微生物学报
Acta Microbiologica Sinica
2016, 56(3): 485-495
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20150613

Research Paper 研究报告

碱性条件下苏云金芽胞杆菌基础代谢分析

考桂伟[1]，邱丽丽[1,2]，彭琦[1]，张杰[1]，李杰[2]，宋福平[1*]

[1] 中国农业科学院植物保护研究所，植物病虫害生物学国家重点实验室，北京 100193
[2] 东北农业大学生命科学学院，黑龙江 哈尔滨 150030

摘要：【目的】探索苏云金芽胞杆菌(*Bacillus thuringiensis*)形成转录差异的碱性条件，明确*B. thuringiensis*在该条件下的基础代谢途径变化。【方法】采用半定量RT-PCR技术及实时荧光定量PCR技术，确定碱刺激下参考基因*pspA*存在表达差异的碱性处理条件。在该条件下提取RNA进行Agilent定制*B. thuringiensis*表达谱芯片杂交，对芯片数据进行差异表达分析、GO富集分析及生物途径富集分析等。【结果】通过检测*pspA*表达变化，将对数生长中期的菌体加入终浓度为28 mmol/L的NaOH并诱导培养10 min，作为*B. thuringiensis*响应碱刺激的研究条件。富集分析表明碳代谢、脂肪酸合成代谢、氨基酸合成代谢途径变化明显。细胞糖酵解途径至少19个酶促基因上调表达，三羧酸循环中催化α-酮戊二酸转化为苹果酸的大部分酶蛋白编码基因上调2倍以上。【结论】本研究发现在碱性条件下*B. thuringiensis*基础代谢明显增强，细胞可能通过大量合成酸性物质如乳酸、苹果酸等来提高细胞对于碱性环境的适应能力。

关键词：苏云金芽胞杆菌，耐碱适应，基础代谢，苹果酸

苏云金芽胞杆菌(*Bacillus thuringiensis*)是昆虫病原菌，由于其能产生杀虫伴胞晶体(Cry和Cyt毒素蛋白)，已成为农业领域广泛应用的微生物农药。在活体实验中*B. thuringiensis*晶胞混合物毒杀大蜡螟(*Galleria mellonella*)活性明显高于晶体蛋白[1]，说明营养体可能影响晶体蛋白的杀虫效果。鳞翅目昆虫中肠肠道是碱性环境[2]，*B. thuringiensis*在昆虫肠道能否适应生存尚不可知，但碱性环境对*B. thuringiensis*杀虫效果的发挥至关重要[3]。碱性条件作为胁迫刺激对细菌的生长和繁殖均有较大影响，研究表明在体外实验中，蜡样芽胞杆菌族细胞能够在碱性条件下适应生存[4–5]。

大肠杆菌*Escherichia coli*遇到胁迫环境时，一种编码应激蛋白的基因*pspA*诱导表达[6]；在枯草芽胞杆菌*Bacillus subtilis*中，碱性条件下*pspA*上调表达，该基因是*B. subtilis*对碱性条件响应的参考基因[7]。目前关于细菌耐碱适应机制的研究大多围绕细胞表面蛋白及膜离子泵等方面，碱性条件

基金项目：国家自然科学基金(31530095)
***通信作者**。Tel：+86-10-62896634，E-mail：fpsong@ippcaas.cn
收稿日期：2015-12-25；**修回日期：**2016-01-21；**网络出版日期：**2016-02-02

下*B. subtilis*中δ^W控制的多个与环境应答有关的基因差异表达[7]，其中包括许多表面抗原、双组份信号系统感受蛋白、逆向转运蛋白等；在*E. coli*中发现，外膜蛋白OmpA及膜结合氧化调节蛋白DsbA在感受外界pH变化时较为敏感[8]，说明膜蛋白对细胞适应外界pH变化较为重要。另外，胞内离子浓度变化和离子转运可能也参与了细胞对碱刺激响应[9−10]，高pH条件下乳酸粪肠球菌*Streptococcus faecalis*负责离子转运的Na^+-ATPase的缺失影响了细胞的正常生长[11]；在*B. subtilis*中发现$K^+(Na^+)/H^+$转运蛋白活动加强[12−13]。目前除了细菌对碱刺激应答响应的机制研究外，在原核生物中并未发现胞内基础代谢变化影响细胞耐碱能力的相关报道。

