微生物学通报
Microbiology China
http://journals.im.ac.cn/wswxtbcn

Nov. 20, 2023, 50(11): 4988−5014
DOI: 10.13344/j.microbiol.china.230193

研究报告

不同遗传背景下 QsvR 调控副溶血弧菌基因表达的转录组分析

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WANG Jian, XUE Xingfan, ZHANG Miaomiao, LI Xue, YANG Wenhui, HU Lingfei, ZHOU Dongsheng, LU Renfei, ZHANG Yiquan. Transcriptome analysis of QsvR regulating gene expression of *Vibrio parahaemolyticus* with different genetic backgrounds[J]. Microbiology China, 2023, 50(11): 4988-5014.

摘　要：【背景】OpaR 是副溶血弧菌群体感应系统的核心调控因子；QsvR 是 AraC 家族转录调控因子，与 OpaR 之间具有相互调控作用；此外，QsvR 对基因表达的调控作用受 OpaR 的影响，但是影响程度并未完全阐明。【目的】探究在野生株(wild-type, WT)和 *opaR* 基因突变株(Δ*opaR*)的遗传背景下 QsvR 的转录调控元，分析 OpaR 对 QsvR 基因表达调控的影响。【方法】分别以 WT 和 Δ*opaR* 为参照，采用 Illumina HiSeq 测序平台进行比较转录组学研究，分析生物膜形成条件下 *qsvR* 基因突变株(Δ*qsvR*)和 Δ*qsvR*Δ*opaR* 的基因表达情况。【结果】在 WT 遗传背景下，QsvR 共调控 1 735 个基因的转录(调控元 1)，其中被激活的基因有 855 个，被抑制的基因有 880 个；在 Δ*opaR* 遗传背景下，QsvR 共调控 1 187 个基因的转录(调控元 2)，其中被激活的基因有 533 个，被抑制的基因有 654 个。调控元 1 和调控元 2 之间共有 517 个重叠基因，且 QsvR 对绝大多数重叠基因的调控关系相反。基因属性分类(gene ontology, GO)数据库富集分析结果显示，调控元 1 和调控元 2 中分别有 467 个和 204 个基因注释到分子功能、细胞组分和生物学过程 3 个一级分类和 30 个二级分类；京都基因和基因组百科全书(Kyoto encyclopedia of genes and genomes, KEGG)对代谢途径的分析结果显示，调控元 1 和调控元 2 中分别有 372 和 678 个基因归到 30 个代谢通路中(Q value<0.05)，调控元 1 中的

资助项目：国家自然科学基金(82072239)；南通市科学技术局基础科学研究计划(JC2021027)；南通市卫生健康委员会科研课题(QN2022044)

This work was supported by the National Natural Science Foundation of China (82072239), the Natural Science Research Project of Nantong Science and Technology Bureau (JC2021027), and the Research Project of Nantong Health Commission (QN2022044).

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Received: 2023-03-13; Accepted: 2023-04-22; Published online: 2023-05-22

基因主要集中在代谢、基因信息处理和环境信息处理上，而调控元 2 中的基因主要集中在细胞代谢通路上。蛋白相邻类的聚簇(cluster of orthologous groups of proteins, COG)数据库分类结果显示，调控元 1 和调控元 2 中的基因主要涉及氨基酸转运与代谢、信号转导、能量产生与转换、一般功能预测基因和未知功能基因等。此外，调控元 1 和调控元 2 中还含有大量调控因子基因和推定的 c-di-GMP 代谢基因，以及若干极生鞭毛基因、荚膜多糖基因、胞外多糖合成基因、IV 型菌毛合成基因、III 型分泌系统基因和 VI 型分泌系统基因等。【结论】QsvR 是副溶血弧菌中的全局性调控因子，控制着大量基因的转录。QsvR 对靶基因的调控关系及 QsvR 调控元组成均受 OpaR 的影响。
关键词：副溶血弧菌；调控元；转录组；QsvR；OpaR

Transcriptome analysis of QsvR regulating gene expression of *Vibrio parahaemolyticus* with different genetic backgrounds

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Abstract: [Background] OpaR is the master quorum sensing regulator of *Vibrio parahaemolyticus*. QsvR, an AraC-type transcriptional regulator, has reciprocal regulatory activities with OpaR. The regulatory effect of QsvR on gene expression is affected by OpaR, while their relationship in gene regulation remains to be fully elucidated. **[Objective]** To investigate QsvR regulons in the wild type (WT) and the *opaR* mutant (Δ*opaR*), and thus determine the effect of OpaR on the gene regulation of QsvR in *V. parahaemolyticus*. **[Methods]** Illumina HiSeq was employed to mine the differentially expressed genes in the *qsvR* mutant (Δ*qsvR*) or Δ*qsvR*Δ*opaR* relative to that in WT or Δ*opaR* under the biofilm formation growth condition. **[Results]** QsvR regulated 1 735 genes (regulon 1) in the WT background, including 855 genes activated and 880 genes repressed. QsvR regulated 1 187 genes (regulon 2) in the Δ*opaR* background, including 533 genes activated and 654 genes repressed. There were 517 common genes shared by regulons 1 and regulons 2, and most of these genes were oppositely regulated by QsvR between the two regulons. According to the results of gene ontology (GO) annotation, 467 and 204 genes from regulons 1 and regulons 2 were respectively annotated to three categories (biological process, molecular function, and cellular component) and thirty sub-categories. The results of Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment showed that 372 and 678 genes from regulons 1 and regulons 2 were respectively enriched in 30 signaling pathways (Q value<0.05). The genes in regulon 1 were mainly enriched in cellular metabolism, genetic information processing, and environmental information processing, and those in regulon 2 in cellular metabolism. The classification results obtained with

the cluster of orthologous groups of proteins (COG) showed that the genes in regulons 1 and regulons 2 were mainly involved in amino acid transport and metabolism, signal transduction mechanisms, energy production and conversion, general function prediction only, and function unknown. In addition, the regulons 1 and regulons 2 contained a large number of regulatory genes and putative c-di-GMP metabolism-associated genes, as well as some polar flagellar genes, capsular polysaccharide genes, exopolysaccharide synthesis genes, type IV pili-associated genes, type III secretion system genes, and type VI secretion system genes. **[Conclusion]** QsvR was a global regulator in *V. parahaemolyticus*, controlling the transcription of a large number of genes. OpaR affected the regulatory actions of QsvR on its target genes and the composition of QsvR regulon.
Keywords: *Vibrio parahaemolyticus*; regulon; transcriptome; QsvR; OpaR

副溶血弧菌(*Vibrio parahaemolyticus*)是一种革兰氏阴性嗜盐弧菌，广泛分布于海洋环境中，是沿海地区细菌性食物中毒的首要病原菌[1]。副溶血弧菌能表达多种毒力因子，主要包括直接耐热溶血素(thermostable direct hemolysin, TDH)、TDH 相关溶血素(TDH-related hemolysin, TRH)、III 型分泌系统(type III secretion system, T3SS)、VI 型分泌系统(type VI secretion system, T6SS)等[2]。TDH 和 TRH 具有溶血活性、肠毒性和细胞毒性[1,3]。副溶血弧菌大、小基因组上各含有一套 T3SS 基因位点，分别称为 T3SS1 和 T3SS2，前者具有细胞毒性，而后者与肠毒性有关[4]。副溶血弧菌还含有两套 T6SS，即 T6SS1 和 T6SS2，二者均具有细胞黏附活性，此外 T6SS1 还具有抑菌活性[5-6]。一些其他因子，如脂多糖、外膜蛋白和胞外蛋白酶等也和副溶血弧菌毒力有一定的关系[2,7]。

生物膜是细菌在固体表面形成的具有一定立体结构的菌落集群，是细菌抵御不利环境的一种生存策略[8]。副溶血弧菌的生物膜形成需要胞外多糖(exopolysaccharide, EPS)、IV 型菌毛、鞭毛等结构的参与，而且是一个被紧密调控的过程[9-12]。EPS 是生物膜基质的主要成分，占生物膜干重的 50%以上[13-14]。*cpsA-K* 和 *scvA-O* 负责 EPS 的合成(分别称为 Cps 多糖和 Scv 多糖)，二者与副溶血弧菌生物膜形成能力呈正相关[15-16]。IV 型菌毛介导菌体黏附到固体表面，也与生物膜形成呈正相关[10]。副溶血弧菌表达的甘露糖敏感血凝素IV型菌毛(mannose-sensitive haemagglutinin type IV pili, MSHA)和几丁质调节菌毛(chitin-regulated pili, ChiRP)均属 IV 型菌毛[17-18]。鞭毛介导的运动性有利于生物膜三维结构的形成[10]。副溶血弧菌具有极生鞭毛和侧生鞭毛，分别介导菌体在液体中游动(swimming)和在固体表面群集性爬动(swarming)[19-20]。此外，胞外 DNA、膜蛋白、荚膜多糖(capsular polysaccharide, CPS)等也能影响生物膜形成[21-22]。

环二鸟苷酸(c-di-GMP)是一种普遍存在于细菌中的小分子第二信使，对生物膜形成、毒力基因表达、运动性等都具有转录后调控作用[23]。鸟苷酸环化酶(含有 GGDEF 结构域)以 GTP 为底物催化生成 c-di-GMP，而磷酸二酯酶(含有 EAL 或 HD-GYP 结构域)则能将 c-di-GMP 降解成 pGpG 或者 GMP[23]。副溶血弧菌能表达 50 多个含有 EAL 和/或 GGDEF 结构域的蛋白质[17]。其中只有少数几个被详细研究。比如，ScrC 和 ScrG 都含有 EAL 和 GGDEF 结构域，但在生理条件下只有 EAL 结构域具有活性，起降解 c-di-GMP 和抑制生物膜形成的作用[24-25]。ScrO、ScrJ、ScrL 和 GefA 都含有 GGDEF 结构域，而 LafV

