Transcriptome Analysis Reveals Multiple Pathways of Lobelia chinensis in Inhibiting Streptococcus pyogenes

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Abstract: Clinically, Lobelia chinensis has the potential to treat Streptococcus pyogenes (GAS) infections. This study demonstrated that Lobelia chinensis and penicillin have comparative inhibitory effects when their concentration was 12 mg/mL. To uncover the possible pathways of inhibition of GAS by Lobelia chinensis, transcriptome analysis was used to explore significantly changed genes when GAS was cultured under Lobelia chinensis. Lobelia chinensis could induce alterations of 366 genes in expression level, mainly involving biosynthesis process, translation, cytoplasm, and lipid, carbohydrate metabolic process. In addition, penicillin only induced 17 genes alteration and no GO/KEGG pathway enrichment. Therefore, Lobelia chinensis showed more modes of regulating GAS than penicillin. The regulatory modes of Lobelia chinensis may be the inhibition of cell replication and growth of GAS. This study indicated that Lobelia chinensis is a potential drug for the treatment of GAS infection due to its considerable inhibition effects and multiple inhibition modes.

Keywords: Lobelia chinensis, Streptococcus pyogenes, Penicillin, Transcriptome

1. Introduction

Streptococcus pyogenes (group A Streptococcus, GAS) is a Gram-positive human pathogen that causes an estimated 500,000 deaths globally per year [1, 2]. Diseases associated with GAS infection include pharyngitis, streptococcal toxic shock syndrome, acute rheumatic fever, and rheumatic heart disease [3]. Unfortunately, there is no vaccine against GAS infections due to more than 220 streptococcal M protein variants, which are the surface protein, vaccine antigen and virulence factor [4, 5]. In the past few decades, penicillin has been used as the first-line drug for GAS infections in most parts of the world and there is no better choice. However, the study of penicillin-resistant Streptococcus pyogenes is rapidly and world-widely reported [6, 7]. Therefore, more effects are needed to find new drugs for treatment of GAS infections.

Traditional Chinese Medicine (TCM) may be an alternative way to explore antibacterial drugs. Previous studies have reported the antibacterial activities of some herbal extracts [8, 9] or monomers [10]. The main ingredients of Lobelia chinensis are alkaloids and flavones [11], which are often used as antibacterial drugs against infection. Experimental studies show that Lobelia chinensis is effective against gram-positive and gram-negative bacteria [12, 13]. According to the diameter of the antibacterial circle and the minimal inhibitory concentration (MIC), Lobelia chinensis has a better inhibitory effect on Streptococcus pyogenes.

As we all known, there are many kinds of antibacterial pathways for TCM. The transcriptomics analysis may effectively reveal the anti-bacterial pathways of TCM. Therefore, this study used RNA-seq sequencing to explore the whole gene expression in resting cells of Streptococcus pyogenes under extracts of Lobelia chinensis.

2. Materials and Methods

2.1. Microbial Strains, Culture Conditions and Drug Treatment

The experiments used Streptococcus pyogenes ATCC21059 strain. The strain was initially cultured in
Luria Broth medium under aerobic conditions at 37°C to obtain logarithmic phase cells of *S. pyogenes* at 18 h. After washing, the cells were resuspended by 0.9% NaCl to OD 600nm = 1.0. Cell resuspension solution was in resting cultured conditions at 37°C.

The aqueous extracts of *Lobelia chinensis* were first freeze-dried into powder and then resuspended in 0.9% NaCl solution to 0.5 g/mL. Penicillin was stored in 0.5 g/mL solution. Three concentrations (3.0 mg/mL, 6.0 mg/mL and 12.0 mg/mL) of *Lobelia chinensis* or penicillin were placed in a resting culture of cell resuspension to study the cell death rates, cell membrane permeability and bacterial virulence after 18 h of culture. To perform transcriptome analysis of *S. pyogenes*, the concentrations of *Lobelia chinensis* or penicillin was 12.0 mg/mL and total RNA of *S. pyogenes* was extracted after 2 h of culture. "Bc" represents blank control, "Pc" represents penicillin treatment, and "Lc" represents *Lobelia chinensis* treatment.

### 2.2. Determination of Lactate Dehydrogenase Activity

Lactate dehydrogenase could be released into liquid supernatant due to cell membrane disruption. Therefore, the lactate dehydrogenase activity of the supernatant can reflect the cell death rate. 5 mL culture medium was collected and centrifuged at 10000 rpm for 10 min under 4°C. After centrifugation, the total supernatant was filtered using a 0.22 µm filter. The total supernatant was then lyophilized to remove water, and 1 mL PBS (pH 7.4, 0.01M) was added to dissolve residual components. The above solution was tested for lactate dehydrogenase activity using a lactate dehydrogenase assay kit (Nanjing Jiancheng Bioengineering Institute). Supernatant without drug treatment served as blank control.

### 2.3. Determination of Cell Membrane Permeability

When the bacterial cells are inhibited, the permeability of the cell membrane increases. 2 mL cell supernatant were collected and added with 2.9 µmol/L propidium iodide (PI). The culture medium was placed in darkness at 37°C for 60 min and then centrifuged at 10000 rpm for 10 min. After centrifugation, the bacterial cells were washed twice and resuspended in PBS (pH 7.4, 0.01M). The cell resuspension solution was detected at 495 nm. If cell membrane permeability increases, PI could enter cell and insert the double-stranded DNA. Therefore, the amount of PI embedding double-stranded DNA could emit fluorescence at 495 nm light. The fluorescence of PI in the cell solution treated at 63°C for 30 min was considered to be 100% fluorescence.

### 2.4. Determination of Bacterial Virulence

The virulence of *streptococcus pyogenes* was reflected by its hemolytic ability. 5 mL of cell supernatant was collected and centrifuged at 12000 rpm for 5 min. After centrifugation, the supernatant was removed and the bacterial cells were resuspended with 8 mL PBS (pH 7.4, 0.01 M) and 1 mL aseptic defibrinous rabbit blood. The suspension was placed at 37°C for 60 min and then centrifuged at 8000 rpm for 1 min. The supernatant was detected at 543 nm and the absorbance could reflect the degree of hemolysis. In general, the low absorbance of the supernatant indicates that *streptococcus pyogenes* has high hemolysis capacity.

