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

Xiaoying Lin, Xiangyu Kong, Chengping Wen, Zhixing He*

College of Basic Medical Science, Zhejiang Chinese Medical University, Hangzhou, China

Email address:
hzx2015@zcmu.edu.cn (Zhixing He)

*Corresponding author

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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 chinensi*. *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 chinens* 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 50000 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_{600}=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 0.5g/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 lodide (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 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 defibrous 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 Log2 $^{(N+1)}$ 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 1E-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 indictors 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.

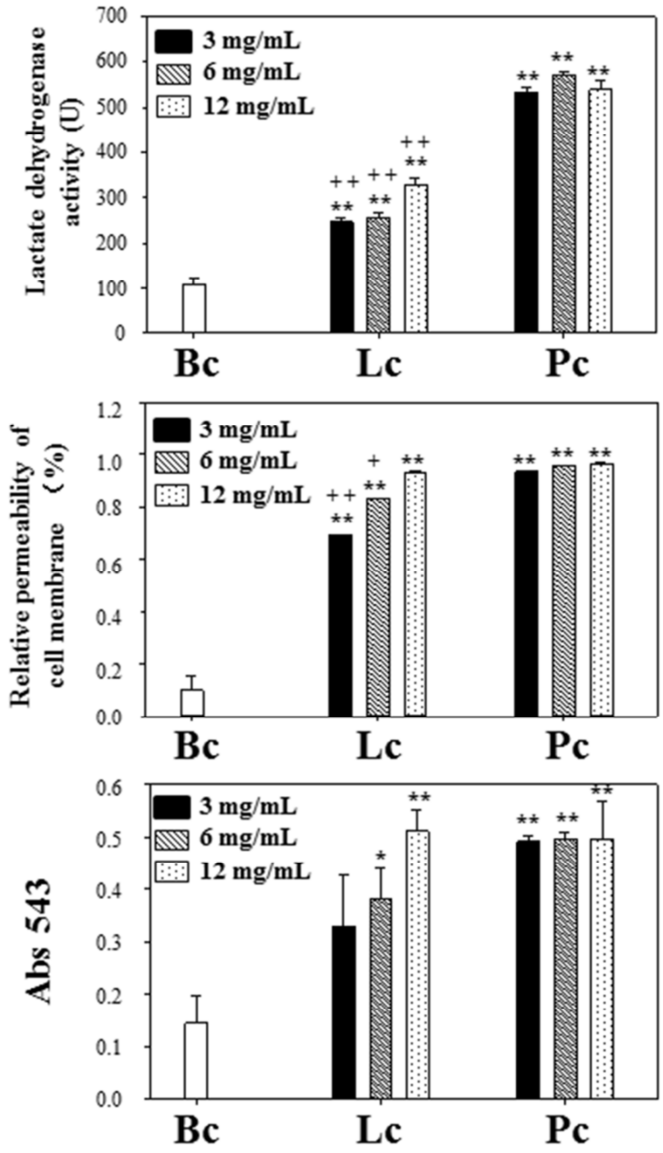

Figure 1. *Inhibitory effects of Lobelia chinensis and penicillin on Streptococcus pyogenes which were reflected by lactate dehydrogenase activity, permeability of cell membrane, and bacterial virulence. Significance was analyzed by the Student's t-test (n=3, "*": p<0.05 compared with Bc; "**": p<0.01 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.