和 TpdA 都含有 EAL 结构域，它们均可调节副溶血弧菌生物膜形成[26-29]。

QsvR 是 AraC 家族转录调控因子，由操纵子 VPA0607-*qsvR* 编码[30]。QsvR 与群体感应系统核心调控因子 AphA 和 OpaR 之间具有相互调控作用，并共同调控众多细胞通路，包括关键毒力基因表达、生物膜形成、运动性和 c-di-GMP 代谢[12,31-37]。根据 CPS 表达与否，副溶血弧菌菌落可呈透明(translucent, TR)或不透明(opaque, OP)状[38]。*opaR* 突变株的菌落呈 TR 状，而野生株的菌落呈 OP 状[39]。TR 菌株(*opaR* 突变株)和 OP 菌株(野生株)都可以形成生物膜，但结构不同[21]。当以 TR 菌株为亲本，*qsvR* 突变株对表面的黏附活性增强，但不能形成成熟的生物膜；若以 OP 菌株为亲本，*qsvR* 突变株的早期生物膜形成能力增强[21]。QsvR 与 OpaR 协同抑制副溶血弧菌生物膜形成，且 QsvR 能回补 *opaR* 突变所导致的生物膜表型变化，反之亦然[33]。然而，QsvR 的调控作用与 OpaR 的关系并未完全阐明。本文利用 RNA 高通量测序(RNA-Seq)技术分别研究了 QsvR 在野生株和 *opaR* 突变株遗传背景下的转录调控元，分析 OpaR 对 QsvR 基因表达调控的影响，以期阐明 QsvR 对靶基因的调控关系及 QsvR 调控元组成是否受 OpaR 的影响。

1 材料与方法

1.1 材料

1.1.1 菌株

副溶血弧菌 RIMD2210633 (wild type, WT)、*qsvR* 非极性突变株(Δ*qsvR*)、*opaR* 非极性突变株(Δ*opaR*)以及 *qsvR* 和 *opaR* 双基因突变株(Δ*qsvR*Δ*opaR*)均为课题组前期构建[31,33,40]，并保存在南通大学附属南通第三医院检验科。

1.1.2 主要试剂和仪器

TRIzol Reagent，Invitrogen 公司；2×*Taq* PCR Master Mix、SuperReal 彩色荧光定量预混试剂(SYBR Green)和 FastKing 一步法除基因组 cDNA 第一链合成预混试剂，天根生化科技(北京)有限公司。NanoDrop 2000 超微量分光光度计，Thermo Scientific 公司；Illumina HiSeq 测序平台，Illumina 公司；实时荧光定量 PCR 仪，Roche 公司；细菌培养箱，北京六一生物科技有限公司。

1.1.3 培养基

M 肉汤培养基和 HI 肉汤培养基，BD Bioscience 公司。

1.2 菌株培养

分别取 20 μL 甘油保存的 WT、Δ*qsvR*、Δ*opaR* 和 Δ*qsvR*Δ*opaR* 接种于 5 mL HI 肉汤中，37 ℃、200 r/min 培养 12 h。按体积比 1:50 将培养产物转接至 5 mL 新鲜的 HI 肉汤中，置相同条件下培养至 OD_{600} 为 1.4。按体积比 1:50 稀释转接至 10 mL M 肉汤中，充分混匀后分装至 24 孔细胞培养板中，每孔 1.5 mL，30 ℃、150 r/min 培养 48 h，此时应形成了成熟的生物膜结构[33,41]。收集生物膜中的菌体和液体中的游离菌体，供后续试验使用。

1.3 RNA 提取与转录组测序(RNA-Seq)

将 1.2 中收集到的适量菌体(即 WT、Δ*qsvR*、Δ*opaR* 和 Δ*qsvR*Δ*opaR*，各 3 个生物学重复)溶解在 1 mL TRIzol 试剂中，送至苏州金唯智生物技术有限公司进行后续处理，包括 RNA 提取、RNA 样品质量检测、cDNA 文库构建、文库纯化等。采用 Illumina HiSeq 300PE 高通量测序平台对文库进行双末端测序。

1.4 测序数据质量评估和序列比对

利用软件 Bcl2fastq (v2.17.1.14)进行图像碱基识别(base calling)，获得原始测序数据(pass

filter fata)。采用软件 FastQC (v0.10.1)分析测序数据质量，碱基的质量值(quality scores, Q)以 $-10\log_{10}$ (e)计算，e 为错误率。使用 Cutadapt (v1.9.1)软件过滤接头序列、3′-末端碱基等低质量序列。采用 bowtie2 (v2.2.6)软件对过滤后的测序片段(clean data)和参考基因组(副溶血弧菌 RIMD2210633)进行比对，对完全匹配的序列(total mapped reads)进行统计定位，计算 reads 数，并取 $\log_2$ 值。

1.5 差异表达基因分析

利用 HTSeq 软件(v0.6.1)和 FPKM (fragments per kilo bases per million reads)方法计算基因表达量，进而使用 Bioconductor 软件包的 DESeq2 (v1.6.3)进行基因的差异表达分析[42-44]。差异显著性表达基因(differentially expressed gene, DEG)的筛选条件为：基因表达变化倍数在 2 倍以上且 *Q* value (fdr, padj)≤0.05。以差异基因的 FPKM 值为表达水平做层次聚类(hierarchical clustering)分析。利用基因本体(gene ontology, GO)数据库分析 DEG 的分子功能(molecular function)、细胞组分(cellular component)及参与的生物过程(biological process)。通过京都基因和基因组百科全书(Kyoto encyclopedia of genes and genomes, KEGG)数据库分析 DEG 参与的最主要代谢通路(pathway)。利用蛋白相邻类聚簇(cluster of orthologous groups of proteins, COG)数据库对 DEG 进行注释和功能分析。

1.6 实时定量 PCR (real-time quantitative PCR, RT-qPCR)

用 TRIzol Reagent 提取副溶血弧菌的总 RNA，再利用 FastKing 一步法除基因组 cDNA 第一链合成预混试剂盒将其逆转录成 cDNA，进而利用 SuperReal 荧光定量预混试剂彩色版试剂盒进行 RT-qPCR 分析。以 16S rRNA 基因的表达量为内参，采用经典的 $2^{-\Delta\Delta C_t}$ 法对靶基因的转录水平进行相对定量[34]。RT-qPCR 反应体系按 SuperReal 彩色荧光定量预混试剂(SYBR Green)盒说明书配制，即：2×SuperReal Color PreMix 10 μL，正、反向引物(10 μmol/L)各 0.6 μL，cDNA 模板(10 ng/mL) 2 μL，去离子水补足 20 μL。RT-qPCR 扩增条件：95 ℃ 15 min；95 ℃ 30 s，54 ℃ 15 s，72 ℃ 15 s，55 个循环；65 ℃ 5 s；95 ℃ 5 s，绘制溶解曲线。所用引物如表 1 所示。RT-qPCR 试验至少重复 3 次，每次 3 个生物学重复，试验结果用平均值±标准差(standard deviation, SD)表示。表达变化倍数在 2 倍以上且双尾成对 *t* 检验的结果值小于 0.01 ($P<0.01$)，为具有显著性差异的筛选标准。

2 结果与分析

2.1 RNA-Seq 数据质量分析

一共测序获得 9 个 Illumina 文库，每个文库都含有超过 1 496 万的原始测序片段(raw reads)。原始数据(raw data)已上传至 NCBI 数据库(登录号为 PRJNA913656)中。利用 Cutadapt (v1.9.1)软件过滤获得合格序列(clean reads)，进而与副溶血弧菌 RIMD2210633 的基因组进行比对，结果如表 2 所示：总匹配率(total mapped)、单一匹配率(uniquely mapped)和多重匹配率(multiple mapped)分别在 99.242%–99.739%、96.798%–97.942%和 1.634%–2.444%之间，说明合格序列满足分析要求。

2.2 差异表达基因分析

以 WT 中的基因表达水平为参照，利用 DESeq2 (v1.6.3)分析 Δ*qsvR* 中的差异表达基因(称为调控元 1)，或者以 Δ*opaR* 中的基因表达水平为参照，分析 Δ*qsvR*Δ*opaR* 中的差异表达基因(称为调控元 2)，以差异表达变化 2 倍及以上

表 1　本研究所用引物

Table 1　Primers used in this study

Gene	Primer sequence (5′→3′)	Amplicon size (bp)
VP0117	Forward: GACCACCTCAATAGTTATCTG	117
	Reverse: TAAGTAGGCTTGGACATCTC	
VP0218	Forward: CTCTAAGCGCATCAACTGCAT	139
	Reverse: CTGTCTGACGCTGCAACTGCTA	
calR	Forward: GCACTCAATGTTAGAGAAG	182
	Reverse: CCACGGCATTACTTACTG	
VP0699	Forward: CTGACACATCGTGATACTTC	147
	Reverse: TTGATGTTGCAGCTCTTG	
VP1212	Forward: ACACTCCGGTCGTGATCCTA	163
	Reverse: ATGACCGGAGAAGCGTTACC	
scrG	Forward: CTCATCTCTGTTGCCAGTAAGG	164
	Reverse: CCGAACTCGTCCATGTAGAAG	
VP1483	Forward: TCAAAGTGATCGACGGACCA	101
	Reverse: AATTACCTCGTCACCCGTTG	
VP1678	Forward: TATAGCGGCACTTATTCTAC	134
	Reverse: GCTCAGCTAATGCTTTTCCG	
VP1881	Forward: AGAATCAACCAACACACGAA	150
	Reverse: CACAATACTGTTGATGGCGTA	
VP2366	Forward: AAAGCCAAATTGACGCTCTC	180
	Reverse: CTAAGACATAATCTCCCGCATC	
VP2972	Forward: GTTAAAACCCACCTCGCCAA	185
	Reverse: AACAGTTGTAACGCTAACACC	
VP2979	Forward: GCAACTCTCAAGTCATCATC	130
	Reverse: CAACAACCGTCTTCTATGG	
VPA0009	Forward: GTATCGGTAACCAGCTTATG	145
	Reverse: AGGTAGCTCGACTCCACTTG	
VPA0609	Forward: GCACAGAACTTATCGAAAGCC	133
	Reverse: ATCAAAAGATCATTCGAGATCGC	
VPA1130	Forward: GAAAATGGCGAGCTACTGCG	171
	Reverse: GATCGCGTTCATTGGAGTGC	
scrA	Forward: CACACCACGAACACATTGC	151
	Reverse: TCAATAGCGTCACGGAATGC	
VPA1735	Forward: AAAAGACTACCAACGCGCTGA	138
	Reverse: CTCTCATCCATGTGCGGGAA	
16S rRNA	Forward: GACACGGTCCAGACTCCTAC	179
	Reverse: GGTGCTTCTTCTGTCGCTAAC	

且 *Q* value (fdr, padj)≤0.05 为筛选依据。调控元 1 共包含 1 735 个基因，其中 855 个下调基因，880 个上调基因(图 1A)；调控元 2 共包含 1 187 个基因，其中 533 个下调基因，654 个上调基因(图 1B)。