### 2.5. RNA Isolations, Library Construction and Sequencing

Total RNA of each sample was isolated using RNeasy Mini Kit (Cat#74106, Qiagen) according to the manufacturer’s instructions. The quantity and quality of total RNA were evaluated using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE), gel electrophoresis and an Agilent 2100 analyzer (Agilent technologies, Santa Clara, CA, US). The total RNA with absorbance 260/280 ratio between 1.9 and 2.0 and a content of greater than 50 ng was used for removing ribosomal RNA. The depletion of ribosomal RNA was performed with the Ribo-Zero kit for meta-bacteria (Epicentre Biotechnologies, Madison, WI, USA).

Random oligonucleotides and SuperScript III were used to synthesize the first strand cDNA. Second strand cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase treatment. A paired-end library was constructed from the cDNA synthesized using a Genomic Sample Prep Kit (Illumina). cDNA fragments around 300 bp in length were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15 cycle PCR reaction. The products were purified with the AMPure XP system and quantified using the Agilent 2100 system (Agilent). The multiplexed DNA libraries were then mixed in equal volumes at a normalized concentration of 10 mM. The library was then sequenced on the Illumina HiSeq 1500 platform (by the Shanghai Personal Biotechnology Co., Ltd. Shanghai, China).

### 2.6. RNA-seq Data Analysis

Raw reads of all samples were mixed together to perform filtration using the following criteria: (1) reads with adaptor were removed; (2) reads containing more than 50 bases with low quality (Q20) were removed; (3) reads with more than 3 N bases were removed; (4) low quality bases or assigned as N bases at the 3’ tail were removed; (5) reads shorter than 20 bp were also removed. All the bases in these sequences were defined. De novo transcriptome assembling was carried out step by step as Trinity software performed.

Then high quality reads of each sample were remapped to transcripts to estimate the abundance of transcripts. Those transcripts with no reads mapped in all samples were considered errors and removed. All the transcripts were searched against the *streptococcus pyogenes* reference genome using a CLC genomics Workbench 8.0. The count data of expression values were then analyzed using a DESeq package of R/Bioconductor. The differentially expressed
genes were identified by performing a negative binomial test using the DESeq software, with the cut-off fold-change larger than 2. The raw sequence reads were normalized by dividing with size factors, then $\log_2(N+1)$ transformed.

The sequences were BLAST searched and annotation against the NCBI non-redundant (nr) databases, Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and gene ontology (GO) database, with a cut-off E-value of $1\times10^{-5}$. Functional annotations were implied by sequence similarity against the nr database and the annotations of first sequence with highest sequence similarity and clear functional annotation were associated with the corresponding sequences. Functional annotation by GO was analyzed against the GO database, and the pathways annotations were retrieved using the internal KEGG information of hits in the GO database.

3. Results

3.1. Inhibition of Streptococcus Pyogenes by Lobelia Chinensis

As shown in Figure 1, three indicators were used to reflect the inhibitory effects of Lobelia chinensis and penicillin on Streptococcus pyogenes. The Lobelia chinensis could markedly promote the release of lactate dehydrogenase, increase cell membrane permeability, and reduce the virulence of Streptococcus pyogenes. Additionally, the above inhibitory effects were positively correlated with the concentration of Lobelia chinensis. Although penicillin had better inhibition than Lobelia chinensis, there was no difference in inhibition among three concentrations of penicillin. When the concentration was 12 mg/mL, the inhibitory effect of Lobelia chinensis on the cell membrane permeability and virulence of Streptococcus pyogenes were almost consistent with that of penicillin.

![Figure 1](image-url). Inhibitory effects of Lobelia chinensis and penicillin on Streptococcus pyogenes which were reflected by lactate dehydrogenase activity, permeability of cell membrane, and bacterial virulence. Significance was analyzed by the Student's t-test ($n=3$, “*”: $p<0.05$ compared with Bc; “**”: $p<0.01$ compared with Bc; “+”: $p<0.05$ compared with Pc; “++”: $p<0.01$ compared with Pc).
3.2. Lobelia Chinensis Induced Multiple Genes Expression Changes in Streptococcus Pyogenes

To reveal the inhibitory pathways of Lobelia chinensis or penicillin on Streptococcus pyogenes, transcriptome analysis was used to reveal differentially expressed genes (DEGs). As shown in Figure 2, the different expressed genes were defined with a threshold of the absolute value (> 1) of Log2 (Fold Change). There were 366 DEGs between Bc and Lc. The detailed information of DEGs has been listed in Table 1. Among the 366 DEGs of Streptococcus pyogenes, Lobelia chinensis induced 201 up-regulated genes, and 165 down-regulated genes. The results of GO and KEGG enrichment analysis showed the 201 up-regulated genes involved in lipid metabolic process, carbohydrate metabolic process, and metabolism of terpenoids and polyketides, the 165 down-regulated genes involved in biosynthetic process, structural molecule activity, RNA binding, organelle, intracellular, cytoplasm, translation, methyltransferase activity, and translation (Figure 3). The number of DEGs in Bc vs Pc was 17 (Table 2). Compared with the Bc, penicillin induced up-regulation of 3 genes and down-regulation of 14 genes in Streptococcus pyogenes. However, there was no GO and KEGG enrichment pathway being identified in the DEGs in Bc vs Pc.

Figure 2. The volcano plot of differentially expressed genes (DEGs) from Streptococcus pyogenes between Lc and Bc, between Bc and Pc. Blue dot represents DEGs, red dot represent non-DEGs.