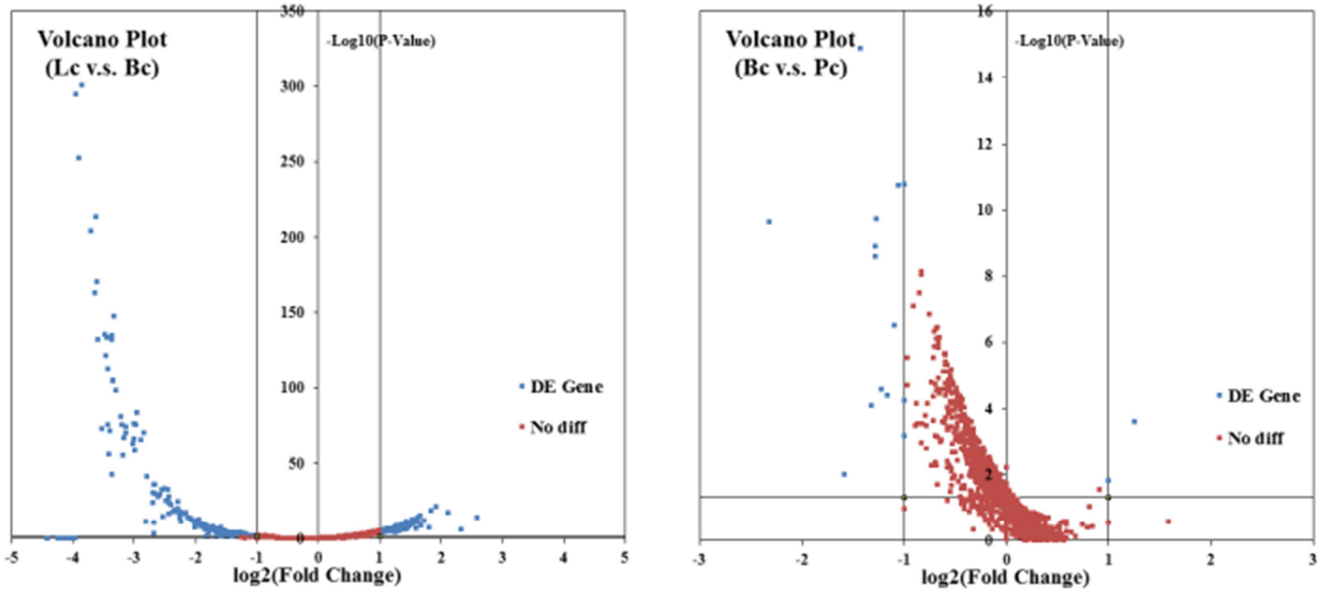

Figure 2. *The volcano plot of differentially expressed genes (DEGs) from Streptococcus pyogenes between Lc and Bc, between Bc and Pc. Blue dot represents DEGs, red dot represent non-DEGs.*

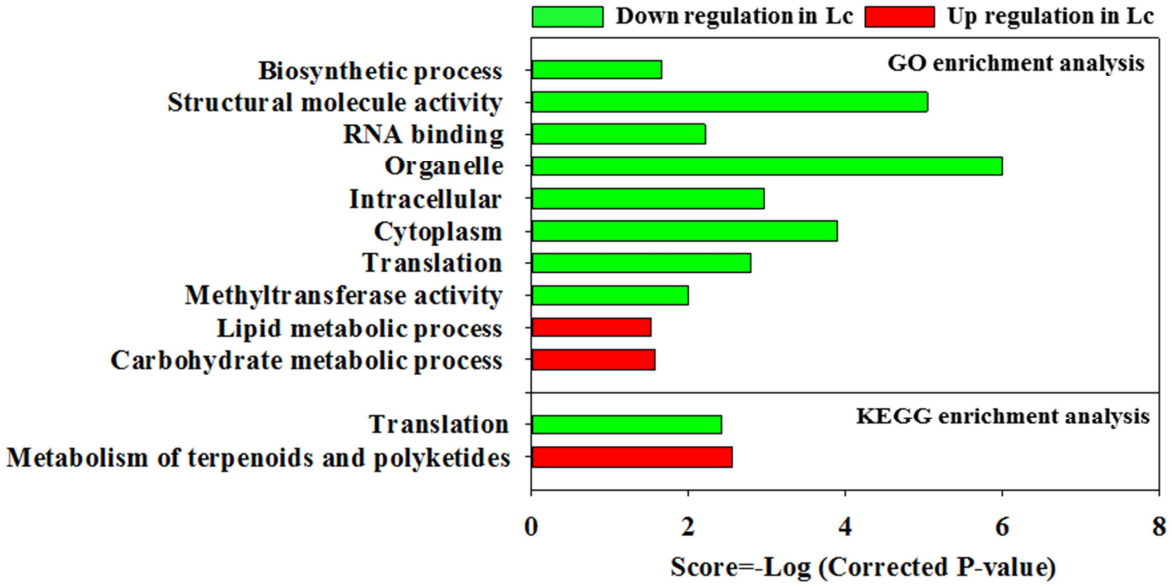

Figure 3. *Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment results for DEGs from Streptococcus pyogenes between Lc and Bc.*

Table 1. *Different expression genes of Streptococcus pyogenes between Bc and Lc.*

