微生物学报
Acta Microbiologica Sinica
2018, 58(3): 380-390
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20170184

Research Article 研究报告

苏云金芽胞杆菌 Sigma54 和 CcpA 共同调控的基因鉴定

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摘要：【目的】通过综合分析苏云金芽胞杆菌(*Bacillus thuringiensis*) HD73 菌株 Sigma54 缺失突变体的转录组数据和蜡样芽胞杆菌(*Bacillus cereus*) ATCC 14579 菌株 CcpA 缺失突变体的转录组数据，并进行启动子与 CcpA 蛋白的体外结合验证，明确 Bt HD73 菌株中 Sigma54 和 CcpA 共同调控的基因，丰富了对微生物的代谢调控网络的认识。【方法】以转录组测序结果为基础，通过基因同源性的比对在 Bt HD73 菌株中寻找受 Sigma54 和 CcpA 共同调控的基因，在这些基因中找到具有 *cre* 序列的启动子，通过凝胶阻滞验证这些启动子与 CcpA 蛋白的结合。【结果】Bt HD73 菌株中有 31 个基因受 Sigma54 和 CcpA 共同调控，其中 14 个基因的启动子序列包含 *cre* 序列，这些启动子都可以与 CcpA 蛋白发生体外结合。【结论】Bt HD73 菌株中有 14 个基因直接受 CcpA 的调控，同时其转录受 Sigma54 的控制。

关键词：苏云金芽胞杆菌，Sigma54，CcpA，转录调控

CcpA (catabolite control protein A) 是 LacI-GalR 家族的蛋白，在革兰氏阳性菌中主要调控碳源分解代谢(carbon catabolite repression/carbon catabolite activation，CCR/CCA)[1−2]，如变异链球菌(*Streptococcus mutans*)的乙酸盐代谢[3]、金黄色葡萄球菌(*Staphylococcus aureus*)的谷氨酸盐代谢[4]、发酵乳杆菌(*Lactobacillus fermentum*)的木糖和葡萄糖代谢[5]等。CcpA 是多效的调控因子，调控细菌的许多重要生理功能，如细菌与噬菌体的互作[6]、几丁质酶的活性[7]、生物膜的形成[8]、环境适应性[9]、毒力基因的表达[10]、芽胞形成[11]等。CcpA 通过与启动子上的保守 *cre* (catabolite responsive element)序列结合而发挥调控功能[12]，不同细菌中的 *cre* 位点表现出多样性，如枯草芽胞杆菌(*Bacillus subtilis*)的保守 *cre* 序列为 TGWAANCGNTNWCA[13]，猪链球菌(*Streptococcus suis*)有 2 个保守的 *cre* 序列(WWGAAARCGYTTTCWW 和 TTTTYHWDH HWWTTTY)[14]，丙酮丁醇梭杆菌(*Clostridium acetobutylicum*)的 *cre* 序列表现出高度的灵活性，6 个碱基的重复序列中间是多变的碱基

基金项目：国家自然科学基金(31530095)
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收稿日期：2017-04-13；**修回日期：**2017-05-15；**网络出版日期：**2017-05-25

(TGTAAA-Yx-TTTACA)[15]。

Sigma 因子是 RNA 聚合酶的亚基，它通过识别目标基因的启动子，并与 RNA 聚合酶核心酶结合从而起始转录。根据功能不同分为 Sigma70 型和 Sigma54 型，区别在于 Sigma70 识别启动子的–35/–10 区，Sigma54 识别启动子的–24/–12 区，同时需要增强子结合蛋白(enhance binding protein, EBPs)的激活才能起始转录[16]。不同细菌中 Sigma54 因子控制的基因参与多种生理功能，如碳氮源代谢[17–18]、生物膜形成[19]、环境适应能力[20–21]、毒力作用[22–24]等。通过转录组测序发现更多 Sigma54 控制的基因，大肠杆菌(*Escherichia coli*)有 30 个依赖于 Sigma54 的操纵子，其中一半以上参与氮源代谢[25]；霍乱弧菌(*Vibrio Cholerae*)有 82 个操纵子由 Sigma54 控制[26]；单核细胞增多性李斯特菌(*Listeria Monocytogenes*)有 77 个基因的转录由 Sigma54 控制，其中大多数与碳源代谢相关[27]；本实验室前期对苏云金芽胞杆菌(*Bacillus thuringiensis*，简称 Bt)的 Sigma54 转录组分析发现 47 个基因的转录由 Sigma54 控制，大多数参与碳氮源的代谢[28]，包括 γ-氨基丁酸代谢[29–30]、肌氨酸代谢[31]和赖氨酸代谢[32]等。目前关于 Bt 中 Sigma54 控制基因与 CcpA 调控基因间的关系仍不清楚，对于碳源代谢调控网络的认识仍不够。