Figure 3. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment results for DEGs from Streptococcus pyogenes between Lc and Bc.
| ID           | Bc expression | Lc expression | p Value          | Annotation                                                                 |
|--------------|---------------|---------------|------------------|-----------------------------------------------------------------------------|
| DP15_RS00060 | 110           | 28            | 4.47E-10         | DNA gyrase subunit A                                                        |
| DP15_RS00065 | 153           | 28            | 3.72E-26         | class A sortase                                                              |
| DP15_RS00070 | 378           | 26            | 1.07E-301        | glyoxalase                                                                  |
| DP15_RS00075 | 280           | 27            | 2.38E-133        | hypothetical protein                                                         |
| DP15_RS00080 | 7964          | 1243          | 3.04E-04         | lipid kinase VegS/Rv2252/BmrU family                                          |
| DP15_RS00248 | 298           | 79            | 9.33E-49         | alanlytransferase                                                            |
| DP15_RS00250 | 646           | 34            | 0                | heme ABC transporter ATP-binding protein                                     |
| DP15_RS00255 | 743           | 39            | 0                | membrane protein                                                             |
| DP15_RS00260 | 617           | 37            | 0                | pyridoxamine kinase                                                         |
| DP15_RS00270 | 540           | 217           | 5.71E-04         | hypothetical protein                                                         |
| DP15_RS00275 | 181           | 73            | 0.00218302       | cardiolipin synthase                                                         |
| DP15_RS00285 | 124           | 27            | 1.35E-17         | lipote–protein ligase A                                                     |
| DP15_RS00340 | 151           | 42            | 4.56E-09         | sugar ABC transporter permease                                              |
| DP15_RS00380 | 233           | 101           | 0.007660396      | two-component sensor histidine kinase                                       |
| DP15_RS00425 | 913           | 87            | 1.28E-72         | 23S rRNA methyltransferase                                                  |
| DP15_RS00430 | 413           | 37            | 4.38E-136        | inositol monophosphatase                                                    |
| DP15_RS00435 | 206           | 49            | 2.22E-14         | hypothetical protein                                                         |
| DP15_RS00440 | 152           | 28            | 4.54E-29         | regulatory protein Spx                                                       |
| DP15_RS00445 | 93            | 24            | 2.34E-11         | bifunctional riboflavin kinase/FMN adenyltransferase                        |
| DP15_RS00535 | 177           | 52            | 4.34E-08         | hypothetical protein                                                         |
| DP15_RS00600 | 929           | 74            | 1.81E-164        | membrane protein                                                             |
| DP15_RS00615 | 46            | 19            | 6.08E-04         | 4-alpha-glucanotransferase                                                  |
| DP15_RS00645 | 238           | 100           | 8.93E-04         | transposase                                                                 |
| DP15_RS00655 | 112           | 53            | 0.009042071      | D-alamyl-telopeichoic acid biosynthesis protein DhtD                         |
| DP15_RS00695 | 193           | 69            | 1.16E-04         | PTS cellbiose transporter subunit IIC                                        |
| DP15_RS00735 | 53            | 23            | 0.001011901      | hypothetical protein                                                         |
| DP15_RS00770 | 481           | 61            | 1.80E-76         | integrase                                                                   |
| DP15_RS00795 | 822           | 67            | 2.04E-171        | thi oesterase                                                               |
| DP15_RS00875 | 112           | 34            | 1.90E-07         | hypothetical protein                                                         |
| DP15_RS00885 | 444           | 43            | 1.21E-135        | membrane protein                                                             |
| DP15_RS00890 | 93            | 25            | 9.39E-10         | uridine kinase                                                               |
| DP15_RS00900 | 339           | 139           | 0.008173083      | deoacylase                                                                  |
| DP15_RS00915 | 26            | 10            | 2.63E-04         | phosphocarrier protein HPr                                                   |
| DP15_RS00955 | 134           | 60            | 0.004320379      | CAAX amino protease                                                         |
| DP15_RS01030 | 939           | 44            | 0                | hypothetical protein                                                         |
| DP15_RS01080 | 191           | 82            | 0.013642265      | potassium transporter Kup                                                   |
| DP15_RS01230 | 77            | 35            | 0.016337765      | phage tail tape measure protein                                             |
| DP15_RS01305 | 165           | 73            | 0.002370695      | hypothetical protein                                                         |
| DP15_RS01335 | 11            | 5             | 0.007029435      | hypothetical protein                                                         |
| DP15_RS01400 | 19            | 6             | 4.21E-06         | excisionase                                                                 |
| DP15_RS01420 | 645           | 169           | 1.23E-10         | hypothetical protein                                                         |
| DP15_RS01430 | 302           | 30            | 1.62E-148        | transcriptional regulator                                                   |
| DP15_RS01440 | 37            | 15            | 5.34E-04         | phage repressor protein                                                     |
| DP15_RS01455 | 2256          | 204           | 1.73E-122        | DNA-binding protein HU                                                       |
| DP15_RS01480 | 92            | 42            | 0.006537327      | DNA repair protein RecN                                                      |
| DP15_RS01485 | 272           | 76            | 1.84E-49         | ArgR family transcriptional regulator                                       |