2.3　GO 富集分析

利用 GO 数据库对 DEGs 进行功能分类统计，并将富集最显著的前 30 个 GO term 进行展示。对于调控元 1 (图 2A)，在分子功能(molecular function)方面，富集数量靠前的为核糖体结构

表 2　合格序列与参考基因组匹配统计

Table 2　Statistical results of clean reads mapping with the reference genome

Sample	Total mapped	Uniquely mapped	Multiple mapped
WT-1	16 705 599 (99.328%)	16 324 794 (97.064%)	380 805 (2.264%)
WT-2	16 423 203 (99.463%)	16 052 438 (97.217%)	370 765 (2.245%)
WT-3	17 110 878 (99.242%)	16 689 514 (96.798%)	421 364 (2.444%)
Δ*qsvR*-1	16 007 242 (99.470%)	15 744 339 (97.837%)	262 903 (1.634%)
Δ*qsvR*-2	16 413 379 (99.477%)	16 124 022 (97.723%)	289 357 (1.754%)
Δ*qsvR*-3	16 540 668 (99.468%)	16 233 786 (97.622%)	306 882 (1.845%)
Δ*opaR*-1	17 533 364 (99.516%)	17 156 247 (97.376%)	377 117 (2.140%)
Δ*opaR*-2	14 891 479 (99.677%)	14 558 292 (97.447%)	333 187 (2.230%)
Δ*opaR*-3	15 693 957 (99.549%)	15 346 058 (97.342%)	347 899 (2.207%)
Δ*qsvR*Δ*opaR*-1	16 401 982 (99.427%)	16 126 622 (97.758%)	275 360 (1.669%)
Δ*qsvR*Δ*opaR*-2	16 036 555 (99.339%)	15 753 521 (97.586%)	283 034 (1.753%)
Δ*qsvR*Δ*opaR*-3	17 342 068 (99.739%)	17 029 712 (97.942%)	312 356 (1.796%)

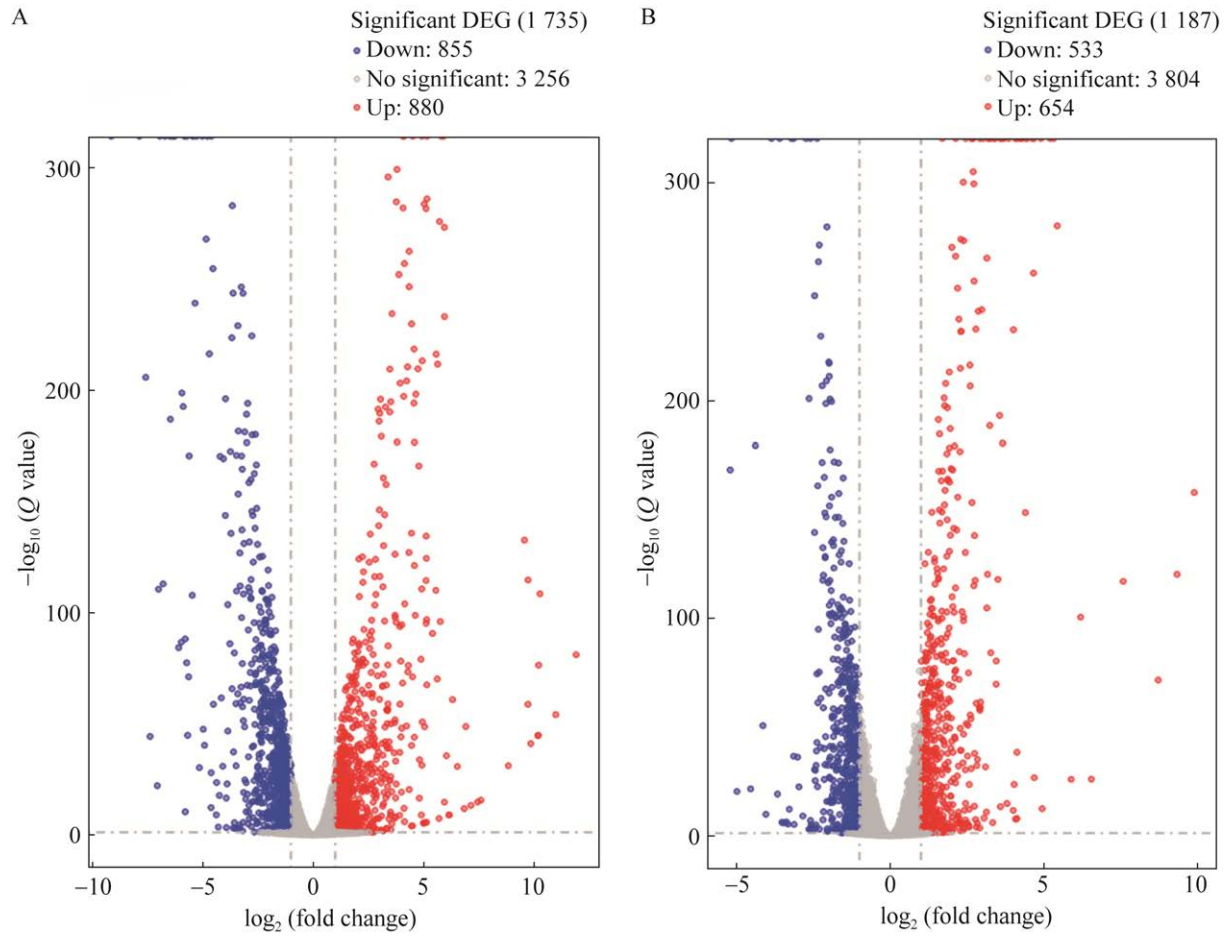

图 1　QsvR 在 WT (A)和 Δ*opaR* (B)背景下的调控元

Figure 1　QsvR regulons in the WT (A) and Δ*opaR* (B) backgrounds.

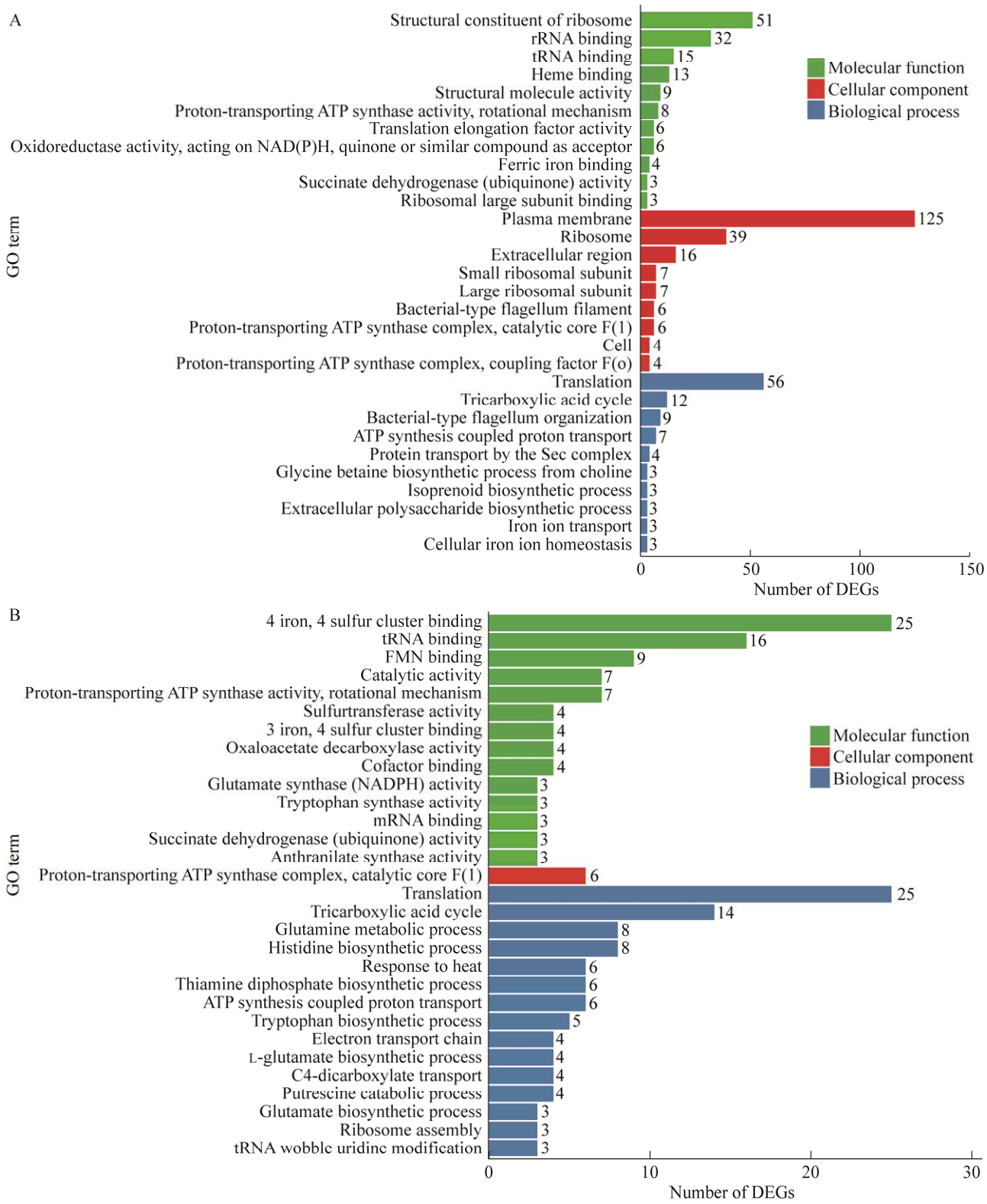

图 2　调控元 1 (A)和调控元 2 (B)中显著性差异表达基因的 GO 富集分布图

Figure 2　The results of GO enrichment of DEGs in regulon 1 (A) and regulon 2 (B).