Bt 是革兰氏阳性细菌，在形成芽胞的同时能产生对多种农林害虫具有特异杀虫活性的伴胞晶体(主要成分是杀虫晶体蛋白，即 Cry 蛋白)，因其具有环境友好、生物安全等特点，在害虫的生物防治领域获得了广泛的应用[33]。而从代谢调控的角度对 Bt 芽胞和晶体蛋白形成研究的报道极少。转录组分析表明，Sigma54 和 CcpA 都调控许多与芽胞形成相关基因的表达[28,34]，并且 Bt HD73 菌株中 Sigma54 的缺失影响晶体蛋白的产量(待发表)，但作用机制不清楚。本文通过综合分析蜡样芽胞杆菌属遗传距离最近的两种细菌 Bt HD73 菌株 Sigma54 缺失突变体的转录组数据[28]和蜡样芽胞杆菌(*Bacillus cereus*) ATCC 14579 菌株 CcpA 缺失突变体的转录组数据[34] (两个菌株的基因组相似性 90%以上)，并进行启动子与 CcpA 蛋白的体外结合验证，明确 Bt HD73 菌株中共同受 Sigma54 和 CcpA 调控的基因，为从代谢调控的角度研究芽胞和晶体形成提供线索，并丰富对 Bt 的代谢调控网络的认识。

1 材料和方法

1.1 材料

1.1.1 菌株、质粒和培养基：所用菌株和质粒见表 1。大肠杆菌(*Escherichia coli*，*E. coli*)和 Bt 的培养使用 LB 培养基(Tryptone 1.0%，Yeast extract 0.5%，NaCl 1.0%，pH 7.2)。Bt 在 30 °C、220 r/min 条件下培养；*E. coli* 在 37 °C、220 r/min 条件下培养，氨苄青霉素终浓度为 100 μg/mL。

1.1.2 主要仪器和材料：限制性内切酶、PrimeSTAR® HS DNA 聚合酶和 T4 DNA 连接酶均购自宝生物工程(大连)有限公司；*Taq* DNA 聚合酶购自北京博迈德科技发展有限公司；质粒提取、DNA 回收和 PCR 产物纯化试剂盒购自 Axygen 公司。镍亲和层析柱填料(chelating sepharose fast flow)购自 GE 公司。poly(dI:dC)[Poly(ethylene glycol) di-(4-hydroxyphenyl)diphenylphosphine] 购自 Sigma 公司。Gel Shift Assay Systems 购自 Promega 公司。其他生化试剂和抗生素均为进口或国产生化或分析纯级试剂。

1.1.3 引物合成及序列测定：根据 Bt HD73 基因组[36]序列设计引物，引物合成由生工生物工程公司北京合成部完成，序列测定由北京六合华大基因科技股份有限公司完成，引物名称及序列见表 2。

表 1. 菌株与质粒

Table 1. Strains and plasmids

Strains and plasmids	Characterization	Resource
Strains		
E. coli TG1	Δ(*lac-proAB*) *supE thi hsd*-5 (*F′ traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ*ΔM15), general purpose cloning host	This lab
BL21(DE3)	*E. coli B*, *F*$^-$, *dcm*, *ompT hsdS(rB-mB-)*, *gal*, *λ(DE3)*	[35]
BL21(pET*ccpA*)	BL21 (DE3) strain containing plasmid pETccpA	This study
BLpET	BL21 strain carrying pET21b	This lab
HD73	*B. thuringiensis* subsp. *kurstaki* carrying the *cry1Ac* gene	This lab
Plasmids		
pET21b	Expressional vector, Amp^r, 5.4 kb	This lab
pET*ccpA*	pET21b containing *ccpA* gene, Amp^r	This study