本实验室前期研究发现在液体培养*B. thuringiensis*菌株时加入NaOH，之后培养基pH逐渐下降；*B. thuringiensis*比*B. subtilis*具有更强的碱适应能力，*B. thuringiensis*胞外pH下降至8.9–9.1时菌体即可恢复生长，而*B. subtilis*菌株需胞外pH下降至8.2–8.4才能恢复生长[14]，说明*B. thuringiensis*适应碱性环境可能具有自身的特点。本文通过探索*B. thuringiensis*对碱刺激条件的响应，利用表达谱芯片研究*B. thuringiensis* HD73菌株的全基因组表达情况，重点分析了碱刺激胁迫下*B. thuringiensis* HD73基础代谢变化可能对细胞耐碱能力产生的影响。

1 材料和方法

1.1 菌种和培养条件

供试菌株为本实验室保存的*B. thuringiensis* serovar. *kurstaki* HD73菌株，在LB培养基中按照1%接种量接种，30 °C、220 r/min条件下培养。

1.2 半定量RT-PCR、实时荧光定量PCR

HD73菌株在LB培养基中培养至对数生长中期，在处理组菌体中加入终浓度为28 mmol/L的NaOH，对照组菌体不进行加碱处理，继续培养10 min后快速取样，低温离心弃上清，沉淀立即用预冷的TRIzol重悬，RNA提取按照Qiagen Easy RNA kit说明。RNA纯化后电泳检测，–70 °C保存备用。以纯化后的RNA为模板合成cDNA，RT-PCR试剂盒为Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo公司)，实时荧光定量qRT-PCR试剂盒为Go Taq qPCR Master Mix (Promega公司)，所用引物见表1。

1.3 Agilent定制苏云金芽胞杆菌表达谱芯片杂交

实验芯片杂交前采用Agilent表达谱芯片配套试剂盒和标准操作流程对样品total RNA中的mRNA进行放大和标记，并用RNeasy mini kit纯化标记产物cRNA。随后取600 ng cRNA进行芯片杂交，在滚动杂交炉(Hybridization Oven)中65 °C、10 r/min滚动杂交17 h，在洗缸中进行洗片处理。最后对完成杂交的芯片进行Agilent Microarray Scanner扫描，用Feature Extraction software 10.7读取数据并通过Gene Spring Software 11.0完成归一化处理。

1.4 GO富集分析和KEGG分析

HD73基因组[15]和对应蛋白序列均下载于NCBI数据库。为了获取更精确的GO注释信息，利用Blast2GO软件对HD73的蛋白序列进行比对和注释。HD73基因以及基础代谢途径注释来自于KEGG数据库(http://www.genome.jp/kegg/)。后续富集分析采用本地编写的Perl程序完成。

表 1. 引物序列

Table 1. Primers used in this study

Primer names	Sequences (5′→3′)
16S rRNA5′	ATCTTCCGCAATGGACGAAAGTC
16S rRNA3′	GGTCTTGCAGCTCTTTGTACCGT
pspA5′	CCGCCAGCAGTACGAAATAA
pspA3′	GCCGCATTTAACCTTGAAACA

2 结果和分析

2.1 *pspA*基因在碱性条件下诱导表达

在HD73基因组中发现了*B. subtilis*碱刺激响应参考基因*pspA*的同源基因HD73_1073，蛋白序列比对Identity约为42.9%，以该基因表达情况作为衡量*B. thuringiensis*细胞对碱刺激响应的标准。对处理组和对照组所提取的RNA进行半定量RT-PCR实验，发现细胞对数生长中期时加入终浓度为28 mmol/L的NaOH并诱导培养10 min后，在内参基因16S rRNA表达量相同的情况下，*pspA*基因的转录表达发生明显变化(图1-A)。利用qRT-PCR验证同样条件下*pspA*差异表达情况，碱性处理组的*pspA*表达量明显上调(图1-B)。因此将上述时期作为碱刺激处理条件，提取RNA进行基因芯片杂交。对NaOH处理的实验组和不采用NaOH处理的对照组分别设置3个生物学重复。