组成相关(51 个)、rRNA 结合(32 个)、tRNA 结合(15 个)和血红素结合(13 个)；在细胞组分(cellular component)方面，富集结果主要集中在细胞膜相关(125 个)、核糖体相关(39 个)和胞外区相关(16 个)；在生物过程(biological process)方面，富集数量靠前的为翻译相关(56 个)、三羧酸循环相关(12 个)和鞭毛结果相关(9 个)。对于调控元 2 (图 2B)，在分子功能方面，富集数量靠前的为铁硫簇结合(25 个)、tRNA 结合(16 个)和黄素单核苷酸结合(9 个)；在细胞组分方面，只富集出 6 个基因，涉及 ATP 合酶；在生物过程方面，富集数量靠前的为翻译相关(25 个)、三羧酸循环相关(14 个)、谷氨酰胺代谢(8 个)和组氨酸代谢(8 个)。

2.4 KEGG 富集分析

利用KEGG数据库对DEGs参与的pathway进行注释解析，以确定 DEGs 参与的主要代谢途径和信号通路，并对富集最显著的前 30 条进行展示。对于调控元 1 (图 3A)，DEGs 主要集中在有机系统(organismal systems)、细胞代谢(metabolism)、人类疾病(human diseases)、基因信息处理(genetic information processing)、环境信息处理(environmental information processing)和细胞进程(cellular processes)上，分别包含 13、130、23、55、128 和 23 个基因。对于调控元 2 (图 3B)，DEGs 主要集中在细胞代谢、人类疾病和基因信息处理上，分别包含 654、14 和 10 个基因。

2.5 COG 注释分析

利用COG数据库对DEGs编码的蛋白或蛋白集合进行注释和分类分析。对调控元1 (图4A)，位于前 10 的 COG 注释依次集中在一般功能预测基因(general function prediction only)、未知功能基因(function unknown)、氨基酸转运与代谢(amino acid transport and metabolism)、信号转导(signal transduction mechanisms)、能量产生与转换(energy production and conversion)、转录(transcription)、无机离子的转运与代谢(inorganic ion transport and metabolism)、细胞壁/膜/包膜的生物生成(cell wall/membrane/envelope biogenesis)、翻译/核糖体结构及生物生成(translation, ribosomal structure and biogenesis)、碳水化合物的转运与代谢(carbohydrate transport and metabolism)。对于调控元 2 (图 4B)，位于前 10 的 COG 注释依次集中在一般功能预测基因、氨基酸转运与代谢、能量产生与转换、未知功能基因、翻译/核糖体结构及生物生成、转录、无机离子的转运与代谢、信号转导、碳水化合物的转运与代谢、辅酶转运与代谢。在前 10 个 COG 分类中，细胞壁/膜/包膜的生物生成相关 DEGs 是调控元 1 所特有的，而辅酶转运与代谢相关 DEGs 是调控元 2 所特有的。

2.6 关键基因分析

在调控元 1 和调控元 2 中，未知功能基因和一般功能预测基因占比最多，但也包含许多已知功能基因。如表 3 所示，调控元 1 包含 89 个推定的调控因子基因、24 个推定的 c-di-GMP 代谢基因、19 个 CPS 基因、10 个 EPS 多糖基因、6 个 Scv 多糖基因(全部上调)、15 个 IV 型菌毛基因、35 个极生鞭毛基因、1 个侧生鞭毛蛋白基因、20 个 T3SS1 基因、8 个 T3SS2 基因、6 个 T6SS1 基因和 19 个 T6SS2 基因。在调控元 2 (表 4)共包含 51 个推定的调控因子基因、4 个推定的 c-di-GMP 代谢基因、2 个 CPS 基因、1 个 Scv 多糖基因、7 个 IV 型菌毛基因、14 个极生鞭毛基因、2 个 T3SS1 基因、17 个 T3SS2 基因、7 个 T6SS1 基因和 2 个 T6SS2 基因。此外，调控元 1 和调控元 2 之间共有 517 个重叠基因，其中包括 20 个推定的调控因子基因、2 个推定的 c-di-GMP 代谢基因、8 个 CPS 基因、7 个

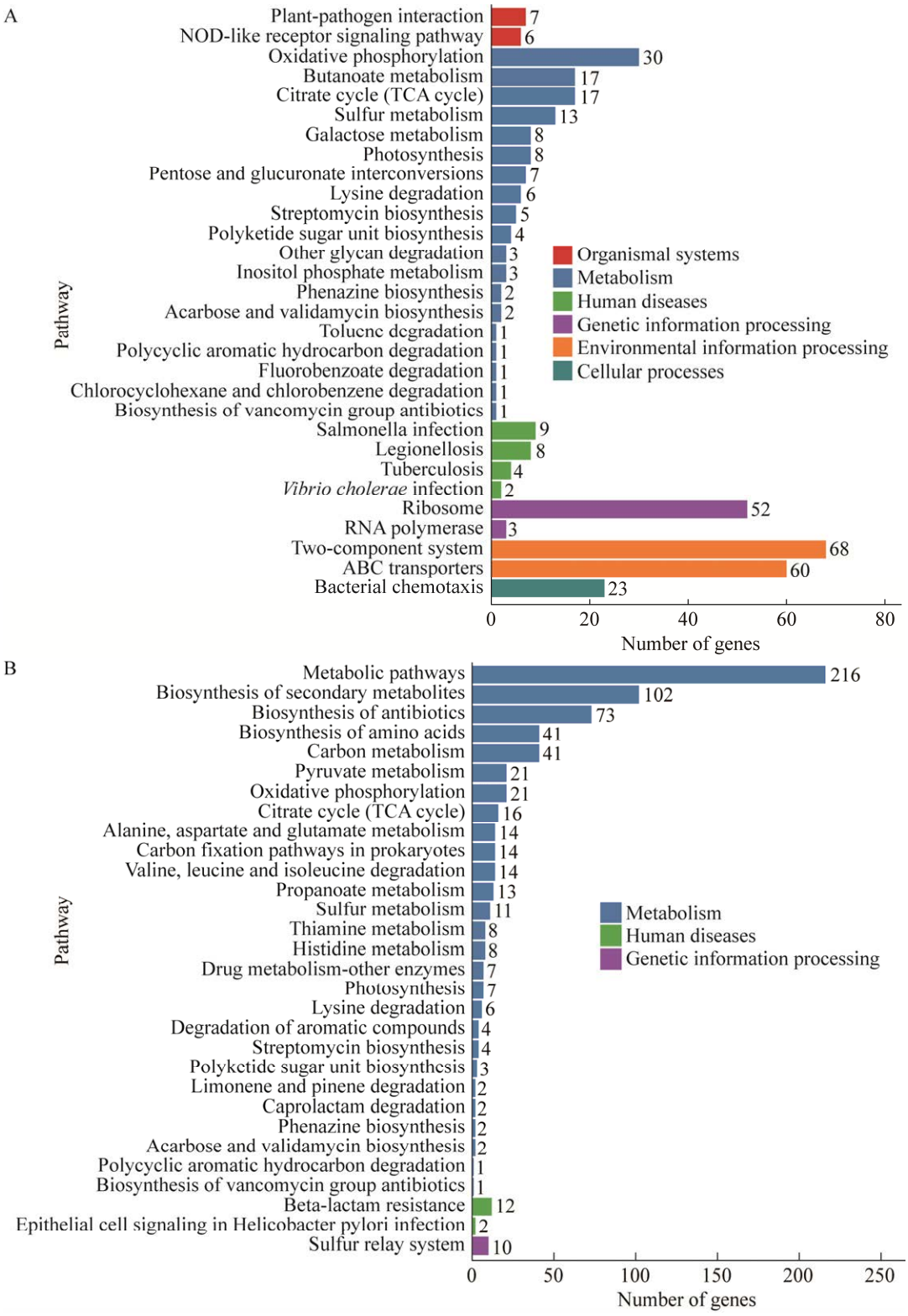

图 3　调控元 1 (A)和调控元 2 (B)中显著性差异表达基因的 KEGG 富集分析

Figure 3　KEGG enrichment analysis of DEGs in regulon 1 (A) and regulon 2 (B).

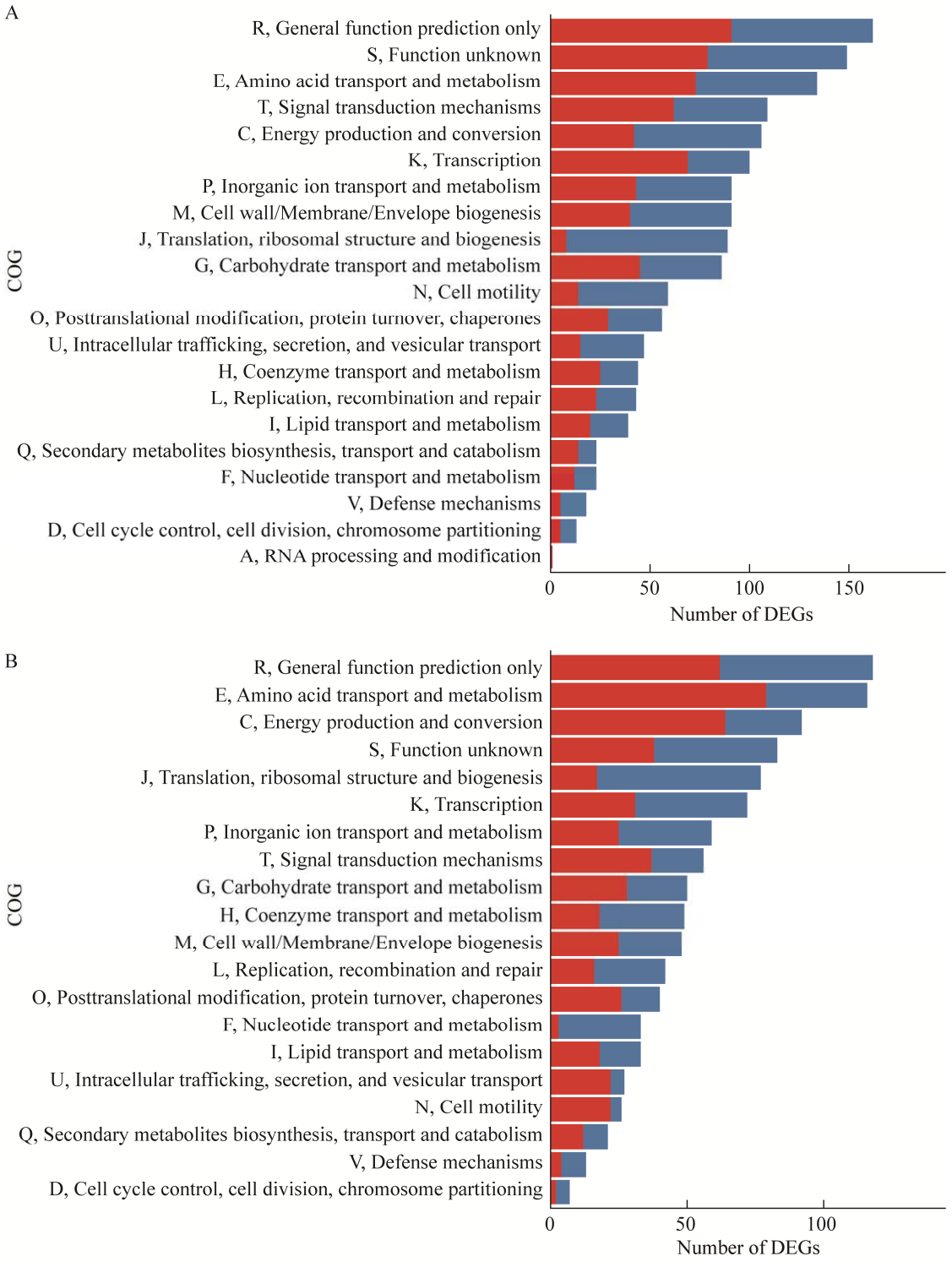

图 4　调控元 1 (A)和调控元 2 (B)中显著性差异表达基因的 COG 功能分类　红色柱：上调基因；蓝色柱：下调基因

Figure 4　COG analysis of DEGs in regulon 1 (A) and regulon 2 (B). Red bars: Up-regulated DEGs; Blue bars: Down-regulated DEGs.