表 2. 引物序列

Table 2. Primers used in this study

Primer name	Sequence (5′→3′)
ccpA-F	CGGGATCCGATGAACGTAACAATCTATG
ccpA-R	ACGCGTCGACTTACTTCGTTGAATCTCTAAA
P0460-F	CAAAGAATTGTGCGATAA
P0460-R	GGTTTCCCCTCCACACC
P0493-F	GCTGGTACTATTATTTCAA
P0493-R	AAAATTCCCTCTCTCTTGC
P0737-F	CGTAGTAGGTGCCATCTTT
P0737-R	TCTCTTTTCTCCTCATTTCA
P1239-F	GAAGATGGATCATATGGAGATG
P1239-R	GAAATTCTCCCCCTTTGCA
P1416-F	CTCTGATAATTTAGAAAAGTG
P1416-R	CATGCTCATCGCTACTGTTG
P1464-F	GCATAATGAAACGTTTACAAAGG
P1464-R	GTCCTTCACCTCGCTCTG
P1489-F	TATTCCGTTATATTGTTG
P1489-R	TTGTTTAAACCTCCCAATG
P2061-F	GTTATGTATGAATGATAGGAG
P2061-R	ATTGTATGGTCAATTAACTTTGC
P4468-F	GCGTTAGGAATTTCCGTTCGAA
P4468-R	ATTTTGTAATCAACCCTTTCCGTCG
P4900-F	GCAAGTAATACAAGCGGATAAG
P4900-R	CGTCATTCCCCTTTCTTCTTC
P5854-F	TACTGAAATTCAGCCATGGTT
P5854-R	TTTGGCCACCTCTTTCGATTT
P0668-F	GAAATCGGGATAATCATTTAGGA
P0668-R	TATAGCTCACCTCTTTAATTG
P4302-F	TCTTTATTCCGCCATATTGGG
P4302-R	TTTCATTACCCCTTCTATAAGT
P5768-F	GTGGTTCCATTCCTCTACATA
P5768-R	TTTTTCCTCTCCTATTCTTC
PcwlX-F	TTGCAGAGGTTGAAAGAAGCTGTGAAGG
PcwlX-R	CCTCCGTGCAAACTATATCTAGCCAT

1.2 CcpA 表达菌株构建

根据 GenBank 中 Bt HD73 菌株(GenBank 登录号：CP004069)的 *ccpA* (HD73_4987)基因序列及 pET21b 质粒的酶切位点，设计扩增 *ccpA* 基因 ORF 的引物 *ccpA*-F 和 *ccpA*-R (表 2)，以 HD73 基因组为模板扩增 *ccpA* 基因，PCR 产物纯化后经 *Bam*H I 和 *Sal* I 双酶切，连接含有 His 标签的 pET21b 质粒 *Bam*H I 和 *Sal* I 双酶切片段上，转化 *E. coli* TG1 菌株，获得重组质粒 pET*ccpA*。重组质粒经 PCR、酶切和测序鉴定，转化至 *E. coli* BL21 (DE3)菌株，获得表达菌株 BL21 (pET*ccpA*)。

1.3 CcpA 蛋白的表达与纯化

将 BL21 (pET*ccpA*)菌株在含有 100 μg /mL 氨苄青霉素的新鲜 LB 培养基中，37 °C、220 r/min 培养至 OD_{600}=0.5–0.6，加入终浓度为 0.5 mmol/L IPTG (isopropyl-β-D-thiogalactopyranoside)，18 °C、150 r/min 诱导 12 h。8000 r/min 低温离心 15 min 收集菌体，用 50 mmol Tris-HCl 缓冲液(pH 8.0)悬浮菌体，将悬浮液置于冰上超声破碎 6 min (CP750 COLE-PARMER，超声功率 40%，超声 3 s，暂停 5 s)。12000 r/min 低温离心 10 min 分离上清和沉淀，上清中含有可溶性 CcpA 蛋白。

