbe in *S. epidermidis* was measured. Results showed that these four genes were differentially expressed after treatment.

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**Fig. 2.** Distribution of the differentially expressed genes in a sulfur metabolism pathway. Compared to the control group, the enzyme marked by a green frame is related to a down-regulated gene product. The number in the frame represents the enzyme code.

**Fig. 3.** Comparison of fold changes detected by RNA-seq (RPKM) and q-PCR.

Bars represent the log₂ value of the fold changes of the gene expressions between the control and TASA treatment groups. On the top of the bars are the abbreviations of enzymes encoded by the nine genes.
with TASA at 1/2 MIC for 12 h. However, these four genes did not appear in the 282 DEGs obtained by transcriptome sequencing due to the DEGs screening criteria (fold change ≥ 2 and FDR < 0.01). It was suggested that genes related to biofilm formation did have differential expression, but they may not be directly regulated by TASA. Instead, their expression may be indirectly affected by the changes of other metabolic pathways (i.e. sulfur metabolism).

For a long time, the formation of biofilm has been widely recognized as a dynamic process and regulated by some genes including ica, sigB, agr and fbe etc. Now, it was shown that there is a relationship between sulfur metabolism and biofilm formation in S. epidermidis. Based on the results of transcriptome analysis, it was suggested that TASA inhibitory effects on S. epidermidis and its biofilm formation is mainly due to the regulation of the sulfur metabolism pathway.

The regulatory mechanism of TASA has not been analyzed due to its multi-channeled and multi-targeted actions. In the study, the effect of TASA on S. epidermidis was comprehensively analyzed by using RNA-seq. The enrichment of DEGs showed that metabolism, genetic information processing and environmental information processing were greatly influenced by TASA. The sulfur metabolism pathway was the most significant with all of the key enzymes in this pathway being down-regulated. Given the important physiological role of sulfur metabolism in bacteria and its effect on biofilm formation, it is concluded that the inhibitory effect of TASA on S. epidermidis and its biofilm formation are mainly due to its actions on this pathway.

In conclusion, according to the analysis of differential expression and metabolic pathway enrichment, the molecular mechanism of TASA regulation of pathogenicity, virulence and metabolism of S. epidermidis was further characterized. The sulfur metabolism pathway was identified and results could provide valuable information for follow-up studies to examine the regulatory mechanism of TASA on S. epidermidis, and could serve as the basis for exploring potential drug targets.

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