表 3　调控元 1 中的部分基因

Table 3　Selected DEGs from regulon 1

Gene ID	Gene name	Fold change	Regulation	Product
Putative regulators				
VP0154	*ompR*	2.067	Negative	Osmolarity response regulator
VP0350	*calR*	7.170	Negative	Leucine transcriptional activator
VP0358		3.084	Negative	DeoR family transcriptional regulator
VP0361		2.851	Negative	Response regulator
VP0514	*cpsR*	0.229	Positive	Sigma-54 dependent regulator
VP0529		2.070	Negative	Transcriptional activator HlyU
VP0569	*phoB*	0.090	Positive	Response regulator PhoB
VP0825	*rfaH*	2.082	Negative	Transcriptional activator RfaH
VP0877		2.431	Negative	LysR family transcriptional regulator
VP1093		2.121	Negative	Transcriptional regulator
VP1101	*cysB*	2.259	Negative	Transcriptional regulator CysB
VP1136		2.186	Negative	Transcription regulator TxR
VP1172		2.255	Negative	*psp* operon transcriptional activator
VP1190		5.196	Negative	Transcription regulator
VP1212		3.189	Negative	DNA-binding response regulator
VP1244		0.335	Positive	Response regulator
VP1245		0.427	Positive	Response regulator
VP1316		2.036	Negative	LysR family transcriptional regulator
VP1482		0.289	Positive	Response regulator
VP1649		2.167	Negative	GntR family transcriptional regulator
VP1676		2.274	Negative	Transcriptional regulator
VP1699	*exsA*	0.359	Positive	Transcriptional regulator ExsA
VP1993		3.237	Negative	Transcriptional regulator
VP2183		7.714	Negative	Response regulator
VP2357		11.400	Negative	Transcriptional activator ChrR
VP2393		0.485	Positive	LacI family transcription regulator
VP2402		0.433	Positive	Transcriptional repressor EbgR
VP2424		49.910	Negative	AraC family transcriptional regulator
VP2450		2.232	Negative	MarR family transcriptional regulator
VP2516	*opaR*	0.002	Positive	OpaR protein
VP2710		0.320	Positive	LuxR family transcriptional regulator
VP2762	*aphA*	4.079	Negative	Hypothetical protein
VP2808		2.276	Negative	Transcriptional repressor NsrR
VP2817	*hfq*	0.467	Positive	RNA-binding protein Hfq
VP2836		3.759	Negative	TetR family transcriptional regulator
VP2858		3.572	Negative	Transcriptional regulator CpxR
VP2859	*cpxA*	2.373	Negative	Two-component sensor protein
VP2885	*fis*	0.215	Positive	DNA-binding protein Fis
VP2894	*zntR*	2.087	Negative	Zinc-responsive transcriptional regulator
VP2941		0.428	Positive	Transcriptional repressor FabR
VP2971		2.475	Negative	ArsR family transcriptional regulator
VP3009		7.942	Negative	AraC family transcriptional regulator

(待续)

(续表 3)

Gene ID	Gene name	Fold change	Regulation	Product
VP3020		2.621	Negative	LysR family transcriptional regulator
VPA0021		0.460	Positive	Response regulator
VPA0076		2.308	Negative	Regulator
VPA0107		2.030	Negative	Transcriptional regulator
VPA0115		4.210	Negative	Helix-turn-helix domain-containing protein
VPA0118		2.787	Negative	Transcriptional regulator
VPA0148		0.483	Positive	Transcriptional regulator CpxR
VPA0216		2.997	Negative	Transcriptional regulator
VPA0249		0.117	Positive	Transcriptional activator
VPA0251		34.833	Negative	LysR family transcriptional regulator
VPA0293		2.317	Negative	Transcriptional regulator
VPA0303		2.209	Negative	Regulatory protein
VPA0331		3.732	Negative	Transcriptional regulator
VPA0335		2.380	Negative	SoxR protein
VPA0355		0.133	Positive	Transcriptional regulator
VPA0358		0.017	Positive	LuxR family transcriptional regulator
VPA0359		0.022	Positive	Putative DNA binding protein
VPA0381		2.305	Negative	Transcriptional regulator
VPA0456		6.734	Negative	Transcriptional regulator
VPA0531		2.271	Negative	AraC family transcriptional regulator
VPA0593		5.523	Negative	Transcriptional regulator
VPA0619		18.791	Negative	Transcriptional regulator
VPA0662		2.016	Negative	MerR family transcriptional regulator
VPA0663		2.635	Negative	AraC family transcriptional regulator
VPA0682		4.964	Negative	LysR family transcriptional regulator
VPA0692		2.310	Negative	LysR family transcriptional regulator
VPA0733		3.198	Negative	LysR family transcriptional regulator
VPA0740		2.428	Negative	Transcriptional regulator
VPA0806		2.763	Negative	TetR family transcriptional regulator
VPA0830		2.143	Negative	AraC family transcriptional regulator
VPA0852		2.167	Negative	LysR family transcriptional regulator
VPA0961		0.188	Positive	Transcriptional regulator
VPA1049		42.489	Negative	Two-component response regulator
VPA1060		3.920	Negative	Two-component response regulator
VPA1114		3.160	Negative	Transcriptional regulator BetI
VPA1130		7.428	Negative	Response regulator
VPA1289	*cspA*	0.204	Positive	Cold shock transcriptional regulator CspA
VPA1365		2.307	Negative	Two-component response regulator
VPA1391		194.249	Negative	Helix-turn-helix domain-containing protein
VPA1446	*cpsQ*	0.399	Positive	LuxR family transcriptional regulator
VPA1447	*cpsS*	0.228	Positive	LuxR family transcriptional regulator
VPA1516		2.552	Negative	Two-component response regulator
VPA1562		2.350	Negative	Transcriptional regulator
VPA1563		2.855	Negative	Transcriptional regulator

(待续)

(续表 3)

Gene ID	Gene name	Fold change	Regulation	Product
VPA1607		2.772	Negative	Transcriptional regulator
VPA1727		2.878	Negative	AraC family transcriptional regulator
VPA1747		2.012	Negative	LysR family transcriptional regulator
c-di-GMP metabolism				
VPA1513	*scrA*	0.485	Positive	Aminotransferase ScrA
VP0117		9.883	Negative	GGDEF/EAL-type protein
VP0376		2.855	Negative	EAL-type protein
VP0699		2.264	Negative	GGDEF-type protein
VP1377	*scrG*	0.328	Positive	GGDEF/EAL-type protein
VP1483		0.318	Positive	GGDEF-type protein
VP1637		0.488	Positive	GGDEF-type protein
VP1754		0.459	Positive	GGDEF/EAL-type protein
VP1768		0.494	Positive	EAL-type protein
VP1881		7.077	Negative	EAL-type protein
VP1979		10.550	Negative	EAL-type protein
VP2366		3.913	Negative	GGDEF-type protein
VP2972		3.169	Negative	EAL-type protein
VP2979		0.221	Positive	EAL-type protein
VPA0198		2.627	Negative	GGDEF-type protein
VPA0360	*scrM*	0.064	Positive	GGDEF-type protein
VPA0476		4.230	Negative	GGDEF-type protein
VPA0518		3.687	Negative	GGDEF/EAL-type protein
VPA0609		34.797	Negative	GGDEF/EAL-type protein
VPA0818		0.371	Positive	EAL-type protein
VPA0927		2.296	Negative	GGDEF-type protein
VPA1115	*scrJ*	0.450	Positive	GGDEF-type protein
VPA1324		10.175	Negative	EAL-type protein
VPA1735		0.267	Positive	GGDEF/EAL-type protein
Capsule polysaccharide				
VP0215		0.380	Positive	OtnG protein
VP0217		0.485	Positive	Putative regulator
VP0218		0.342	Positive	Hypothetical protein
VP0220	*wbfF*	0.461	Positive	Capsule assembly protein
VP0221	*wzz*	0.482	Positive	Polysaccharide chain length determinant
VP0222	*rmlB*	0.339	Positive	dTDP-glucose 4,6 dehydratase
VP0223	*rmlA*	0.347	Positive	D-glucose-1-phosphate thymidylyltransferase
VP0224	*rmlD*	0.392	Positive	dTDP-4-dehydrorhamnose reductase
VP0225		0.367	Positive	Capsular polysaccharide biosynthesis protein
VP0226		0.295	Positive	Putative rhamnosyl transferase
VP0227		0.351	Positive	Hypothetical protein
VP0228		0.247	Positive	Putative integral membrane protein
VP0229	*rmlC*	0.266	Positive	dTDP-4-dehydrorhamnose 3,5-epimerase
VP0230		0.294	Positive	Glycosyltranferase
VP0231		0.471	Positive	UDP-galactose phosphate transferase

(待续)

(续表 3)