| DP15_RS01490 | 196           | 79            | 5.85E-04         | cell division protein FixJ                                                  |
| DP15_RS01495 | 208           | 66            | 5.47E-07         | polypropynyl synthetase                                                     |
| DP15_RS01500 | 291           | 119           | 5.78E-04         | exodeoxyribonuclease 7 small subunit                                        |
| DP15_RS01505 | 86            | 27            | 3.20E-07         | exodeoxyribonuclease VII large subunit                                       |
| DP15_RS01555 | 592           | 92            | 1.82E-11         | isoleucine–tRNA ligase                                                      |
| DP15_RS01640 | 596           | 248           | 0.008564199      | peptidase                                                                   |
| DP15_RS02000 | 1322          | 129           | 5.18E-106        | hypothetical protein                                                         |
| DP15_RS02100 | 152           | 60            | 4.97E-04         | membrane protein                                                             |
| DP15_RS02130 | 879           | 139           | 6.85E-37         | hypothetical protein                                                         |
| DP15_RS02140 | 16            | 8             | 0.009363103      | bacteriophage peptidoglycan hydrolase                                       |
| DP15_RS02585 | 1040          | 86            | 2.32E-133        | fatty acid-binding protein DegV                                             |
| DP15_RS02595 | 386           | 149           | 0.001138288      | haloacid dehalogenase                                                       |
| DP15_RS02605 | 455           | 88            | 1.11E-18         | tagatose 1,6-diphosphate aldolase                                           |
| DP15_RS02610 | 243           | 100           | 5.90E-04         | tagatose-6-phosphate kinase                                                 |
| DP15_RS02615 | 296           | 46            | 4.18E-37         | galactose-6-phosphate isomerase                                             |
| DP15_RS02620 | 368           | 42            | 3.25E-75         | galactose-6-phosphate isomerase                                             |
| DP15_RS02625 | 188           | 62            | 9.01E-06         | PTS galactitol transporter subunit IIC                                       |
| DP15_RS02630 | 199           | 86            | 0.001680541      | PTS fructose transporter subunit IIB                                        |
| DP15_RS02665 | 744           | 68            | 1.74E-134        | ribosome-binding factor A                                                   |
| ID          | Bc expression | Lc expression | p Value       | Annotation                                      |
|------------|---------------|---------------|---------------|------------------------------------------------|
| DP15_RS02755 | 250           | 92            | 8.12E-05      | hypothetical protein                            |
| DP15_RS02760 | 514           | 109           | 1.89E-12      | PTS mannose transport subunit EIIAB             |
| DP15_RS02765 | 676           | 104           | 1.71E-31      | PTS mannose/fructose/sorbos transporter subunit IIC |
| DP15_RS02770 | 897           | 155           | 1.24E-15      | PTS mannose transporter subunit IID             |
| DP15_RS02775 | 927           | 60            | 1.19E-29      | PTS mannose transporter accessory protein ManO   |
| DP15_RS02780 | 983           | 91            | 9.74E-77      | serine-5'-RNA ligase                           |
| DP15_RS02875 | 96            | 46            | 0.016878501   | D-alanyl-D-alanine carboxypeptidase              |
| DP15_RS02880 | 44            | 9             | 1.54E-25      | phosphoglycerate mutase                        |
| DP15_RS02885 | 194           | 27            | 2.03E-71      | membrane protein                               |
| DP15_RS02935 | 664           | 73            | 5.53E-56      | haloacid dehalogenase                          |
| DP15_RS03030 | 89            | 21            | 1.12E-13      | mannose-6-phosphate isomerase                   |
| DP15_RS03035 | 196           | 77            | 4.62E-04      | fructokinase                                   |
| DP15_RS03060 | 405           | 161           | 5.16E-04      | N utilization substance protein B homolog       |
| DP15_RS03100 | 424           | 27            | 0             | 30S ribosomal protein S18                       |
| DP15_RS03175 | 521           | 58            | 3.37E-68      | DNA polymerase IV                               |
| DP15_RS03205 | 69            | 22            | 1.02E-06      | multidrug MFS transporter                       |
| DP15_RS03220 | 2169          | 212           | 2.08E-10      | transcriptional regulator                       |
| DP15_RS03245 | 285           | 97            | 1.14E-05      | hypothetical protein                            |
| DP15_RS03395 | 36            | 12            | 2.77E-08      | Apu family transcriptional regulator           |
| DP15_RS03410 | 55            | 14            | 1.14E-12      | hypothetical protein                            |
| DP15_RS03415 | 47            | 20            | 2.72E-04      | methyltransferase                               |
| DP15_RS03480 | 10            | 4             | 0.002676136   | IstB-like ATP-binding protein                   |
| DP15_RS03530 | 20            | 8             | 6.71E-05      | oxidoreductase                                 |
| DP15_RS03615 | 264           | 94            | 3.67E-05      | 2-deoxyribose-5-phosphate aldolase             |
| DP15_RS03630 | 328           | 163           | 0.037653154   | GntR family transcriptional regulator           |
| DP15_RS03635 | 203           | 26            | 6.60E-05      | 30S ribosomal protein S14                       |
| DP15_RS03725 | 87            | 21            | 8.69E-13      | CTP synthetase                                 |
| DP15_RS03735 | 58            | 15            | 1.19E-13      | DNA-directed RNA polymerase subunit delta       |
| DP15_RS03745 | 491           | 93            | 2.15E-23      | hypothetical protein                            |
| DP15_RS03750 | 412           | 42            | 1.86E-99      | membrane protein                               |
| DP15_RS03755 | 212           | 42            | 1.83E-20      | hydroxymethylpyrimidine/ phosphomethylpyrimidine kinase |
| DP15_RS03760 | 269           | 45            | 5.50E-30      | trRNA pseudouridine(38,39,40) synthase TruA     |
| DP15_RS03765 | 198           | 54            | 4.66E-10      | competence protein ComX                         |
| DP15_RS03820 | 893           | 68            | 5.13E-20      | hypothetical protein                            |
| DP15_RS03855 | 38            | 16            | 0.001181859   | lanthiotic salivaricin A                        |
| DP15_RS03890 | 48            | 21            | 4.43E-04      | galactose-6-phosphate isomerase                 |