将上清置于平衡好的镍亲和层析柱中，使含有 His 标签的 CcpA 蛋白与柱料充分结合，弃去流穿的液体；用 5 倍柱体积的平衡缓冲液(20 mmol/L Tris-HCl pH 8.5，0.5 mol/L NaCl，20 mmol/L 咪唑)洗脱杂蛋白；用 5 倍柱体积的洗脱缓冲液(20 mmol/L Tris-HCl pH 8.5，0.5 mol/L NaCl，250 mmol/L 咪唑)洗脱目的蛋白并收集；SDS-PAGE 检测洗脱的蛋白样品。对纯化的蛋白用 pH 8.0 Tris-HCl 进行透析。

1.4 凝胶迁移实验

以苏云金芽胞杆菌 HD73 为模板，用带有 FAM (羧基荧光素)标记的引物(表 2)扩增带有 *cre* 序列的 DNA 片段。凝胶阻滞实验(EMSA，electrophoresis mobility shift assays)确定 DNA 片段与蛋白的结合：20 μL 反应体系中含有 0.05 μg 或 0.1 μg 带标记的 DNA、不同浓度的 CcpA 蛋白、结合缓冲液(10 mmol/L Tris-HCl，0.5 mmol/L dithiothreitol (DTT)，50 mmol/L NaCl，500 ng poly (dI:dC)，pH 7.5 和 4% (*V*/*V*) glycerol)，25 °C 反应 20 min。用 5% (*W*/*V*)非变性聚丙烯酰胺胶在 TBE 缓冲液(90 mmol/L Tris-base，90 mmol/L 硼酸，2 mmol/L EDTA，pH 8.0)中电泳检测反应产物(Mini-PROTEAN system (Bio-Rad)，160 V，4 °C，1 h)。用荧光凝胶成像系统(Fujifilm，FLA Imager FLA-5100)对非变性胶进行扫描(Laser：473 nm，电压：900 V，Filter：526-000/01)。

2 结果和分析

2.1 Sigma54 和 CcpA 共同调控的基因分析

转录组测序分析表明，Bt HD73 菌株中有 121 个基因在 *sigL* (编码 Sigma54 因子)突变体中上调表达，255 个基因下调表达[28]；Bc ATCC 14579 菌株中，有 173 个基因在 *ccpA* 突变体中上调表达，80 个基因下调表达[34]。根据 Bc ATCC 14579 菌株 CcpA 缺失突变体的转录组，在 HD73 基因组中找到同源基因(一致性 90%以上)，与 Bt HD73 菌株 Sigma54 缺失突变体的转录组进行比较，共发现 31 个基因同时出现在 CcpA 缺失突变体和 Sigma54 缺失突变体的转录组数据中(表 3)。在 *ccpA* 突变体中上调表达的基因中，有 11 个基因在 *sigL* 突变体中上调表达，16 个基因下调表达；在 *ccpA* 突变体中下调表达的基因中，有 3 个基因在 *sigL* 突变体中上调表达，2 个基因下调表达。对 Sigma54 与 CcpA 共同调控的 31 个基因的启动子区域(ATG 上游 500 bp 到 ATG 下游 100 bp)在 DBTBS 数据库(http://dbtbs.hgc.jp/)进行序列分析，发现 14 个基因(或基因所在的操纵子)的启动子序列具有保守的 *cre* 序列(图 1)。

2.2 CcpA 蛋白的表达与纯化

为了进一步验证 14 个具有 *cre* 位点的启动子在体外是否与 CcpA 蛋白结合，构建 Bt HD73 菌株的 CcpA 表达纯化载体，Bt HD73 *ccpA* 基因 ORF 区全长为 999 bp，氨基酸序列与 Bc ATCC 14579 菌株的 CcpA 相似性为 100%。PCR 扩增 *ccpA* 基因全长并与含有 His 标签的 pET21b 载体连接，转化 *E. coli* TG1 菌株，获得重组质粒 pET*ccpA*，经 PCR 鉴定得到 999 bp 的条带(图 2-A)，*Bam*H I 和 *Sal* I 双酶切鉴定得到 5.4 kb 大小的载体条带和 1000 bp 左右大小的目的片段(图 2-A)，将重组质粒送生物公司进行测序分析，序列比对表明重组质粒 pET*ccpA* 构建正确。重组质粒转化至 *E. coli* BL21 菌株，获得 BL21 (pET*ccpA*)表达菌株，经 IPTG 诱导表达，超声破碎细胞，离心收集可溶性组分，对 CcpA-His 融合蛋白进行 Ni^{2+}螯合琼脂