Gene ID	Gene name	Fold change	Regulation	Product
VP0234		0.355	Positive	Amino transferase
VP0235		0.497	Positive	Putative epimerase
VP0236	*wcvB*	0.252	Positive	UDP-glucose 6-dehydrogenase
VP0237		0.457	Positive	UTP-glucose-1-phosphate uridylyltransferase
Cps exopolysaccharide				
VPA1403	*cpsA*	13.120	Negative	Glycosyltransferase
VPA1404	*cpsB*	11.876	Negative	Hypothetical protein
VPA1406	*cpsD*	29.953	Negative	Exopolysaccharide biosynthesis protein
VPA1407	*cpsE*	32.171	Negative	Hypothetical protein
VPA1408	*cpsF*	17.645	Negative	Lipopolysaccharide biosynthesis protein
VPA1409	*cpsG*	142.158	Negative	Hypothetical protein
VPA1410	*cpsH*	61.651	Negative	Hypothetical protein
VPA1411	*cpsI*	71.342	Negative	Putative glycosyltransferase
VPA1412	*cpsJ*	0.345	Positive	Polysaccharide biosynthesis related protein
VPA1413	*cpsK*	0.233	Positive	Hypothetical protein
Scv exopolysaccharide				
VP1466	*scvH*	6.438	Negative	Hypothetical protein
VP1467	*scvG*	3.620	Negative	Putative galactosyltransferase
VP1468	*scvF*	7.212	Negative	Putative hexosyltransferase
VP1473	*scvD*	3.243	Negative	Capsular polysaccharide biosynthesis
VP1474	*scvC*	4.086	Negative	Capsule transport protein OtnA
VP1475	*scvB*	8.718	Negative	Hypothetical protein
Type IV pili				
VP2692	*mshQ*	0.391	Positive	Hypothetical protein
VP2693	*mshP*	0.291	Positive	MshP protein
VP2694	*mshO*	0.322	Positive	Putative type IV prepilin, MshO
VP2695	*mshD*	0.448	Positive	Putative MSHA pilin protein MshD
VP2697	*mshA*	0.248	Positive	Putative MSHA pilin protein MshA
VP2698	*mshB*	0.146	Positive	Putative MSHA pilin protein MshB
VP2699	*mshF*	0.448	Positive	MSHA biogenesis protein MshF
VP2700	*mshG*	0.094	Positive	MSHA biogenesis protein MshG
VP2701	*mshE*	0.134	Positive	MSHA biogenesis protein MshE
VP2702	*mshN*	0.060	Positive	MSHA biogenesis protein MshN
VP2703	*mshM*	0.069	Positive	MSHA biogenesis protein MshM
VP2704	*mshL*	0.081	Positive	MSHA biogenesis protein MshL
VP2705	*mshK*	0.117	Positive	MSHA biogenesis protein MshK
VP2706	*mshJ*	0.079	Positive	MSHA biogenesis protein MshJ
VP2707	*mshI*	0.162	Positive	MSHA biogenesis protein MshI
T3SS1				
VP1656	*vopD*	0.251	Positive	Translocator protein PopD
VP1657	*vopB*	0.108	Positive	Translocator protein PopB
VP1658	*vcrH*	0.099	Positive	Low calcium response locus protein H
VP1659	*vcrV*	0.396	Positive	Hypothetical protein
VP1664	*vscX*	0.082	Positive	Type III secretion protein

(待续)

(续表 3)

Gene ID	Gene name	Fold change	Regulation	Product
VP1667	*vopN*	0.383	Positive	Outer membrane protein PopN
VP1670	*vscP*	0.248	Positive	Translocation protein in type III secretion
VP1671	*vscQ*	0.196	Positive	Type III secretion system protein
VP1676		2.274	Negative	Transcriptional regulator
VP1677		5.762	Negative	Hypothetical protein
VP1678		4.565	Negative	Dienelactone hydrolase
VP1679		2.511	Negative	Hypothetical protein
VP1682	*vecA*	0.241	Positive	Hypothetical protein
VP1686	*vopS*	2.768	Negative	Adenosine monophosphate-protein Transferase
VP1689	*vscK*	0.405	Positive	Type III secretion protein
VP1690	*vscJ*	0.412	Positive	Type III secretion lipoprotein
VP1692	*vscH*	0.465	Positive	Type III export protein
VP1694	*vscF*	0.495	Positive	Type III export protein YscF
VP1698	*exsD*	0.224	Positive	Hypothetical protein
VP1699	*exsA*	0.359	Positive	Transcriptional regulator ExsA
T3SS2				
VPA1321	*vopC*	2.300	Negative	Cytotoxic necrotizing factor
VPA1322		2.320	Negative	Zinc finger protein
VPA1325		6.288	Negative	Hypothetical protein
VPA1327	*vopT*	3.262	Negative	Exoenzyme T
VPA1328		2.848	Negative	Hypothetical protein
VPA1329		5.356	Negative	TraA protein
VPA1344		9.018	Negative	Hypothetical protein
VPA1365		2.307	Negative	Two-component response regulator
T6SS1				
VP1386		2.104	Negative	Hypothetical protein
VP1388		0.241	Positive	Hypothetical protein
VP1389		0.439	Positive	Hypothetical protein
VP1390		0.449	Positive	Hypothetical protein
VP1409		5.330	Negative	Hypothetical protein
VP1413		0.486	Positive	Hypothetical protein
T6SS2				
VPA1024		0.069	Positive	Hypothetical protein
VPA1025		0.082	Positive	Hypothetical protein
VPA1026	*vgrG2*	0.064	Positive	Hypothetical protein
VPA1027	*hcp2*	0.005	Positive	Hypothetical protein
VPA1028	*clpV2*	0.276	Positive	ClpA/B-type chaperone
VPA1029		0.310	Positive	Hypothetical protein
VPA1030		0.148	Positive	Hypothetical protein
VPA1031		0.067	Positive	Hypothetical protein
VPA1032		0.033	Positive	Hypothetical protein
VPA1033		0.044	Positive	Hypothetical protein
VPA1034	*vipB2*	0.023	Positive	Hypothetical protein
VPA1035	*vipA2*	0.020	Positive	Hypothetical protein

(待续)

(续表 3)

Gene ID	Gene name	Fold change	Regulation	Product
VPA1036		0.048	Positive	Hypothetical protein
VPA1038		0.018	Positive	Hypothetical protein
VPA1039		0.043	Positive	Hypothetical protein
VPA1040		0.020	Positive	Hypothetical protein
VPA1041		0.011	Positive	Hypothetical protein
VPA1042		0.008	Positive	Hypothetical protein
VPA1044		0.038	Positive	Hypothetical protein
Motility				
VP0772	*flgA*	2.035	Negative	P-ring biosynthesis protein FlgA
VP0777	*flgD*	0.324	Positive	Flagellar basal body rod modification protein
VP0778	*flgE*	0.368	Positive	Flagellar hook protein FlgE
VP0780	*flgF*	0.468	Positive	Flagellar basal body rod protein FlgF
VP0785	*flgK*	0.414	Positive	Flagellar hook-associated protein FlgK
VP0786	*flgL*	0.413	Positive	Flagellar hook-associated protein FlgL
VP0788	*flaC*	0.409	Positive	Flagellin
VP0790	*flaD*	0.254	Positive	Flagellin
VP0791	*flaE*	0.336	Positive	Flagellin
VP2224	*orf3*	0.259	Positive	Hypothetical protein
VP2225	*cheW*	0.408	Positive	Chemotaxis protein CheW
VP2226	*orf2*	0.301	Positive	Hypothetical protein
VP2227	*orf1*	0.308	Positive	Soj-like protein
VP2228	*cheB*	0.316	Positive	Chemotaxis-specific methylesterase
VP2229	*cheA*	0.433	Positive	Chemotaxis protein CheA
VP2234	*flhF*	0.370	Positive	Flagellar biosynthesis regulator FlhF
VP2235	*flhA*	0.389	Positive	Flagellar biosynthesis protein FlhA
VP2236	*flhB*	0.387	Positive	Flagellar biosynthesis protein FlhB
VP2238	*fliQ*	0.269	Positive	Flagellar biosynthesis protein FliQ
VP2239	*fliP*	0.326	Positive	Flagellar biosynthesis protein FliP
VP2241	*fliN*	0.343	Positive	Flagellar motor switch protein
VP2242	*fliM*	0.451	Positive	Flagellar motor switch protein FliM
VP2243	*fliL*	0.439	Positive	Flagellar basal body protein FliL
VP2244	*fliK*	0.382	Positive	Hook-length control protein FliK
VP2245	*fliJ*	0.361	Positive	Flagellar biosynthesis chaperone
VP2246	*fliI*	0.331	Positive	Flagellum-specific ATP synthase
VP2247	*fliH*	0.425	Positive	Flagellar assembly protein H
VP2249	*fliF*	0.495	Positive	Flagellar MS-ring protein
VP2254	*fliS*	0.296	Positive	Flagellar protein FliS
VP2256	*fliD*	0.195	Positive	Flagellar capping protein
VP2257	*flaG*	0.208	Positive	Flagellar protein FlaG
VP2258	*flaA*	0.236	Positive	Flagellin
VP2259	*flaB*	0.261	Positive	Flagellin
VP2261	*flaF*	0.328	Positive	Flagellin
VP2811	*motX*	0.381	Positive	Sodium-type polar flagellar protein MotX
VPA0263	*flgA*	2.135	Negative	P-ring biosynthesis protein FlgA

表 4　调控元 2 中的部分基因

Table 4　Selected DEGs from regulon 2

Gene ID	Gene name	Fold change	Regulation	Product
Putative regulators				
VP0040		0.394	Positive	TetR family transcriptional regulator
VP0358		0.278	Positive	DeoR family transcriptional regulator
VP0367		2.330	Negative	DNA-binding transcriptional regulator DhaR
VP0529		2.194	Negative	Transcriptional activator HlyU
VP0553		0.484	Positive	Trp operon repressor
VP0595	*iscR*	0.344	Positive	Transcriptional regulator IscR
VP0678	*nrdR*	0.369	Positive	Transcriptional regulator NrdR
VP0713		0.429	Positive	Winged helix-turn-helix domain-containing protein
VP0833	*fur*	0.437	Positive	Ferric uptake regulator
VP0914		2.565	Negative	Transcriptional regulator
VP0947		2.187	Negative	AsnC family transcriptional regulator
VP1190		6.706	Negative	Transcription regulator
VP1212		0.402	Positive	DNA-binding response regulator
VP1244		6.333	Negative	Response regulator
VP1245		2.555	Negative	Response regulator
VP1316		2.629	Negative	LysR family transcriptional regulator
VP1382		0.488	Positive	LysR family transcriptional regulator
VP1649		0.382	Positive	GntR family transcriptional regulator
VP1711		4.852	Negative	Response regulator
VP1876		0.447	Positive	Hypothetical protein
VP1907		2.076	Negative	LuxR family transcriptional regulator
VP1962		2.962	Negative	Transcriptional regulator
VP2009		2.819	Negative	Tetrathionate reductase complex: response regulator
VP2075		0.354	Positive	Regulatory protein
VP2357		2.564	Positive	Transcriptional activator ChrR
VP2387		0.499	Positive	DeoR family transcriptional regulator
VP2450		0.435	Positive	MarR family transcriptional regulator
VP2520	*pdhR*	0.334	Positive	Transcriptional regulator PdhR
VP2885	*fis*	0.320	Positive	DNA-binding protein Fis
VP2894	*zntR*	2.305	Negative	Zinc-responsive transcriptional regulator
VPA0021		2.224	Negative	Response regulator
VPA0065		0.471	Positive	LysR family transcriptional regulator
VPA0132		2.434	Negative	Transcriptional activator
VPA0148		0.192	Positive	Transcriptional regulator CpxR
VPA0183		2.296	Negative	C4-dicarboxylate transport transcriptional regulator
VPA0331		2.050	Negative	Transcriptional regulator
VPA0358		4.949	Negative	LuxR family transcriptional regulator
VPA0369		0.426	Positive	LuxR family transcriptional regulator
VPA0741		0.425	Positive	Fimbrial protein Z transcriptional regulator