| DP15_RS04025 | 988           | 330           | 6.19E-04      | FMN reductase                                   |
| DP15_RS04115 | 121           | 28            | 3.41E-15      | exodeoxyribonuclease III                        |
| DP15_RS04320 | 407           | 85            | 1.27E-14      | glycerol dehydrogenase                         |
| DP15_RS04390 | 1049          | 98            | 5.32E-57      | hypothetical protein                            |
| DP15_RS04415 | 117           | 54            | 0.018115652   | Clp protease ClpX                               |
| DP15_RS04420 | 77            | 14            | 2.84E-33      | transcriptional regulator                       |
| DP15_RS04510 | 161           | 74            | 0.017745345   | endopeptidase                                  |
| DP15_RS04745 | 618           | 179           | 3.03E-05      | membrane protein                               |
| DP15_RS04810 | 674           | 240           | 0.002894797   | DUF368 domain-containing protein               |
| DP15_RS04815 | 362           | 52            | 2.24E-42      | coenzyme A pyrophosphatase                      |
| DP15_RS04890 | 238           | 30            | 2.23E-59      | L-serine dehydratase                            |
| DP15_RS04960 | 187           | 48            | 9.14E-11      | sugar transporter                               |
| DP15_RS05345 | 30            | 10            | 8.03E-07      | transcriptional regulator                       |
| DP15_RS05355 | 48            | 23            | 0.005849542   | protein-tyrosine-phosphatase                    |
| DP15_RS05380 | 305           | 116           | 0.002550178   | MATE family efflux transporter                  |
| DP15_RS05725 | 89            | 43            | 0.010263903   | membrane protein                               |
| DP15_RS05740 | 366           | 45            | 6.09E-64      | Single-stranded DNA-binding protein 1           |
| DP15_RS05930 | 2057          | 324           | 9.03E-31      | hypothetical protein                            |
| DP15_RS06010 | 935           | 352           | 0.033608351   | membrane protein                               |
| DP15_RS06100 | 40            | 15            | 1.75E-04      | DNA repair protein RadA                        |
| DP15_RS06110 | 70            | 12            | 3.02E-33      | TIGR00266 family protein                       |
| DP15_RS06180 | 122           | 46            | 7.41E-05      | N-acetylneuraminate lyase                       |
| DP15_RS06185 | 162           | 30            | 3.83E-25      | hypothetical protein                            |
| DP15_RS06260 | 57            | 22            | 2.92E-04      | glyceraldehyde-3-phosphate dehydrogenase       |
| DP15_RS06365 | 3130          | 173           | 0             | transposase                                    |
| DP15_RS06400 | 86            | 26            | 5.63E-09      | competence protein ComX                        |
| DP15_RS06430 | 36            | 15            | 5.29E-05      | ribosome silencing factor RsfS                  |
| DP15_RS06480 | 237           | 81            | 4.06E-06      | potassium transporter Trk                       |
| DP15_RS06570 | 891           | 282           | 0.004199357   | membrane protein insertase YidC                 |
| DP15_RS06770 | 702           | 114           | 8.17E-29      | copper homeostasis protein CuxC                 |
| DP15_RS06780 | 487           | 152           | 2.42E-05      | exodeoxyribonuclease III                        |
| ID          | Be expression | Lc expression | p Value     | Annotation                                      |
|------------|---------------|---------------|-------------|------------------------------------------------|
| DP15_RS06850 | 1207          | 56            | 0           | hypothetical protein                            |
| DP15_RS06910 | 3260          | 317           | 1.96E-43    | MarR family transcriptional regulator          |
| DP15_RS07100 | 250           | 44            | 2.38E-34    | bacteriocin                                     |
| DP15_RS07105 | 400           | 54            | 5.98E-67    | bacteriocin                                     |
| DP15_RS07160 | 86            | 43            | 0.03069606  | transcriptional regulator                      |
| DP15_RS07205 | 136           | 62            | 0.00634067  | pyrrolidone-carboxylate peptidase               |
| DP15_RS07305 | 28            | 14            | 0.02899345  | metallo-hydrolase                              |
| DP15_RS07335 | 76            | 35            | 0.00520858  | L-glutamate ligase                              |
| DP15_RS07345 | 79            | 26            | 2.85E-06    | S-adenosylmethionine synthetase                 |
| DP15_RS07440 | 6             | 2             | 0.042715349 | exfoliative toxin                               |
| DP15_RS07455 | 100           | 30            | 3.97E-08    | asparagine ligase A                             |
| DP15_RS07480 | 249           | 52            | 4.50E-19    | transposase                                     |
| DP15_RS07505 | 176           | 19            | 1.72E-76    | transcription antiterminator BglG               |
| DP15_RS07510 | 377           | 58            | 9.60E-25    | PTS beta-glu cose transporter subunit EII BCA   |
| DP15_RS07515 | 586           | 34            | 0           | 6-phospho-beta-glu cosidase                    |
| DP15_RS07570 | 7152          | 1016          | 4.30E-12    | membrane protein                                |
| DP15_RS07585 | 131           | 53            | 1.59E-04    | hypothetical protein                            |
| DP15_RS07605 | 264           | 55            | 3.74E-18    | phosphoglycerate mutase                        |
| DP15_RS07660 | 73            | 15            | 1.11E-20    | triose-phosphate isomerase                      |
| DP15_RS07665 | 356           | 99            | 8.83E-07    | peptidoglycan branched peptide synthesis protein |
| DP15_RS07700 | 187           | 75            | 6.05E-04    | transcriptional regulator                      |
| DP15_RS07755 | 49            | 16            | 6.11E-08    | haloaicid dehalogenase                         |
| DP15_RS07780 | 263           | 98            | 1.11E-04    | hypothetical protein                            |
| DP15_RS07785 | 129           | 16            | 1.93E-67    | acetoin reductase                               |
| DP15_RS07840 | 778           | 201           | 1.79E-11    | 50S ribosomal protein L31 type B               |
| DP15_RS07875 | 94            | 21            | 3.87E-18    | DNA gyrase subunit B                            |
| DP15_RS07880 | 186           | 20            | 1.02E-81    | DNA gyrase subunit B                            |
| DP15_RS07885 | 229           | 26            | 1.51E-71    | septation ring formation regulator EzzrA        |
| DP15_RS07890 | 673           | 192           | 5.36E-08    | hypothetical protein                            |