表 3. Sigma54 与 CcpA 共同调控的基因

Table 3. Sigma54 and CcpA regulated genes

Bt HD73	Bc ATCC 14579	Identify/%	In *sigL* mutant	In *ccpA* mutant	Annotation
HD73_0460	BC0378	97	Up-regulated	Up-regulated	5-Methylthioribose kinase
HD73_0493	BC0410	99	Up-regulated	Up-regulated	Crp family transcriptional regulator
HD73_0742	BC0664	97	Down-regulated	Up-regulated	RbsB (Ribose ABC transporter)
HD73_1144	BC0999	94	Down-regulated	Up-regulated	Hypothetical protein
HD73_1238	BC1082	99	Up-regulated	Up-regulated	Acetyltransferase
HD73_1239	BC1083	99	Up-regulated	Up-regulated	Transcriptional regulator LacI
HD73_1412	BC1181	99	Down-regulated	Up-regulated	Oligopeptide transport system permease oppC
HD73_1413	BC1182	99	Down-regulated	Up-regulated	Oligopeptide ABC transporter ATP-binding protein
HD73_1416	BC1185	99	Up-regulated	Up-regulated	Peptide/nickel transport system substrate-binding protein
HD73_1455	BC1224	99	Down-regulated	Up-regulated	Acetyltransferase
HD73_1456	BC1225	99	Down-regulated	Up-regulated	Hypothetical protein
HD73_1467	BC1235	97	Down-regulated	Up-regulated	Indole-3-glycerol phosphate synthase
HD73_1469	BC1237	94	Down-regulated	Up-regulated	Tryptophan synthase beta chain
HD73_1488	BC1251	98	Down-regulated	Up-regulated	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex
HD73_2025	BC1776	98	Down-regulated	Up-regulated	Branched-chain amino acid aminotransferase
HD73_2063	BC1822	98	Down-regulated	Up-regulated	Pyrimidine-nucleoside phosphorylase
HD73_3012	BC2961	90	Up-regulated	Up-regulated	AI-2 transport system permease protein
HD73_3011	BC2962	94	Up-regulated	Up-regulated	AI-2 transport system permease protein
HD73_3010	BC2963	99	Up-regulated	Up-regulated	ABC transporter
HD73_3901	BC3627	96	Down-regulated	Up-regulated	Acetyl-CoA C-acetyltransferase
HD73_4119	BC3834	99	Down-regulated	Up-regulated	Succinyl-CoA synthetase beta subunit
HD73_4205	BC3921	98	Up-regulated	Up-regulated	Putative N-acetyltransferase
HD73_4466	BC4161	98	Down-regulated	Up-regulated	Branched-chain-fatty-acid kinase
HD73_4468	BC4163	99	Down-regulated	Up-regulated	Phosphate butyryltransferase
HD73_4889	BC4594	99	Up-regulated	Up-regulated	Citrate synthase 2
HD73_4900	BC4606	99	Down-regulated	Up-regulated	Hypothetical protein
HD73_5854	BC5439	99	Up-regulated	Up-regulated	Murein hydrolase exporter
HD73_0668	BC0595	99	Up-regulated	Down-regulated	Cadmium efflux system accessory protein
HD73_1412	BC1181	99	Up-regulated	Down-regulated	Oligopeptide transport system permease protein
HD73_3222	BC2771	88	Up-regulated	Down-regulated	Hypothetical protein
HD73_4302	BC4017	98	Down-regulated	Down-regulated	Hypothetical protein
HD73_5768	BC5359	99	Down-regulated	Down-regulated	Aminopeptidase YwaD

Consensus sequence	WTGNAANCGNWNNCW
HD73_0460	TAGAAAACGCTTACA
HD73_0493	TTGAATACGCTTTCT
HD73_0737–0742	CTGTAAACGGTTACA
HD73_1239–1240	AAGAAAACGTTTTCA
HD73_1416	ATGAAAGGGTTAACA
HD73_1464–1470	ATGAAATCGTTTAAG
HD73_1489–1488	ATGAAAGCGCTTATT
HD73_2061–2063	TAGAATGCGCTTTAT
HD73_4468	TTGAATGCGTTTTCA
HD73_4900–4901	ATAGAAGCGGTTAAT
HD73_5854	ATGTAAACGCTTACT
HD73_0668	GTGGATGCGGTGAAA
HD73_4302	AAGTAAACGTTTACA
HD73_5768	ATGTAGGAGCTTTCA

图 1. 启动子的 *cre* 序列

Figure 1. Consensus sequence of *cre* in promoter region. The underline indicated the conserved base.