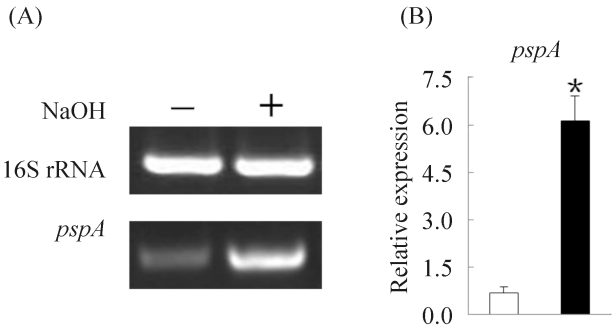

图 1. *B. thuringiensis* HD73响应碱性条件的确定及验证

Figure 1. Expression of *pspA* was confirmed by RT-PCR and qRT-PCR. A: Semi-quantitative RT-PCR analysis of the transcription of *pspA*. The sample treated with NaOH was labeled "+", and sample untreated was labeled "–". B: qRT-PCR analysis of the transcription of *pspA*. The sample treated with NaOH was labeled "T", and sample untreated was labeled "C". The bars represent the mean±S.D (*n*=3).

2.2 表达谱基因芯片代谢途径富集分析

根据Scanner扫描发现共检测到5436个基因表达信号，约占HD73基因总数的87%。为了保证数据可靠性，采用严格的域值(Fold change>2.00或Fold change<0.50，*P*<0.05)筛选差异表达基因。与对照组相比，碱处理后约有1400个基因存在表达差异，其中47% (662个)基因上调表达，53% (739个)基因下调表达，有432个基因为未知功能或功能不明确的基因。

Gene Ontology (简称GO)和KEGG 途径的富集分析是系统性描述表达谱数据变化概况和趋势的工具，能够为进一步挖掘利用表达谱信息提供分析基础。以*P*值小于0.05 (Chi-squared test)为阈值对表达谱进行GO富集分析，发现细胞的物质代谢、有机物合成、氧化还原反应等多个途径明显上调富集(图2)。在KEGG富集分析中，参与细胞基础代谢途径的基因明显上调，包括碳代谢循环、脂肪酸代谢及氨基酸的合成与转运。由于下调基因存在较多未知功能蛋白，分析中并未出现明显抑制富集的途径。

综合以上分析结果可知，*B. thuringiensis*在碱性条件下的基因表达情况并不是单一、独立的改变，而是在细胞基础代谢及信号调控等方面均存在系统性差异。由于基础代谢对于细胞生长是不可或缺的过程，以下重点对碱性条件下基础代谢途径变化进行分析。

2.3 碱性条件对糖酵解途径的影响

糖酵解是为细胞提供能量的主要途径，有研究报道细菌遇到胁迫条件时多糖代谢明显加强[16]，而本研究也发现这一现象。碱刺激胁迫下细胞内糖酵解途径中至少19个编码重要酶类的基因明显上调，涉及到糖酵解多数反应过程(图3-A)，而糖异生的过程并没有明显差异变化，仅有*fruK* (Gene ID: HD73_4002)出现抑制表达，说明此时细胞加快对葡萄糖的利用，糖酵解途径显著加强，丙酮酸激酶编码基因上调表达2倍，促使细胞产生更多丙酮酸。然而在丙酮酸的代谢途径中，催化丙酮