(待续)

(续表 4)

Gene ID	Gene name	Fold change	Regulation	Product
VPA0827		3.092	Negative	Transcriptional regulatory protein PgtA
VPA1049		0.447	Positive	Two-component response regulator
VPA1286		0.366	Positive	Transcriptional regulator
VPA1423		2.126	Negative	Transcriptional regulator
VPA1432		3.554	Negative	Two-component response regulator
VPA1446	*cpsQ*	3.285	Negative	LuxR family transcriptional regulator
VPA1472		4.313	Negative	MerR family transcriptional regulator
VPA1562		2.025	Negative	Transcriptional regulator
VPA1678		2.028	Negative	DNA-binding transcriptional regulator AraC
VPA1682		0.378	Positive	MarR family transcriptional regulator
VPA1729		0.450	Positive	LuxR family transcriptional regulator
VPA1732		2.557	Negative	Two-component response regulator
c-di-GMP metabolism				
VP1289		2.223	Negative	GGDEF-only
VP1483		2.072	Negative	GGDEF-only
VPA0518		0.496	Positive	GGDEF-EAL
VPA0869		2.079	Negative	GGDEF-EAL
Type IV pili				
VP2699	*mshF*	2.824	Negative	MSHA biogenesis protein MshF
VP2700	*mshG*	2.329	Negative	MSHA biogenesis protein MshG
VP2702	*mshN*	2.231	Negative	MSHA biogenesis protein MshN
VP2703	*mshM*	3.972	Negative	MSHA biogenesis protein MshM
VP2704	*mshL*	3.626	Negative	MSHA biogenesis protein MshL
VP2705	*mshK*	2.163	Negative	MSHA biogenesis protein MshK
VP2706	*mshJ*	2.128	Negative	MSHA biogenesis protein MshJ
Capsule polysaccharide				
VP0217		0.467	Positive	Regulator
VP0227		5.483	Negative	Hypothetical protein
Scv exopolysaccharide				
VP1461		0.282	Positive	Glycosyl transferase
T3SS1				
VP1659		2.767	Negative	Hypothetical protein
VP1702	*exsE*	0.421	Positive	Hypothetical protein
T3SS2				
VPA1321	*vopC*	3.808	Negative	Cytotoxic necrotizing factor
VPA1322		3.663	Negative	Zinc finger protein
VPA1327	*vopT*	2.643	Negative	Exoenzyme T
VPA1334	*vocC*	2.543	Negative	Hypothetical protein
VPA1338	*vscN2*	2.035	Negative	ATPase YscN
VPA1343		3.445	Negative	Hypothetical protein
VPA1345		2.269	Negative	Hypothetical protein

(待续)

(续表 4)

Gene ID	Gene name	Fold change	Regulation	Product
VPA1347		3.207	Negative	Hypothetical protein
VPA1348	*vtrB*	2.291	Negative	Transcriptional activator ToxR
VPA1350		3.095	Negative	Hypothetical protein
VPA1354	*vscu2*	2.554	Negative	Type III secretion system EscU protein
VPA1358		3.129	Negative	Dimethyladenosine transferase
VPA1364		4.225	Negative	Hypothetical protein
VPA1365		2.698	Negative	Two-component response regulator
VPA1366		2.774	Negative	Hypothetical protein
VPA1367	*vscJ2*	2.472	Negative	Type III secretion system lipoprotein EprK
VPA1370	*vopL*	2.646	Negative	Hypothetical protein
T6SS1				
VP1388		2.037	Negative	Hypothetical protein
VP1389		2.456	Negative	Hypothetical protein
VP1390		2.269	Negative	Hypothetical protein
VP1399		2.286	Negative	Hypothetical protein
VP1400		0.441	Positive	Hypothetical protein
VP1410		0.378	Positive	Hypothetical protein
VP1411	*fha1*	0.497	Positive	Hypothetical protein
T6SS2				
VPA1027	*hcp2*	3.523	Negative	Hypothetical protein
VPA1034	*vipB2*	2.042	Negative	Hypothetical protein
Cell motility				
VP0784	*flgJ*	2.149	Negative	Flagellar rod assembly protein/muramidase FlgJ
VP0786	*flgL*	2.874	Negative	Flagellar hook-associated protein FlgL
VP2226	*orf2*	4.669	Negative	Hypothetical protein
VP2227	*orf1*	2.750	Negative	Soj-like protein
VP2228	*cheB*	2.707	Negative	Chemotaxis-specific methylesterase
VP2235	*flhA*	0.358	Positive	Flagellar biosynthesis protein FlhA
VP2241	*fliN*	2.016	Negative	Flagellar motor switch protein
VP2245	*fliJ*	4.054	Negative	Flagellar biosynthesis chaperone
VP2246	*fliI*	2.742	Negative	Flagellum-specific ATP synthase
VP2247	*fliH*	2.542	Negative	Flagellar assembly protein H
VP2255	*flaI*	2.913	Negative	Polar flagellar rod protein FlaI
VP2257	*flaG*	2.131	Negative	Flagellar protein FlaG
VP2258	*flaA*	4.835	Negative	Flagellin
VP2259	*flaB*	2.091	Negative	Flagellin

IV 型菌毛基因、12 个极生鞭毛基因、1 个 T3SS1 基因、4 个 T3SS2 基因、3 个 T6SS1 基因和 2 个 T6SS2 基因(图 5，表 5)。

2.7 RT-qPCR 验证 RNA-Seq 数据

为验证 RNA-Seq 数据的可靠性，选取 17 个基因作为研究靶标进行 RT-qPCR 试验，如表 6 所示，虽然 RT-qPCR 数据与 RNA-Seq 结果在变化倍数上有一定的差异，但基因的表达趋势是一致的，这说明转录组测序结果是可靠的。

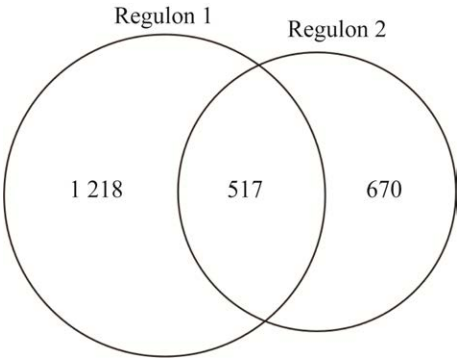

图 5 显著性差异表达基因的文氏图
Figure 5 Venn diagram of DEGs.

3 讨论与结论

QsvR 与 OpaR 协同调控副溶血弧菌毒力和生物膜形成，特别是 QsvR 能回补 *opaR* 突变所导致的生物膜表型变化，反之亦然[31,33]，这说明二者共同调节大量基因的转录。本研究以 WT 为参照，采用比较转录组学方法分析了在生物膜形成条件下 Δ*qsvR* 中的基因表达情况，结果表明共有 1 735 个基因的转录水平发生显著性变化(调控元 1)，包括 855 个下调基因(QsvR 正调控这些基因的转录)和 880 个上调基因(QsvR 负调控这些基因的转录)。当以 Δ*opaR* 为参照时，QsvR 共调控 1 187 个基因的转录(调控元 2)，其中激活 533 个基因、抑制 654 个基因。调控元 1 和调控元 2 中的 DEGs 参与许多细胞代谢通路，包括代谢、毒力、基因表达调控、运动、生物膜形成等。另外，调控元 1 和调控元 2 之间有 517 个重叠基因，QsvR 对其中绝大多数基因的转录调控关系相反，即 QsvR 若在调控元 1 中激活(或抑制)某个基因转录，则在调控元 2 中抑制(或激活)该基因转录。OpaR 对生物膜的抑制强度显著性高于 QsvR[33]，推测 QsvR 对重叠基因的相反调控关系可能与 QsvR 的调控强度弱于 OpaR 有关，当然也有可能存在其他未知的调控因子能在 Δ*qsvR*Δ*opaR* 背景下发挥调控作用。实际上，在调控元 1 和调控元 2 中，分别包含 89 个和 51 个推定的调控因子基因，其中只有极少数几个基因的功能是已知的(如 *calR*[45]、*cpsR*[46]、*exsA*[47-49]、*aphA*[12,32]、*opaR*[50]和 *cpsQ*[21]等)，这些基因是否参与到 QsvR 与 OpaR 的调控网络机制中还有待于后续深入研究。

非生物膜形成条件下，QsvR 负调控极生鞭毛基因的转录，但对侧生鞭毛基因无调控作用[34]。调控元 1 中包含 35 个极生鞭毛基因，其中 34 个被 QsvR 激活(表 3)。在 M 肉汤中 30 ℃、150 r/min 生长 48 h，副溶血弧菌能形成成熟

表 5 调控元 1 和调控元 2 中重叠的基因
Table 5 Selected overlapping DEGs in regulons 1 and regulons 2

Gene ID	Gene name	Fold change		Product
		Regulon 1	Regulon 2	
Putative regulators				
VP0358		3.084	0.278	DeoR family transcriptional regulator
VP0529		2.070	2.194	Transcriptional activator HlyU
VP1190		5.196	6.706	Transcriptional regulator
VP1212		3.189	0.402	DNA-binding response regulator
VP1244		0.335	6.333	Response regulator
VP1245		0.427	2.555	Response regulator
VP1316		2.036	2.629	LysR family transcriptional regulator
VP1649		2.167	0.382	GntR family transcriptional regulator

(待续)

(续表 5)