糖亲和纯化，SDS-PAGE 结果表明所得蛋白纯度较高，分子量大小约为 37 kDa (图 2-B)。

2.3 CcpA 蛋白与启动子的结合

利用FAM标记的引物(表2)扩增14个基因(操纵子)的启动子片段，与纯化的 CcpA-His 蛋白进行体外结合实验，结果表明(图 3)，凝胶底部的条带为带标记的自由 DNA，上层为 DNA 与蛋白结合的条带，随着蛋白浓度的增加，底部自由 DNA 条带浓度越来越低，上层条带浓度逐渐升高，说明 14 个启动子都与 CcpA 蛋白结合，而对照 DNA 条带(*cwlX* 基因的启动子，360 bp)不与 CcpA 蛋白结合。并且不同浓度的启动子与 CcpA 蛋白结合的亲和力不同。这些结果表明具有 *cre* 序列的 14 个启动子受 CcpA 的直接调控。

3 讨论

苏云金芽胞杆菌是昆虫病原菌，它与人类病原菌炭疽芽胞杆菌 (*Bacillus anthracis*)、机会致病菌蜡样芽胞杆菌(*Bacillus cereus*)同属于蜡样芽胞杆菌族，它们的基因组有 90%以上的相似性。本研究根据 Bt HD73 菌株 Sigma54 缺失突变体的转录组和 Bc ATCC 14579 菌株 CcpA 缺失突变体的转录组数据，在 Bt HD73 基因组中找到 31 个共同受 Sigma54 和 CcpA 调控的基因，并对其中 14 个具有 *cre* 序列的启动子进行体外结合验证，结果表明 CcpA 蛋白可以与这 14 个启动子结合。根据基因组的注释，这 14 个基因及其所在的基因簇参与

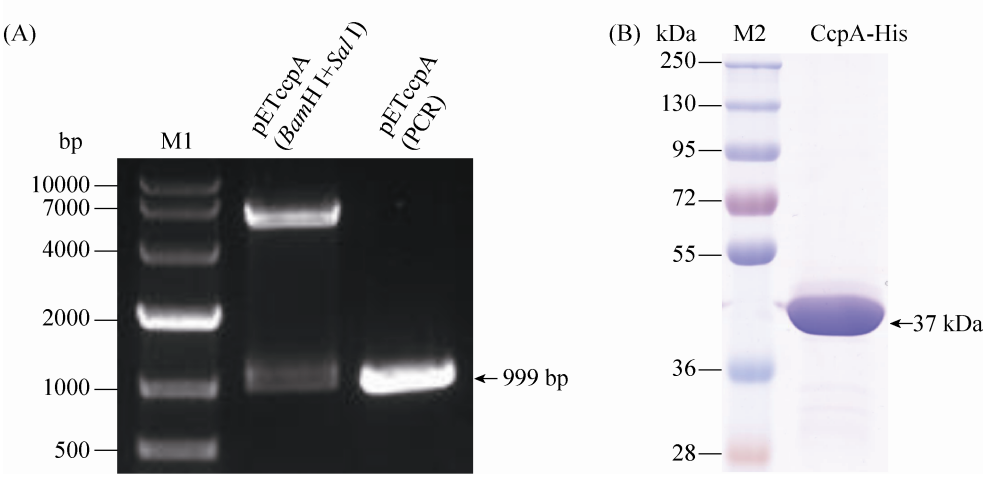

图 2. CcpA 表达载体构建及纯化

Figure 2. Construction and purification of CcpA. A: identification of pETccpA plasmid; M1: DNA marker. B: purification of CcpA-His fusion protein; M2: protein marker.