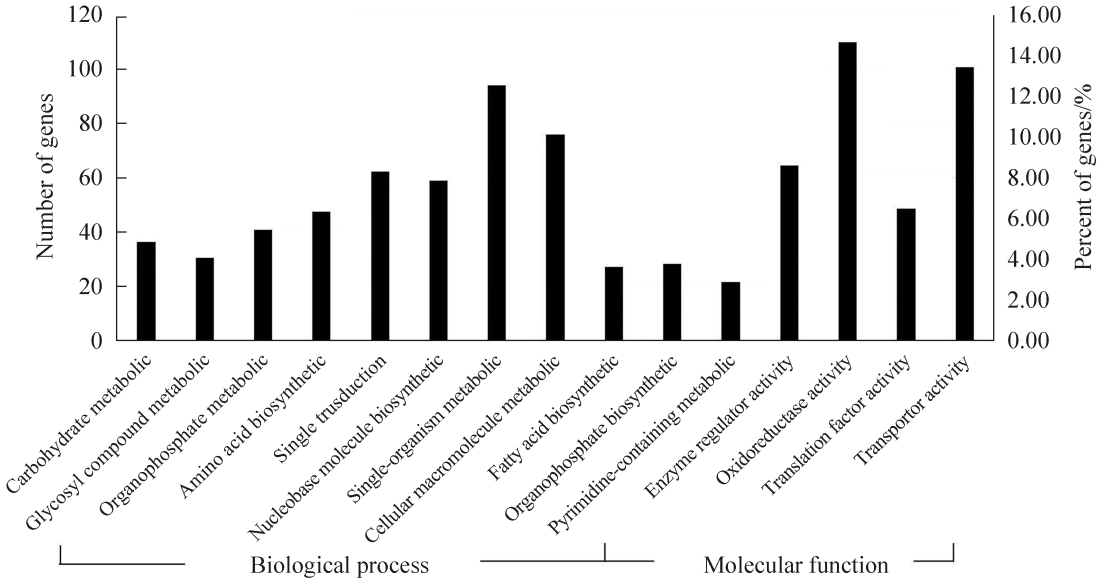

图 2. 碱性条件下上调基因GO富集情况

Figure 2. GO enrichments of up-regulated genes under alkaline stress.

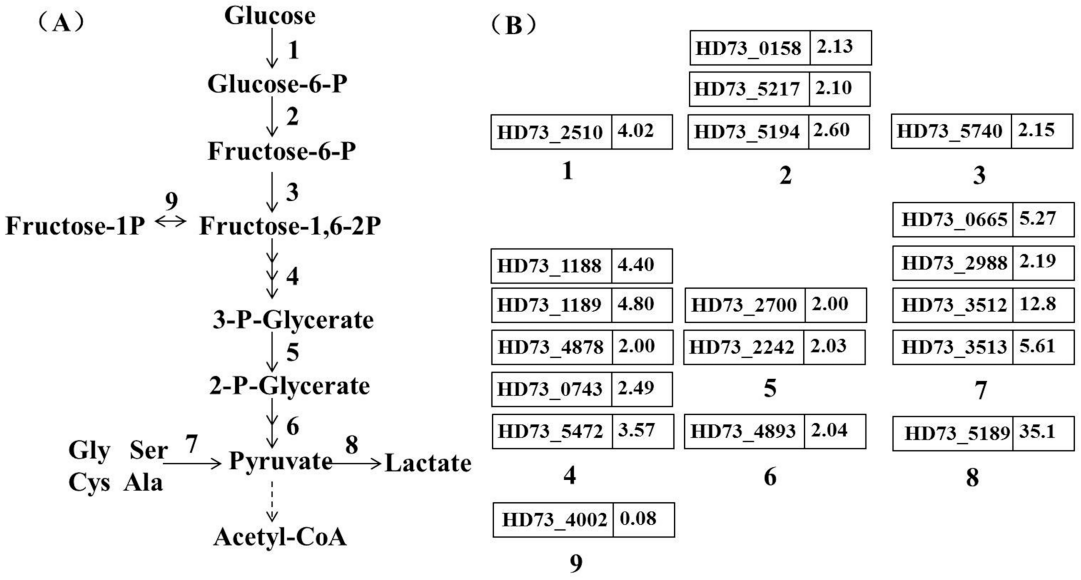

图 3. 碱性条件下糖酵解途径及基因差异表达情况

Figure 3. Glycolytic pathway and involved differential expressed genes of *B. thuringiensis* under alkaline stress. A: The main steps in glycolytic pathway were shown. Numbers 1–9 represent different enzymes. 1: Glucokinase; 2: Glucose phosphate isomerase; 3: Phosphofructokinase; 4: Aldolase, Triose phosphofructokinase, Glyceraldehyde phosphate dehydrogenase, Phosphoglycerate kinase; 5: Phosphoglyceromutase; 6: Pyruvate kinase; 7: Serine dehydratase, Alanine dehydrogenase, Glyoxalase; 8: Lactate dehydrogenase; 9: Phosphofructokinase. B: The fold changes of involved genes of *B. thuringiensis* under alkaline stress.