| DP15_RS07900 | 57            | 23            | 3.74E-04    | ATP synthase subunit gamma                      |
| DP15_RS08035 | 62            | 31            | 0.01197644  | peroxiredoxin                                  |
| DP15_RS08130 | 96            | 39            | 6.88E-04    | alpha-L-Rha alpha-1,3-L-threomosyltransferase   |
| DP15_RS08140 | 122           | 43            | 8.95E-06    | glycosyl transferase family 2                   |
| DP15_RS08170 | 1156          | 265           | 5.02E-15    | ferredoxin                                     |
| DP15_RS08225 | 86            | 22            | 2.06E-11    | glutathione-disulfide reductase                 |
| DP15_RS08425 | 653           | 53            | 7.49E-215   | exosor tase                                    |
| DP15_RS08430 | 388           | 36            | 7.16E-114   | igG-degrading protease                          |
| DP15_RS08475 | 528           | 149           | 0.00306376  | 5'-nucleotidase C-terminal domain protein       |
| DP15_RS08475 | 128           | 16            | 1.59E-77    | GTP pyrophosphokinase                          |
| DP15_RS08480 | 159           | 34            | 1.43E-18    | transcriptional regulator                      |
| DP15_RS08485 | 190           | 64            | 9.52E-06    | two-component sensor histidine kinase           |
| DP15_RS08490 | 180           | 32            | 3.55E-26    | mevalonate kinase                               |
| DP15_RS08495 | 382           | 132           | 2.20E-04    | diphosphomevalonate decarboxylase              |
| DP15_RS08500 | 310           | 93            | 7.44E-07    | phosphomevalonate kinase                       |
| DP15_RS08505 | 558           | 103           | 1.37E-17    | type 2 isopenentyl-diphosphate Delta-isomerase  |
| DP15_RS08550 | 97            | 30            | 2.52E-07    | ribose-5-phosphate isomerase                   |
| DP15_RS08565 | 85            | 26            | 6.60E-08    | purine-nucleoside phosphorylase                 |
| DP15_RS08570 | 152           | 43            | 2.89E-09    | purine-nucleoside phosphorylase                 |
| DP15_RS08580 | 575           | 224           | 0.00712371  | transcriptional regulator                      |
| DP15_RS08625 | 139           | 150           | 0.013133741 | glyc erol-3-phosphate acyltransferase           |
| DP15_RS08730 | 34            | 16            | 0.00868872  | DNA replication protein DnaD                   |
| DP15_RS08740 | 67            | 33            | 0.01450889  | SAM-dependent methyltransferase                |
| DP15_RS08750 | 210           | 75            | 3.95E-05    | FAD-dependent oxidoreductase                   |
| DP15_RS08775 | 243           | 81            | 7.25E-06    | glucose-1-phosphate thymidyltransferase        |
| DP15_RS08760 | 316           | 64            | 6.32E-19    | dTDP-4-dehydrohamnose 3,5-epimerase            |
| DP15_RS08765 | 331           | 82            | 5.03E-11    | dTDP-glucose 4,6-dehydrofate                   |
| DP15_RS08785 | 187           | 80            | 0.00648443  | hypothetical protein                            |
| DP15_RS08840 | 716           | 332           | 0.010351324 | hypothetical protein                            |
| DP15_RS08855 | 764           | 46            | 4.83334224E-315 | lipote–protein ligase A                      |
| DP15_RS08855 | 52            | 21            | 6.81E-05    | PTS mannose transporter subunit II A            |
| DP15_RS08865 | 90            | 44            | 0.02244472  | PTS mannose transporter subunit II C            |
| DP15_RS08970 | 107           | 34            | 2.39E-07    | PTS mannose transporter subunit IID            |
| DP15_RS09010 | 370           | 92            | 3.51E-08    | tRNA modification GTPase                       |
| DP15_RS09130 | 1206          | 65            | 0           | DNA repair protein RadC                       |
| DP15_RS09165 | 96            | 33            | 5.38E-06    | GTP pyrophosphokinase                          |
| DP15_RS09175 | 55            | 27            | 0.016624782 | RNA pseudouridine synthase                     |
| DP15_RS09185 | 888           | 59            | 1.83E-25    | oxidoreductase                                 |
| ID      | Be expression | Lc expression | p Value          | Annotation                                    |
|---------|---------------|---------------|------------------|-----------------------------------------------|
| DP15_RS09230 | 61            | 28            | 0.009164716      | thymidine kinase                              |
| DP15_RS00010 | 26            | 67            | 3.00E-10         | protein-(glutamine-N5) methyltransferase       |
| DP15_RS00100 | 13            | 30            | 3.33E-07         | ribonuclease III                               |
| DP15_RS00120 | 39            | 92            | 3.67E-07         | oxaloacetate decarboxylase                     |
| DP15_RS00145 | 36            | 87            | 1.82E-09         | GntR family transcriptional regulator         |
| DP15_RS00155 | 14            | 40            | 8.88E-11         | hypothetical protein                           |
| DP15_RS00160 | 22            | 48            | 6.17E-06         | acetyl-CoA carboxylase biotin carboxyl carrie  |
| DP15_RS00320 | 28            | 60            | 8.47E-07         | phosphopantothenate--cysteine ligase          |
| DP15_RS00480 | 121           | 263           | 3.15E-06         | TetR family transcriptional regulator         |
| DP15_RS00520 | 31            | 64            | 1.28E-05         | ATP-dependent DNA helicase PerA               |
| DP15_RS00720 | 19            | 48            | 8.63E-10         | membrane protein                               |
| DP15_RS00780 | 18            | 43            | 8.72E-08         | diaminopimelate epimerase                     |
| DP15_RS00800 | 16            | 39            | 1.51E-09         | 23S rRNA (uracil-5-)-methyltransferase RmRNA  |
| DP15_RS00820 | 26            | 52            | 9.97E-06         | 3-phosphoshikimate 1-carboxyvinyltransferase  |
| DP15_RS01005 | 26            | 62            | 1.01E-08         | competence protein CoiA                       |
| DP15_RS01070 | 51            | 117           | 5.45E-07         | methyltransferase                              |
| DP15_RS01075 | 38            | 88            | 6.19E-07         | hypothetical protein                           |
| DP15_RS01180 | 6             | 15            | 2.68E-08         | phage lysis/muramidase                         |
| DP15_RS01210 | 9             | 20            | 9.02E-07         | hypothetical protein                           |
| DP15_RS01215 | 3             | 8             | 1.29E-07         | hyaluronoglucosaminidase                      |
| DP15_RS01220 | 11            | 24            | 1.19E-07         | peptidase                                     |
| DP15_RS01340 | 2             | 7             | 1.48E-08         | hypothetical protein                           |
| DP15_RS01415 | 17            | 49            | 5.04E-12         | hypothetical protein                           |