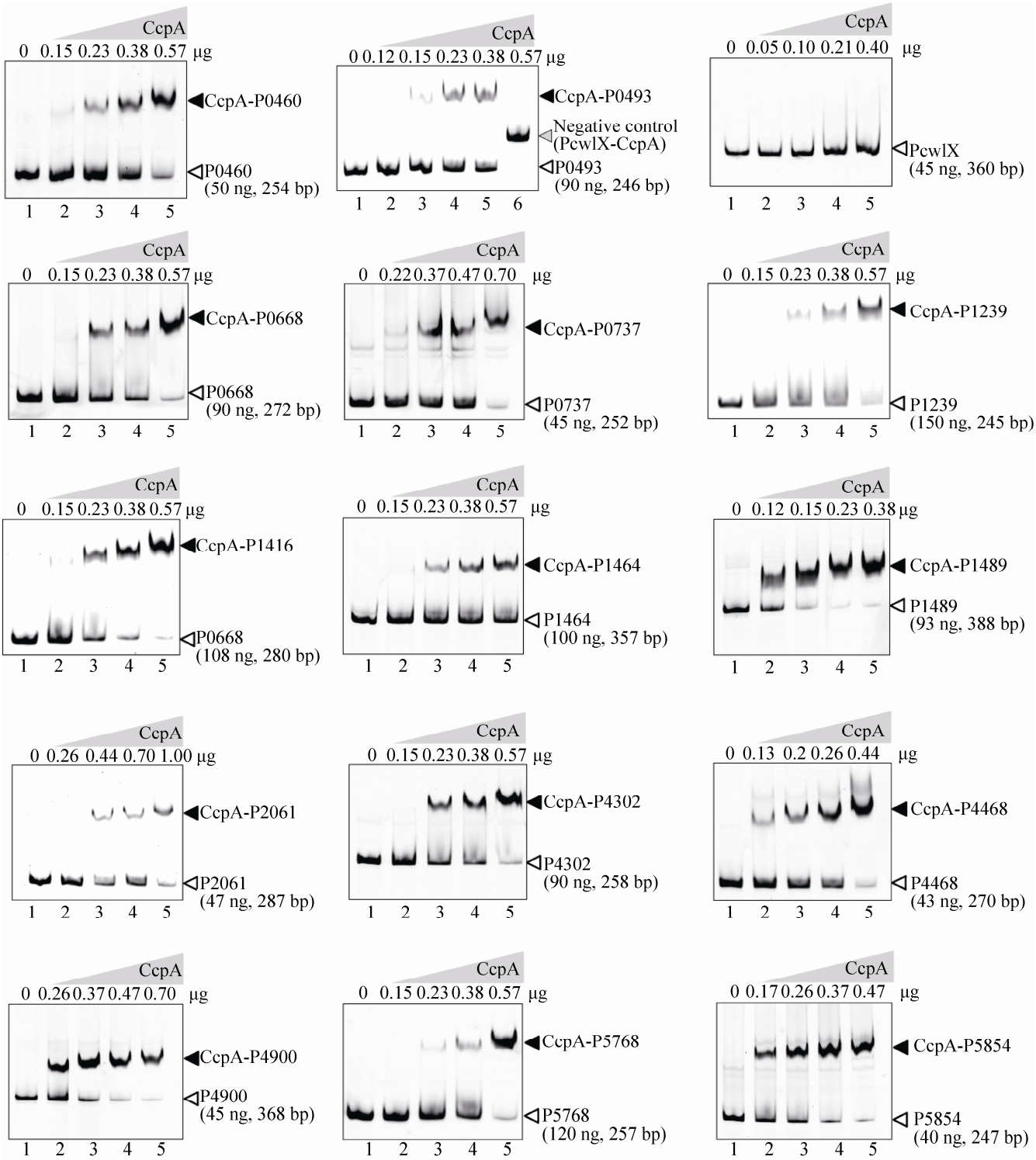

图 3. CcpA 与启动子的结合

Figure 3. Binding of CcpA and promoter. Lane 1: FAM-labeled DNA probe; lanes 2–5: incubation of the probe with increasing concentrations of purified CcpA indicated at the top of the figure.