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 YegS/Rv2252/BmrU family
DP15_RS00245	298	79	9.33E-09	alanyltransferase
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	lipoate--protein ligase A
DP15_RS00340	151	42	4.56E-09	sugar ABC transporter permease
DP15_RS00380	233	101	0.007668396	two-component sensor histidine kinase
DP15_RS00425	913	87	1.28E-72	23S rRNA methyltransferase
DP15_RS00430	414	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 adenylyltransferase
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-alanyl-lipoteichoic acid biosynthesis protein DltD
DP15_RS00695	193	69	1.16E-04	PTS cellobiose 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	thioesterase
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	deacetylase
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-09	ArgR family transcriptional regulator
DP15_RS01490	196	79	5.58E-04	cell division protein FtsJ
DP15_RS01495	208	66	5.47E-07	polyprenyl 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
DP15_RS02735	1280	111	8.80E-74	transporter

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 transporter subunit EIIAB
DP15_RS02765	676	104	1.71E-31	PTS mannose/fructose/sorbose transporter subunit IIC
DP15_RS02770	897	155	1.24E-15	PTS mannose transporter subunit IID
DP15_RS02775	927	60	1.19E-295	PTS mannose transporter accessory protein ManO
DP15_RS02780	983	91	9.74E-77	serine--tRNA 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-105	transcriptional regulator
DP15_RS03245	285	97	1.14E-05	hypothetical protein
DP15_RS03395	36	12	2.77E-08	ArpU 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-85	30S ribosomal protein S14
DP15_RS03725	87	21	8.69E-13	CTP synthetase
DP15_RS03730	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	tRNA pseudouridine(38,39,40) synthase TruA
DP15_RS03765	198	54	4.66E-10	competence protein ComX
DP15_RS03820	893	68	5.13E-205	hypothetical protein
DP15_RS03855	38	16	0.001181859	lantibiotic 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 CutC
DP15_RS06780	487	152	2.42E-05	exodeoxyribonuclease III

ID	Bc 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	0.030669606	transcriptional regulator
DP15_RS07205	136	62	0.006340567	pyrrolidone-carboxylate peptidase
DP15_RS07305	28	14	0.02899345	metallo-hydrolase
DP15_RS07335	76	35	0.005208587	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-glucoside transporter subunit EIIBCA
DP15_RS07515	586	34	0	6-phospho-beta-glucosidase
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	haloacid 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 EzrA
DP15_RS07890	673	192	5.36E-08	hypothetical protein
DP15_RS07990	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-rhamnosyltransferase
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	exosortase
DP15_RS08430	388	36	7.16E-114	igG-degrading protease
DP15_RS08470	528	149	0.003063761	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 isopentenyl-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	0.007123713	transcriptional regulator
DP15_RS08625	319	150	0.013133741	glycerol-3-phosphate acyltransferase
DP15_RS08730	34	16	0.008688712	DNA replication protein DnaD
DP15_RS08740	67	33	0.014508889	SAM-dependent methyltransferase
DP15_RS08750	210	75	3.95E-05	FAD-dependent oxidoreductase
DP15_RS08755	243	81	7.25E-06	glucose-1-phosphate thymidylyltransferase
DP15_RS08760	316	64	6.32E-19	dTDP-4-dehydrorhamnose 3,5-epimerase
DP15_RS08765	331	82	5.03E-11	dTDP-glucose 4,6-dehydratase
DP15_RS08785	187	80	0.006448443	hypothetical protein
DP15_RS08840	716	332	0.010351324	hypothetical protein
DP15_RS08855	764	46	4.833364224E-315	lipoate--protein ligase A
DP15_RS08955	52	21	6.81E-05	PTS mannose transporter subunit IIA
DP15_RS08965	90	44	0.022444472	PTS mannose transporter subunit IIC
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-253	oxidoreductase