酸转换为乙酰-CoA的丙酮酸脱氢酶并未出现差异表达。从图3中可以看到参与途径8中的乳酸脱氢酶基因*ldh2* (Gene ID: HD73_5189)显著上调，诱导倍数高达35倍。随着糖酵解代谢增强而不断积累

的丙酮酸可能并没有及时转换为乙酰-CoA进入TCA循环，而是以合成乳酸的方式完成一部分能量代谢。丙酮酸转化为乳酸能够为胞内提供更多的有机酸类物质以调节胞内pH，且产生的NAD^+能够持续为糖酵解反应提供底物，以此保证糖酵解途径中ATP的生成。

2.4 碱性条件对苹果酸合成途径的影响

从图4中可知参与乙酰-CoA合成α-酮戊二酸的很多酶蛋白编码基因未出现差异表达，与上述推测一致。但在α-酮戊二酸合成苹果酸的过程中，包括*sucB* (Gene ID: HD73_1488)、*sucC* (Gene ID: HD73_4119)、*fumA* (Gene ID: HD73_0553)在内的12个酶促基因明显上调，而由苹果酸转化为草酰乙酸的苹果酸脱氢酶编码基因未出现差异表达，说明在碱性条件下苹果酸的合成与积累明显加强，且产生的苹果酸并没有通过转化为草酰乙酸而降解。从代谢途径13 (图4)中发现，合成α-酮戊二酸的氨基转移酶表现诱导上调，即谷氨酸的降解代谢为细胞提供了进行TCA循环的底物α-酮戊二酸，以上途径帮助细胞合成更多苹果酸。积累的苹果酸可能和乳酸发挥类似作用，这对于细胞响应碱刺激环境胁迫，调节细胞生存微环境中的酸碱平衡具有重要意义。

2.5 碱性条件对脂肪酸合成与代谢途径的影响

色氨酸、赖氨酸、苯丙氨酸能够为脂肪酸合成提供乙酰乙酰-CoA，乙酰乙酰-CoA通过脱酰基作用可变为乙酰-CoA。乙酰-CoA和丙二酸单酰-CoA是脂肪酸合成的底物。乙酰-CoA羧化酶能将乙酰-CoA转化为丙二酸单酰-CoA，在原核生物中乙酰-CoA羧化酶由生物素羧基载体蛋白在内的多个蛋白复合体组成[17]。芯片分析发现与合成生物素相关的*bio*家族基因均诱导上调。在脂肪酸合成的起始阶段中，多个合成酶基因诱导上调，脂肪酸合成速度加快(图5)。与脂肪酸氧化代谢相关的脂酰-CoA脱氢酶的编码基因HD73_3476、脂肪酸去饱和酶编码基因HD73_2987、脂肪酸激酶编码基因HD73_4466均上调表达2倍以上，在碱性条件下脂肪酸合成及代谢反应均明显加强。

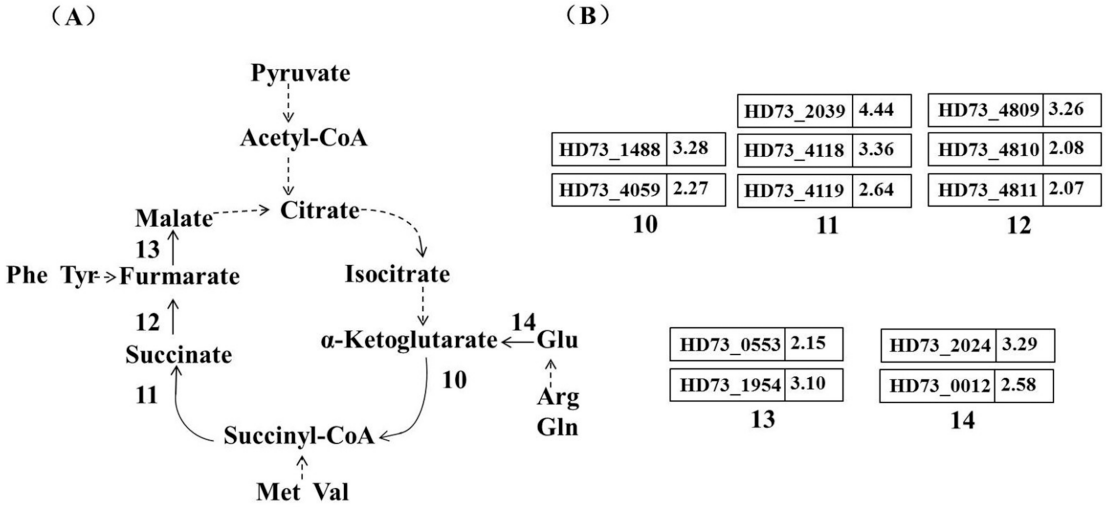

图 4. 碱性条件下HD73的TCA循环途径及基因差异表达情况

Figure 4. TCA cycle and involved differential expressed genes of *B. thuringiensis* under alkaline stress. A: The main steps in TCA cycle were shown. Numbers 10–14 represent different enzymes. 10: α-Ketoglutarate dehydrogenase, Oxoglutarate dehydrogenase; 11: Succinyl-CoA synthetase, Succinate-CoA transferase; 12: Succinate dehydrogenase; 13: Fumarase; 14: Glutamine dehydrogenase. B: The fold changes of involved genes of *B. thuringiensis* under alkaline stress.