的生理功能多样，如碳氮源代谢相关(HD73_0460、HD73_1467、HD73_1469、HD73_1488、HD73_4466、HD73_4468、HD73_5768)、转录调控因子(HD73_0493、HD73_1239)、运输系统(HD73_0742、HD73_1416、HD73_5854、HD73_0668)等。对这些启动子的序列进行分析发现，只有 HD73_4468 基因的启动子有保守的与 Sigma54 结合的–12/–24 区域，而其它启动子无保守序列，说明这些基因的转录并不直接受 Sigma54 的控制，可能存在间接的调控作用。本实验室前期研究表明，HD73_4468 基因所在的 *bkd* 基因簇的转录受 Sigma54 的控制[28,37]，并受 BkdR 的调控，本研究证明 CcpA 蛋白可以与 HD73_4468 的启动子结合，说明 *bkd* 基因簇受 CcpA 的直接调控。这与枯草芽胞杆菌中的报道相似，*bkd* 基因簇在枯草芽胞杆菌中的转录也是受 Sigma54 控制，并同时受 CcpA 的调控[38]，此外还受 CodY 的负调控[18]。枯草芽胞杆菌中的 *bkd* 操纵子编码的酶系统参与支链氨基酸的代谢，在细胞抵御冷刺激条件中发挥作用，Bt 和 Bs 中 *bkd* 基因簇的转录模式具有相似性，因此它们的功能也可能相似，Bt 菌株可能会通过 *bkd* 基因簇的转录使自身具有抵御冷刺激的功能，这也为我们在低温环境下寻找新的 Bt 菌株资源提供了依据。

细菌中受 Sigma54 控制的基因大多数参与碳氮源的代谢，CcpA 主要调控碳源分解代谢，二者调控的基因也参与了细菌毒力作用、生物膜形成、芽胞形成等重要生理过程，它们共同调控的基因参与的生理功能对菌株的生长具有重要意义。目前已报道艰难梭状芽胞杆菌(*Clostridium difficile*)的半胱氨酸代谢同时受 Sigma54 和 CcpA 调控[39]，枯草芽胞杆菌受 Sigma54 控制的启动子(参与多糖代谢的 *lev* 操纵子[40]、3-羟基丁酮的 *aco* 操纵子[13]、精氨酸代谢的 *rocG* 基因[41])也同时受 CcpA 的调控。在 Bt HD73 菌株中，已经证明同源的 *lev* 和 *aco* 操纵子的转录受 Sigma54 的控制[42,28]，并且在这些基因的启动子序列中也存在 *cre* 结合位点，但在 Bc ATCC 14579 菌株 CcpA 的转录组数据中并没有发现这些基因，可能与转录组测序样品的培养条件有关。通过本研究发现的共同受 Sigma54 和 CcpA 调控的基因参与的生理功能不尽相同，这些基因的发现丰富了对 Bt 的代谢调控网络的认识。

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Identification of genes regulated by Sigma54 and CcpA in *Bacillus thuringiensis*

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Abstract: [Objective] We analyzed the Sigma54 mutant regulon data of *Bacillus thuringiensis* (Bt) HD73 strain and CcpA mutant regulon data of *Bacillus cereus* ATCC 14579 strain, and verified the binding of promoter and CcpA protein, to determine Sigma54 and CcpA co-regulated genes in Bt HD73 and enrich the understanding of microbial metabolic regulation network. **[Methods]** Based on the results of transcriptome sequencing, genes regulated by Sigma54 and CcpA were found via the comparison of homology gene in Bt HD73 strain. *cre* sequence of promoter was found by blast and verified the binding with CcpA protein by electrophoresis mobility shift assays. **[Results]** Thirty-one genes were co-regulated by Sigma54 and CcpA in Bt HD73 strain. The fourteen promoters of these genes contained the *cre* sequence, which could bind to the CcpA protein *in vitro*. **[Conclusion]** Fourteen genes were directly regulated by CcpA in Bt HD73, while their transcription was controlled by Sigma 54. This finding provides clues to the study of sporulation and crystalformation through the point of view the metabolic regulation, and enriches the Bt metabolic regulation network.

Keywords: *Bacillus thuringiensis*, Sigma54, CcpA, transcriptional regulation

(本文责编：张晓丽)

Supported by the National Natural Science Foundation of China (31530095)
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Received: 13 April 2017; Revised: 15 May 2017; Published online: 25 May 2017