ID	Bc 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 HII
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 PcrA
DP15_RS00720	19	48	8.63E-10	membrane protein
DP15_RS00780	18	43	8.72E-08	diaminopimelate epimerase
DP15_RS00805	16	39	1.51E-09	23S rRNA (uracil-5-)-methyltransferase RumA
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 lysin/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	MmcQ family protein
DP15_RS01745	9	20	1.92E-07	dihydroneopterin 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_RS02420	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 carboxylase 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	7.68E-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	glycyl 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	7.27E-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 phosphoribosylaminoimidazolecarboxamide formyltransferase
DP15_RS05330	32	93	4.20E-12	5-(carboxyamino)imidazole ribonucleotide synthase
DP15_RS05385	21	55	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	1.33E-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_RS05905	10	21	3.29E-05	BMP family ABC transporter substrate-binding protein
DP15_RS05910	19	38	6.15E-05	transcription termination/antitermination protein NusG
DP15_RS05940	15	46	7.22E-12	leucine--tRNA ligase
DP15_RS05990	19	38	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	glycerol-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	5.58E-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	Tpl 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

ID	Bc expression	Lc expression	*p* Value	Annotation
DP15_RS06875	12	30	4.61E-08	NAD(P)-dependent oxidoreductase
DP15_RS06890	7	16	9.60E-06	ADP-ribose pyrophosphatase
DP15_RS06945	10	28	4.12E-10	50S ribosomal protein L11
DP15_RS06950	7	15	1.71E-06	50S ribosomal protein L1
DP15_RS07145	68	137	3.06E-06	histidine kinase
DP15_RS07235	58	125	4.62E-06	Xaa-Pro dipeptidase
DP15_RS07390	32	80	3.30E-09	hypothetical protein
DP15_RS07485	13	27	9.11E-05	haloacid dehalogenase
DP15_RS07820	13	31	6.19E-08	peptidase C69
DP15_RS07825	10	25	1.72E-08	zinc ABC transporter substrate-binding protein AdcA
DP15_RS08070	17	37	3.22E-06	ABC transporter substrate-binding protein
DP15_RS08075	9	18	1.98E-04	30S ribosomal protein S21
DP15_RS08100	11	25	5.60E-07	NAD(P)-dependent oxidoreductase
DP15_RS08175	24	48	5.77E-05	membrane protein
DP15_RS08190	37	85	2.28E-07	50S ribosomal protein L35
DP15_RS08195	5	15	2.56E-07	50S ribosomal protein L20
DP15_RS08240	24	51	5.33E-07	aminotransferase V
DP15_RS08250	15	30	2.45E-05	metallophosphatase
DP15_RS08265	31	83	5.05E-10	50S ribosomal protein L27
DP15_RS08440	11	27	3.04E-08	transposase
DP15_RS08445	16	39	1.95E-07	hypothetical protein
DP15_RS08600	30	63	1.50E-05	amidase
DP15_RS08615	30	65	4.38E-07	uracil-DNA glycosylase
DP15_RS08800	12	27	4.40E-06	phosphonate ABC transporter ATP-binding protein
DP15_RS08820	13	26	3.53E-06	ABC transporter ATP-binding protein
DP15_RS08900	11	22	8.96E-05	haloacid dehalogenase
DP15_RS08940	30	82	6.51E-10	hypothetical protein
DP15_RS09055	22	45	5.29E-05	pyrophosphokinase
DP15_RS09225	19	50	6.71E-10	tautomerase
DP15_RS01315	0	5	1.45E-28	hypothetical protein
DP15_RS01395	0	1	3.45E-14	transcriptional regulator
DP15_RS03810	0	2	1.68E-10	type I restriction endonuclease
DP15_RS07020	0	2	5.81E-26	bacteriocin ABC transporter

Table 2. *Different expression genes of Streptococcus pyogenes between Pc and Lc.*

ID	Pc expression	Bc expression	*p* Value	Annotation
DP15_RS01355	14	7	0.014623886	hypothetical protein
DP15_RS07900	69	29	2.51E-04	streptolysin S family bacteriocin
DP15_RS01360	1	0	3.81E-115	hypothetical protein
DP15_RS01180	7	15	3.09E-07	phage lysin/muramidase
DP15_RS01315	2	5	8.28E-05	hypothetical protein
DP15_RS01330	1	3	0.009661122	hypothetical protein
DP15_RS01340	3	7	2.72E-05	hypothetical protein
DP15_RS01830	23	48	1.85E-11	shikimate dehydrogenase (NADP+)
DP15_RS03265	1	5	2.28E-10	holin
DP15_RS03530	4	8	6.62E-04	oxidoreductase
DP15_RS03540	4	8	5.75E-05	hypothetical protein
DP15_RS04105	4	9	4.00E-05	hypothetical protein
DP15_RS04135	16	39	1.27E-09	hypothetical protein
DP15_RS04945	33	66	1.72E-11	UTP--glucose-1-phosphate uridylyltransferase
DP15_RS05395	14	34	1.85E-10	50S ribosomal protein L4
DP15_RS05510	16	39	2.44E-09	30S ribosomal protein S11
DP15_RS05970	17	46	1.29E-15	L-ribulose-5-phosphate 4-epimerase

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 ($NADP^+$), holing, 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 regular, glutamine amidotransferase and streptopain (Figure 4).