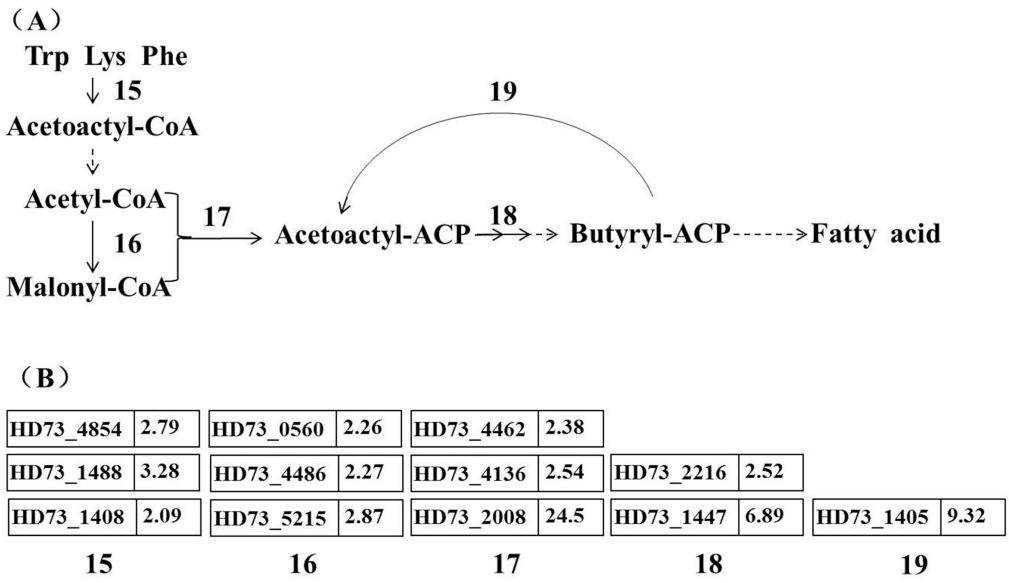

图 5. 碱性条件下HD73中脂肪酸合成及基因差异表达情况

Figure 5. Initial steps of fatty acid synthesis pathway and involved differential expressed genes of *B. thuringiensis* under alkaline stress. A: The initial steps of fatty acid synthesis pathway were shown. Numbers 15–19 represent different enzymes. 15: Trp, PheS, LysS; 16: Acetyl-CoA carboxylase; 17: Lipoamide acyltransferase, Acyl-carrier-protein synthase; 18: Enoyl-reductase, Acyl-CoA thioester hydrolase; 19: Oxoacyl-ACP synthase. B: The fold changes of involved genes of *B. thuringiensis* under alkaline stress.

2.6 碱性条件对氨基酸合成与代谢的影响

上文提到在碱性条件下细胞存在丙酮酸大量合成的情况，根据氨基酸代谢变化发现：可形成丙酮酸的谷丙转氨酶、半胱氨酸合成酶、丝氨酸-tRNA合成酶的多个编码基因上调，说明这些氨基酸的合成明显增强，并可能与丙酮酸的代谢相关，从而实现对外界刺激的适应。

α-酮戊二酸是连接氨基酸代谢和TCA循环的重要中枢分子，能够形成*α*-酮戊二酸的氨基酸有精氨酸、组氨酸、谷氨酸[18]，在芯片数据中负责此类氨基酸降解的诸多基因均明显上调。甲硫氨酸、缬氨酸与琥珀酰-CoA的合成密切相关，其合成酶基因均诱导上调。延胡索酸是合成苹果酸的直接底物，对于苹果酸的合成至关重要，而与延胡索酸有关的苯丙氨酸和酪氨酸的代谢也明显增强。

亮氨酸tRNA合成酶基因、缬氨酸tRNA合成酶基因均诱导表达(表2)，这些氨基酸能够为支链脂肪酸的合成提供前体物质。综上，与糖代谢和脂肪酸代谢途径相关的氨基酸的合成代谢途径都有明显增强，说明细胞可能通过三大类物质的互相转化，保证胞内有机酸合成，为细胞抵抗碱性刺激提供物质基础。