| DP15_RS01635 | 12            | 24            | 2.05E-05         | DNA starvation/stationary phase protection protein |
| DP15_RS01695 | 20            | 50            | 1.52E-09         | ornithine carbamoyltransferase                 |
| DP15_RS01700 | 15            | 38            | 7.17E-10         | acetyltransferase                              |
| DP15_RS01705 | 22            | 46            | 8.26E-07         | arginine deiminase                             |
| DP15_RS01725 | 19            | 38            | 3.60E-06         | penicillin-binding protein 1A                  |
| DP15_RS01745 | 9             | 20            | 1.92E-07         | dihydroxyepiandrosterone aldolase             |
| DP15_RS01830 | 18            | 48            | 2.16E-09         | shikimate dehydrogenase (NADP+)                |
| DP15_RS01840 | 27            | 55            | 2.82E-05         | DNA-binding response regulator                 |
| DP15_RS01880 | 22            | 53            | 1.19E-07         | hyaluronidase                                  |
| DP15_RS01895 | 44            | 107           | 1.82E-05         | alpha-mannosidase                              |
| DP15_RS01905 | 31            | 63            | 1.50E-05         | RNA methyltransferase                          |
| DP15_RS02110 | 15            | 34            | 5.39E-07         | cell cycle protein GpsB                       |
| DP15_RS02125 | 35            | 73            | 8.69E-06         | penicillin-binding protein 1A                  |
| DP15_RS02180 | 1             | 2             | 0.001727         | hypothetical protein                           |
| DP15_RS02410 | 16            | 40            | 2.62E-09         | nicotinate phosphoribosyltransferase           |
| DP15_RS02470 | 11            | 26            | 3.48E-07         | ribosomal RNA small subunit methyltransferase H |
| DP15_RS02510 | 22            | 48            | 5.50E-06         | transaldolase                                  |
| DP15_RS02525 | 21            | 47            | 7.69E-07         | glycerol transporter                           |
| DP15_RS02540 | 8             | 17            | 2.11E-06         | hypothetical protein                           |
| DP15_RS02795 | 36            | 72            | 5.79E-05         | acetyl-CoA carboxylase biotin carboxyl subunit |
| DP15_RS02810 | 9             | 25            | 9.38E-10         | beta-ketoacyl-[acyl-carrier-protein] synthase II |
| DP15_RS02815 | 18            | 38            | 2.34E-05         | beta-ketoacyl-ACP reductase                    |
| DP15_RS03075 | 12            | 28            | 2.71E-07         | hypothetical protein                           |
| DP15_RS03215 | 44            | 103           | 6.14E-06         | Xaa-Pro dipeptidyl-peptidase                   |
| DP15_RS03265 | 1             | 5             | 2.92E-07         | holin                                         |
| DP15_RS03280 | 13            | 35            | 1.91E-12         | phage hyaluronidase                            |
| DP15_RS03285 | 8             | 19            | 2.72E-07         | hyaluronoglucosaminidase                      |
| DP15_RS03545 | 1             | 2             | 0.001618         | antirepressor                                  |
| DP15_RS03590 | 16            | 34            | 8.74E-06         | hypothetical protein                           |
| DP15_RS03600 | 9             | 27            | 2.34E-12         | DNA polymerase III subunit epsilon             |
| DP15_RS03690 | 15            | 33            | 2.49E-06         | protease                                      |
| DP15_RS03905 | 13            | 32            | 2.79E-08         | addiction module toxin RelE                   |
| DP15_RS03920 | 35            | 70            | 3.55E-05         | 50S ribosomal protein L13                     |
| DP15_RS03925 | 9             | 39            | 1.41E-18         | transcriptional regulator                     |
| DP15_RS03950 | 9             | 22            | 7.76E-09         | hypothetical protein                           |
| DP15_RS04010 | 13            | 26            | 3.15E-04         | hypothetical protein                           |
| DP15_RS04070 | 13            | 31            | 3.70E-07         | pullulanase                                    |
| DP15_RS04080 | 11            | 26            | 1.28E-06         | sugar ABC transporter ATP-binding protein      |
| DP15_RS04085 | 31            | 62            | 4.71E-06         | leucine-rich protein                           |
| DP15_RS04100 | 12            | 28            | 9.43E-08         | GTP pyrophosphokinase                          |
| DP15_RS04105 | 4             | 9             | 9.89E-07         | hypothetical protein                           |
| DP15_RS04140 | 43            | 101           | 4.45E-07         | aminobenzoate synthetase                      |
| DP15_RS04145 | 65            | 185           | 1.74E-11         | glutamine amidotransferase                     |
| DP15_RS04150 | 46            | 116           | 2.22E-08         | recombinase RarA                               |
| ID          | Bc expression | Lc expression | p Value | Annotation                                      |
|-------------|---------------|---------------|---------|------------------------------------------------|
| DP15_RS04210| 18            | 37            | 1.93E-05| peptidase C5                                   |
| DP15_RS04225| 1             | 6             | 7.65E-15| M protein, serotype 6                          |
| DP15_RS04240| 5             | 16            | 8.50E-10| hypothetical protein                            |
| DP15_RS04245| 34            | 86            | 1.87E-08| immunogenic secreted protein                   |
| DP15_RS04250| 31            | 71            | 1.02E-06| two-component sensor histidine kinase          |
| DP15_RS04275| 13            | 35            | 2.09E-10| ABC transporter                                |
| DP15_RS04280| 13            | 40            | 9.06E-15| hypothetical protein                            |
| DP15_RS04295| 77            | 191           | 1.16E-09| streptopain                                   |
| DP15_RS04300| 35            | 81            | 3.43E-07| streptopain                                   |
| DP15_RS04325| 8             | 24            | 1.22E-11| fructose-6-phosphate aldolase                 |
| DP15_RS04330| 14            | 50            | 2.54E-19| glycyln radical enzyme                         |
| DP15_RS04405| 15            | 31            | 3.20E-06| molecular chaperone GroEL                      |
| DP15_RS04475| 69            | 146           | 5.24E-06| hypothetical protein                            |
| DP15_RS04500| 6             | 16            | 4.44E-09| 30S ribosomal protein S2                       |
| DP15_RS04505| 10            | 33            | 3.34E-13| elongation factor Ts                           |
| DP15_RS04535| 74            | 160           | 3.48E-06| glyoxalase                                    |
| DP15_RS04595| 13            | 29            | 5.65E-07| damage-inducible protein A                     |