Table 3. *Common differential genes of Streptococcus pyogenes in "Bc vs Lc" and "Bc vs Pc".*

ID	Lc Expression	Pc Expression	Bc Expression	p Value (Bc vs Lc)	p Value (Bc vs Pc)	Annotation
DP15_RS01180	6	7	15	2.68E-08	3.09E-07	phage lysin/muramidase
DP15_RS01315	0	2	5	null	8.28E-05	hypothetical protein
DP15_RS01340	2	3	7	1.48E-08	2.72E-05	hypothetical protein
DP15_RS01830	18	23	48	2.16E-09	1.85E-11	shikimate dehydrogenase (NADP+)
DP15_RS03265	1	1	5	2.92E-07	2.28E-10	holin
DP15_RS03530	20	4	8	6.71E-05	6.62E-04	oxidoreductase
DP15_RS04105	4	4	9	9.89E-07	4.00E-05	hypothetical protein
DP15_RS04945	23	33	66	1.95E-13	1.72E-11	UTP--glucose-1-phosphate uridylyltransferase
DP15_RS05395	9	14	34	1.65E-22	1.85E-10	50S ribosomal protein L4
DP15_RS05510	13	16	39	3.08E-12	2.44E-09	30S ribosomal protein S11

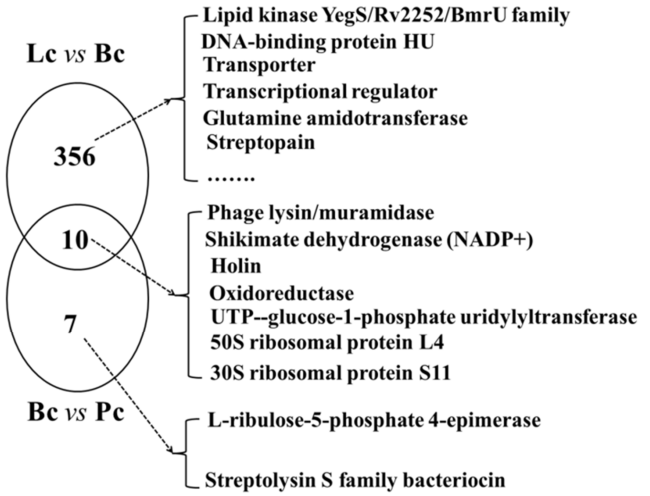

Figure 4. *Venn diagram showing the shared and special DEGs from Streptococcus pyogenes in Lc vs Bc, and Bc vs Pc.*

4. Discussion

Clinically, penicillin is the top-priority drug for the treatment of *Streptococcus pyogenes* infection, but it is susceptible to drug resistance. Drug resistance is mainly due to simplex pathway of penicillin against pathogenic bacteria [14]. According to the transcriptome analysis, penicillin inhibited *Streptococcus pyogenes* by regulating a few genes. Furthermore, the regulatory genes of penicillin could not be enriched in the KEGG or GO pathways. Therefore, this study partially explained the drug resistance of *Streptococcus pyogenes* to penicillin.

In contrast, *Lobelia chinensis* regulated multiple KEGG/GO pathways to inhibit GAS infections. The up-regulated lipid, carbohydrate metabolic process in GAS may be induced by the chemical constituents of *Lobelia chinensis* [11]. The down-regulation of KEGG/GO enrichment pathways in GAS may be the inhibitory pathways of *Lobelia chinensis*. First, *Lobelia chinensis* could inhibit cell replication of GAS based on down-regulation of organelle, intracellular, cytoplasm and structural molecule activity. Blocking cell replication is a normal mode of drugs inhibiting pathogenic bacteria [15, 16]. Second, some KEGG/GO enrichment pathways, such as translation, biosynthetic 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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