3 讨论

HD73在应答碱性胁迫条件时在细胞膜组分、转录调控、物质转运系统等方面均存在诸多差异表达基因，至少15个细胞膜组分基因诱导表达(表3)，其中包括已在*E. coli*耐碱适应中研究过的膜蛋白基因*ompA*[8]的同源基因*romA* (Gene ID: HD73_3311)，有至少18个膜组分基因抑制表达(表4)。在转录调控因子的表达情况中发现一个δ因子编码基因(Gene ID: HD73_5474)上调表达

表 2. 碱性条件下氨基酸合成代谢途径基因差异表达情况

Table 2. Expression of genes involved in the amino acid synthetic pathways under the alkaline stress

Related pathway	Gene ID	Fold change	*P*-value	Gene product
Ketoglutaric acid biosynthesis	HD73_4433	3.37	0.049	Arginine bifunctional protein
	HD73_0012	2.58	0.014	Glutamine amidotransferase
	HD73_0984	2.50	0.044	Glutamate racemase
	HD73_5377	5.01	0.007	L-proline dehydrogenase
Succinyl-CoA biosynthesis	HD73_0036	3.10	0.010	Methionyl-tRNA synthetase
	HD73_5073	2.41	0.004	Adenosylmethionine synthetase
	HD73_2042	3.62	0.008	Methylthioadenosine deaminase
	HD73_1436	3.49	0.030	Methyltransferase
	HD73_4765	2.64	0.001	Valyl-tRNA synthetase
Furmarate biosynthesis	HD73_4854	2.79	0.001	Phenylalanyl-tRNA synthetase
	HD73_1605	2.49	0.002	Aminoacylase
Oxaloacetate biosynthesis	HD73_4172	10.5	0.006	Dihydroorotase
	HD73_0685	4.33	0.029	Aspartate ammonia-lyase
	HD73_4173	8.56	0.008	Aspartate carbamoyltransferase
Fatty acid biosynthesis	HD73_4486	2.10	0.001	Seryl-tRNA synthetase
	HD73_5048	2.33	0.001	Leucyl-tRNA synthetase
	HD73_3511	7.75	0.046	Serine transporter
	HD73_3513	12.8	0.030	L-serine dehydratase
	HD73_4486	2.27	0.002	Acetyl-CoA carboxylase biotin carboxylase

4.94倍，另外有*Crp*、*LacI*、*LysR*、*TetR*等12个转录调控因子的编码基因上调表达，6个与双组份信号系统调节因子编码基因抑制表达。

细菌耐碱适应是一个较为复杂的过程。细胞在感受到酸碱胁迫刺激，完成胞内外信号传导应答后，将快速调整自身基因表达来适应外界胁迫环境的变化，其中通过调节pH稳态是一种较为重要的适应方式[19]。有研究表明，碱性条件下胞质内pH下降，细胞不需要保持胞内中性环境即能维持生长[20]。在真核生物中发现，碱刺激下细胞合成苹果酸的能力增强[21]，但在原核生物中并未发现关于碱刺激下胞内酸性大分子物质合成情况的报道。根据表达谱芯片结果，本研究发现在碱刺激下HD73胞内苹果酸在合成途径上的变化，苹果酸不仅由通常的碳代谢模式合成，而且可能通过加强α-酮戊二酸的合成和代谢逐渐积累。除此之外，细胞内糖酵解途径产生的丙酮酸及丙酮酸代谢合成乳酸的能力均明显增强，这说明*B. thuringiensis*不仅存在苹果酸大量合成的情况，同时也可能通过合成丙酮酸、乳酸等多种酸类物质来适应外界高pH。

在其他基础代谢中，细胞脂肪酸和氨基酸的合成与代谢均有不同程度的加强，尤其与苹果酸合成途径相关的氨基酸合成与代谢的基因多数诱导显著。碱刺激条件使*E. coli*的精氨酸和谷氨酸降解代谢发生变化[22]，本研究结果中也发现这一

表 3. 与膜组分及转录调控相关的上调差异表达基因功能

Table 3. The functions of up-regulated genes involved in membrane component and transcription regulation