Gene ID	Gene name	Fold change		Product
		Regulon 1	Regulon 2	
VP2357		11.400	2.564	Transcriptional activator ChrR
VP2450		2.232	0.435	MarR family transcriptional regulator
VP2885	*fis*	0.215	0.320	DNA-binding protein Fis
VP2894	*zntR*	2.087	2.305	Zinc-responsive transcriptional regulator
VPA0021		0.460	2.224	Response regulator
VPA0148		0.483	0.192	Transcriptional regulator CpxR
VPA0331		3.732	2.050	Transcriptional regulator
VPA0358		0.017	4.949	LuxR family transcriptional regulator
VPA0359		0.022	2.017	Putative DNA binding protein
VPA1049		42.489	0.447	Two-component response regulator
VPA1446	*cpsQ*	0.399	3.285	LuxR family transcriptional regulator
VPA1562		2.350	2.025	Transcriptional regulator
c-di-GMP metabolism				
VP1483		0.318	2.072	GGDEF-type protein
VPA0518		3.687	0.496	GGDEF/EAL-type protein
Capsule polysaccharide				
VP0217		0.485	0.467	Putative regulator
VP0221	*wzz*	0.482	4.179	Polysaccharide chain length determinant
VP0222	*rmlB*	0.339	2.266	dTDP-glucose 4,6 dehydratase
VP0223	*rmlA*	0.347	2.286	D-glucose-1-phosphate thymidylyltransferase
VP0224	*rmlD*	0.392	4.102	dTDP-4-dehydrorhamnose reductase
VP0225		0.367	2.785	Capsular polysaccharide biosynthesis protein
VP0226		0.295	4.043	Putative rhamnosyl transferase
VP0227		0.351	5.483	Hypothetical protein
Type IV pili				
VP2699	*mshF*	0.448	2.824	MSHA biogenesis protein MshF
VP2700	*mshG*	0.094	2.329	MSHA biogenesis protein MshG
VP2702	*mshN*	0.060	2.231	MSHA biogenesis protein MshN
VP2703	*mshM*	0.069	3.972	MSHA biogenesis protein MshM
VP2704	*mshL*	0.081	3.626	MSHA biogenesis protein MshL
VP2705	*mshK*	0.117	2.163	MSHA biogenesis protein MshK
VP2706	*mshJ*	0.079	2.128	MSHA biogenesis protein MshJ
T3SS1				
VP1659	*vcrV*	0.396	2.767	Hypothetical protein
T3SS2				
VPA1321	*vopC*	2.300	3.808	Cytotoxic necrotizing factor
VPA1322		2.320	3.663	Zinc finger protein
VPA1327	*vopT*	3.262	2.643	Exoenzyme T
VPA1365		2.307	2.698	Two-component response regulator
T6SS1				
VP1388		0.241	2.037	Hypothetical protein

(待续)

(续表 5)

Gene ID	Gene name	Fold change		Product
		Regulon 1	Regulon 2	
VP1389		0.439	2.456	Hypothetical protein
VP1390		0.449	2.269	Hypothetical protein
T6SS2				
VPA1027	*hcp2*	0.005	3.523	Hypothetical protein
VPA1034	*vipB2*	0.023	2.042	Hypothetical protein
Motility				
VP0786	*flgL*	0.413	2.874	Flagellar hook-associated protein FlgL
VP2226	*orf2*	0.301	4.669	Hypothetical protein
VP2227	*orf1*	0.308	2.750	Soj-like protein
VP2228	*cheB*	0.316	2.707	Chemotaxis-specific methylesterase
VP2235	*flhA*	0.389	0.358	Flagellar biosynthesis protein FlhA
VP2241	*fliN*	0.343	2.016	Flagellar motor switch protein
VP2245	*fliJ*	0.361	4.054	Flagellar biosynthesis chaperone
VP2246	*fliI*	0.331	2.742	Flagellum-specific ATP synthase
VP2247	*fliH*	0.425	2.542	Flagellar assembly protein H
VP2257	*flaG*	0.208	2.131	Flagellar protein FlaG
VP2258	*flaA*	0.236	4.835	Flagellin
VP2259	*flaB*	0.261	2.091	Flagellin

表 6 RNA-Seq 数据验证的 RT-qPCR 结果(变化倍数)

Table 6 Validation of RNA-Seq data by RT-qPCR (fold change)

Gene	Δ*qsvR*_*vs*._WT		Δ*qsvR*Δ*opaR*_*vs*._Δ*opaR*	
	RT-qPCR	RNA-Seq	RT-qPCR	RNA-Seq
VP0117	6.568±0.706	9.883	0.568±0.018	1.087
VP0218	0.448±0.198	0.342	0.855±0.117	0.796
calR	2.493±0.233	7.170	1.129±0.019	0.848
VP0699	3.033±0.137	2.264	0.605±0.068	1.275
VP1212	5.158±1.086	5.152	0.454±0.107	0.402
scrG	0.674±0.011	0.328	1.15±0.113	1.280
VP1483	0.430±0.035	0.318	4.728±0.804	2.072
VP1678	6.101±0.988	4.565	0.558±0.098	1.134
VP1881	89.193±6.069	7.077	0.353±0.028	0.610
VP2366	2.103±0.141	3.913	0.523±0.059	0.939
VP2972	10.963±1.943	3.169	1.175±0.180	1.161
VP2979	0.477±0.028	0.221	4.553±0.084	1.860
VPA0009	8.712±2.366	4.857	0.748±0.097	0.930
VPA0609	2.797±0.107	34.797	1.147±0.221	0.775
VPA1130	9.568±0.530	7.428	0.355±0.021	0.638
scrA	0.073±0.012	0.485	1.227±0.132	0.685
VPA1735	0.352±0.024	0.267	0.460±0.165	0.598

的生物膜[41]。而 QsvR 对生物膜形成具有负调控作用[33]。鞭毛介导的运动性既有利于生物膜三维结构的形成，又能促进菌体游离生物膜[10]。在成熟生物膜中激活极生鞭毛基因的表达，以促进生物膜解离，可能是 QsvR 抑制生物膜形成的机制之一。此外，调控元 2 中含有 14 个极生鞭毛基因，其中 13 个被 QsvR 抑制(表 4)，说明 QsvR 对极生鞭毛基因的调控关系受菌体中有无 OpaR 的影响。鉴于 OpaR 和 QsvR 协调一致调控生物膜形成[33]，因此在本研究的培养条件下，OpaR 很可能对极生鞭毛基因也具有激活作用。与单基因突变株相比，*qsvR* 和 *opaR* 同时缺失不能进一步增强副溶血弧菌的表型改变[33]，但不排除会有其他调控因子参与进来，促进 Δ*qsvR*Δ*opaR* 中极生鞭毛基因的转录，因而表现出在 Δ*opaR* 背景下，QsvR 抑制极生鞭毛基因的转录。

QsvR 负调控副溶血弧菌生物膜形成，但似乎对胞外多糖基因无调控作用[33]。本研究发现在 WT 的遗传背景下，QsvR 显著性抑制 8 个 EPS 多糖基因和 6 个 Scv 多糖基因的转录(表 2)；而在 Δ*opaR* 的遗传背景下，只有 1 个 Scv 多糖基因受 QsvR 的抑制(表 4)。可见，QsvR 对胞外多糖基因位点的调控关系受细菌培养条件和遗传背景的双重影响。有 15 个 MSHA 基因在调控元 1 中被显著性下调(QsvR 正调控)，而有 7 个在调控元 2 中被显著上调(QsvR 负调控)，出现这种相反调控关系的机制可能类似于 QsvR 对鞭毛基因的调控(表 3–5)。MSHA 主要介导菌体黏附到固体表面，因而可促进生物膜形成，但是 MSHA 缺失株仍可形成成熟生物膜[18]。MSHA 也可介导菌体黏附到真核细胞表面，表明其可能与致病性也有关[51]。此外，在 WT 的遗传背景下，有 19 个 CPS 基因的转录受 QsvR 的激活(表 3)；而在 Δ*opaR* 的遗传背景下，只有 2 个 CPS 基因的转录具有显著性差异(表 4)。这与之前发现的 QsvR 和 OpaR 协同激活 CPS 相关基因转录的结果相一致[33]。另外，调控元 1 和调控元 2 中分别包含 24 个和 4 个推定的 c-di-GMP 代谢基因(表 3 和表 4)，说明 QsvR 可以通过调控 c-di-GMP 代谢来调控副溶血弧菌 OP 和 TR 菌株的生物膜形成。然而，除 *scrA*[52]、*scrG*[25]、*scrM*[26]和 *scrJ*[27]外，其余基因是否参与 c-di-GMP 代谢，还有待于进一步确证。

在关键毒力基因方面，调控元 1 含有 20 个 T3SS1 基因，其中有 15 个是下调的，包括 *exsA* 和 *exsD* (表 3)，这与之前的研究结果[31]相一致。ExsA 能激活 T3SS1 相关基因的转录，而 ExsD 结合 ExsA，阻止 ExsA 的这种激活作用[48]。QsvR 可以通过调控 *exsA* 的转录来调控 T3SS1 相关基因的转录。相比之下，调控元 2 中只有 2 个 T3SS1 基因且分别被上调和下调(表 4)，表明 QsvR 对 T3SS1 的调控作用需要 OpaR 的存在。调控元 1 和调控元 2 中还分别包含 8 个和 17 个 T3SS2 基因，且它们的转录水平均被显著性上调，这与之前 QsvR 正调控 T3SS2 相关基因转录的结果相反[31]，说明 QsvR 对 T3SS2 的调控关系与培养条件有关。此外，调控元 1 中含有 19 个 T6SS2 相关基因(全部下调)和 6 个 T6SS1 相关基因(2 个上调、4 个下调)；而调控元 2 中含有 7 个 T6SS1 相关基因(4 个上调、3 个下调)和 2 个 T6SS2 相关基因(全部上调)。先前已有结果表明 QsvR 正调控 T6SS2 相关基因的转录[53]。然而，T6SS1 (VP1386-1420)和 T6SS2 (VPA1025-1046)都是多基因编码产物，调控其中的个别基因，且调控关系还不一致，并不能得出明确结论[17]。在 Δ*opaR* 遗传背景下，QsvR 是否调控 T6SS1 和 T6SS2 相关基因的转录，以及在 WT 遗传背景下，QsvR 对 T6SS1 相关基因是否具有调控作用，还需要进一步研究。

本文利用 RNA-Seq 技术分别研究了 QsvR 在野生株和 Δ*opaR* 遗传背景下的转录调控元，发现 2 个调控元中均包含 1 000 多个显著性差异表达基因，这些基因参与物质代谢、信号转导、毒力、生物膜形成、运动等各种细胞通路，尤其是 2 个调控元中均包含几十个未知功能的转录调控因子基因，后续对这些基因功能的研究有助于解析副溶血弧菌的基因表达调控网络。

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