| DP15_RS04605| 33            | 79            | 1.27E-08| Holliday junction ATP-dependent DNA helicase RuVA|
| DP15_RS04770| 16            | 36            | 7.2E-06 | 50S ribosomal protein L32                     |
| DP15_RS04945| 23            | 66            | 1.95E-13| UTP-glucose-1-phosphate uridylyltransferase    |
| DP15_RS04950| 65            | 142           | 1.67E-07| hypothetical protein                           |
| DP15_RS05310| 26            | 66            | 1.22E-08| bifunctional phosphoribosylaminomimidazolecarboxamidase |
| DP15_RS05330| 32            | 93            | 4.20E-12| 5-(carboxyamino)imidazole ribonucleotide synthase|
| DP15_RS05385| 55            | 21            | 2.56E-10| 30S ribosomal protein S10                     |
| DP15_RS05395| 9             | 34            | 1.65E-22| 50S ribosomal protein L4                      |
| DP15_RS05400| 14            | 44            | 1.83E-16| 50S ribosomal protein L23                     |
| DP15_RS05410| 8             | 22            | 3.33E-08| 30S ribosomal protein S19                     |
| DP15_RS05415| 14            | 38            | 1.10E-09| 50S ribosomal protein L22                     |
| DP15_RS05430| 7             | 15            | 1.49E-05| 50S ribosomal protein L29                     |
| DP15_RS05465| 13            | 26            | 1.49E-05| 50S ribosomal protein L6                      |
| DP15_RS05470| 9             | 26            | 2.18E-10| 50S ribosomal protein L18                     |
| DP15_RS05510| 13            | 39            | 3.08E-12| 30S ribosomal protein S11                     |
| DP15_RS05520| 13            | 33            | 3.42E-09| 50S ribosomal protein L17                     |
| DP15_RS05715| 51            | 115           | 1.18E-06| pyrroline-5-carboxylate reductase              |
| DP15_RS05720| 31            | 83            | 3.70E-10| glutamyl aminopeptidase                        |
| DP15_RS05760| 1             | 2             | 4.46E-05| hypothetical protein                           |
| DP15_RS05800| 15            | 33            | 3.86E-06| hypothetical protein                           |
| DP15_RS05830| 63            | 139           | 1.82E-06| DNA-binding protein                            |
| DP15_RS05895| 62            | 134           | 5.43E-06| membrane protein                               |
| DP15_RS05900| 47            | 101           | 1.88E-05| peptide binding protein                        |
| DP15_RS05915| 38            | 105           | 1.53E-05| transcription termination/antitermination protein NusG|
| DP15_RS05920| 15            | 46            | 7.22E-12| leucine--tRNA ligase                           |
| DP15_RS05995| 19            | 36            | 1.03E-05| glycine/betaine ABC transporter permease       |
| DP15_RS06015| 21            | 46            | 4.93E-07| tRNA-guanine(34)transglycosylase               |
| DP15_RS06070| 31            | 65            | 3.88E-06| glyceraldehyde-3-phosphate dehydrogenase (NAD(P)(+)) |
| DP15_RS06145| 21            | 51            | 1.08E-08| Jag protein                                   |
| DP15_RS06150| 13            | 35            | 8.39E-08| 50S ribosomal protein L34                     |
| DP15_RS06200| 16            | 32            | 2.85E-05| ribonuclease M5                                |
| DP15_RS06210| 29            | 64            | 1.26E-07| ribosome small subunit-dependent GTPase A       |
| DP15_RS06245| 15            | 36            | 1.04E-08| 30S ribosomal protein S12                     |
| DP15_RS06250| 3             | 8             | 5.85E-07| 30S ribosomal protein S7                      |
| DP15_RS06275| 8             | 19            | 9.34E-05| exfoliative toxin                              |
| DP15_RS06310| 27            | 56            | 7.60E-06| Fe-S cluster assembly protein SufD             |
| DP15_RS06565| 8             | 18            | 1.66E-06| transcription elongation factor GreA           |
| DP15_RS06580| 33            | 75            | 6.38E-08| 23S rRNA methyltransferase                    |
| DP15_RS06610| 32            | 70            | 1.94E-06| phosphodiesterase                             |
| DP15_RS06665| 47            | 96            | 1.56E-06| SAM-dependent methyltransferase               |
| DP15_RS06680| 25            | 50            | 3.91E-06| manganese-dependent inorganic pyrophosphatase |
| DP15_RS06705| 43            | 117           | 1.22E-10| ABC transporter ATP-binding protein            |
| DP15_RS06730| 16            | 49            | 4.43E-14| hypothetical protein                           |
| DP15_RS06740| 12            | 24            | 6.06E-05| DNA polymerase III subunit delta'             |
| DP15_RS06745| 89            | 185           | 1.52E-06| Tlp protein                                  |
| DP15_RS06750| 10            | 25            | 1.86E-06| hypothetical protein                           |
| DP15_RS06755| 15            | 33            | 1.31E-06| initiation-control protein YabA               |
| DP15_RS06795| 21            | 57            | 9.38E-08| peptidase S8                                  |
| DP15_RS06830| 17            | 34            | 1.14E-04| ADP-ribosyltransferase                        |
Figure 4 showed the mutual and distinct DEGs induced by Lobelia chinensis and penicillin, both of which could lead to significant changes in 10 genes in Streptococcus pyogenes. These genes are annotated as phage lysin/muramidase, shikimate dehydrogenase (NADP+), holin, oxidoreductase, 50S ribosomal protein L4, 30S ribosomal protein S11, and UTP--glucose-1-phosphate uridylyltransferase (Table 3). Only the DEGs encoding oxidoreductase was up-regulated by Lobelia chinensis and down-regulated by penicillin. The other 9 DEGs were down-regulated by both Lobelia chinensis and penicillin (Figure 4). In addition, both drugs could regulate different genes in Streptococcus pyogenes. Penicillin specifically induced the up-regulation of streptolysin S family bacteriocin gene and the down-regulation of L-ribulose-5-phosphate 4-epimerase (Figure 4). In Streptococcus pyogenes, genes specifically regulated by Lobelia chinensis mainly were lipid kinase YegS/Rv2252/BmrU family, DNA-binding protein HU, transporter, transcriptional regulator, glutamine amidotransferase and streptopain (Figure 4).
process and methyltransferase activity, indicated that another mode of *Lobelia chinensis* regulating GAS is the inhibition of cell growth. The activity of cell growth is very important for pathogenic ability of bacteria [17, 18]. In summary, two inhibitory modes of *Lobelia chinensis* to GAS were revealed by transcriptome analysis.

### 5. Conclusion

Although the inhibitory effect of *Lobelia chinensis* was weaker than penicillin, its regulatory pathways were more diverse. *Lobelia chinensis* could down-regulate the cell replication and growth pathways of *Streptococcus pyogenes*. Transcriptome analysis has successfully demonstrated that *Lobelia chinensis* is an effective drug for the treatment of GAS infections.

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