Function	Gene ID	Fold change	*P*-value	Gene product
Membrane component	HD73_3311	2.68	0.035	Outer membrane protein romA
	HD73_5566	13.17	0.021	UPF0324 membrane protein
	HD73_3039	4.59	0.036	Integral membrane protein TerC
	HD73_2328	3.79	0.009	Putative Membrane Associated Protein
	HD73_2180	2.68	0.011	Putative membrane protein
	HD73_0451	3.38	0.044	Mandelate racemase/Muconate
	HD73_2850	2.07	0.018	Cell wall anchor domain-containing protein
	HD73_2987	8.28	0.012	Fatty acid desaturase (Membrane bound)
	HD73_4342	2.24	0.017	Beta-lactamase
	HD73_0342	2.07	0.018	Phosphoribosylamine--glycine ligase
	HD73_4083	3.00	0.006	Dihydrodipicolinate synthase
	HD73_4680	4.09	0.030	Cystathionine gamma-synthase
	HD73_2476	5.11	0.029	Hypothetical protein
	HD73_2479	2.54	0.001	Hypothetical protein
	HD73_2897	4.09	0.005	Hypothetical protein
Transcription factor	HD73_0493	21.70	0.027	Crp family transcriptional regulator
	HD73_1240	11.31	0.003	Transcriptional regulator, LacI
	HD73_5474	4.94	0.009	RNA polymerase sigma-54 factor
	HD73_5530	2.11	0.031	Rha family phage regulatory protein
	HD73_4979	2.64	0.039	Response regulator receiver domain protein
	HD73_2097	2.70	0.012	Transcriptional regulator, LysR
	HD73_2836	2.70	0.044	Transcriptional regulator, TetR
	HD73_2314	2.59	0.004	Transcriptional regulator, GntR
	HD73_5854	80.70	0.001	Murein hydrolase exporter
	HD73_2788	2.25	0.002	Hypothetical protein
	HD73_1239	10.40	0.002	Hypothetical protein
	HD73_5853	94.80	0.001	Hypothetical protein

情况，除此之外还有很多与苹果酸合成相关的氨基酸基因诱导表达，这说明碱刺激胁迫促使细胞多种不同途径同时差异变化，以协调酸类物质的合成。综上，尽管在*B. thuringiensis*中可能还存在着其他的耐碱机制，但是本文中发现了*B. thuringiensis*在酸类物质的合成模式、诸多基础代谢途径的变化，为进一步分析和探究菌体耐碱的作用机理提供了新的思路。

表 4. 与膜组分及双组份信号系统相关的下调差异表达基因功能

Table 4. The functions of down-regulated genes involved in membrane component and two-component system

Function	Gene ID	Fold change	*P*-value	Gene product
Membrane component	HD73_1419	0.15	0.001	Putative membrane protein
	HD73_2214	0.19	0.016	Putative membrane protein
	HD73_4460	0.21	0.020	Putative membrane protein
	HD73_5464	0.26	0.006	Membrane protein, putative
	HD73_2313	0.27	0.031	Putative Membrane Spanning Protein
	HD73_4874	0.30	0.047	Membrane attachment protein
	HD73_2141	0.35	0.001	MgtC/SapB family membrane protein
	HD73_0351	0.48	0.022	Putative membrane spanning protein
	HD73_2108	0.15	0.149	DegT/DnrJ/EryC1/StrS
	HD73_2209	0.40	0.041	Hypothetical protein
	HD73_4686	0.38	0.002	Hypothetical protein
	HD73_1886	0.35	0.001	Hypothetical protein
	HD73_3447	0.21	0.015	Sortase
	HD73_5605	0.33	0.002	Teichoic acid linkage unit synthesis
	HD73_5841	0.22	0.008	Glycosyltransferase involved in cell wall biogenesis
	HD73_3575	0.28	0.021	LPXTG-domain-containing protein cell wall anchor domain
	HD73_0603	0.24	0.040	Daunorubicin resistance transmembrane protein
	HD73_5779	0.48	0.012	Membrane protein PfoR
Two-component system	HD73_5882	0.50	0.023	Two-component sensor kinase yycG
	HD73_0642	0.12	0.045	Two-component response regulator
	HD73_5397	0.31	0.001	Two-component response regulator yanRB
	HD73_5163	0.33	0.003	Two component regulator, winged helix
	HD73_1669	0.41	0.009	Two-component response regulator yvqC
	HD73_2137	0.41	0.012	Two component transcriptional regulator
	HD73_1668	0.28	0.002	Sensor histidine kinase
	HD73_0460	0.38	0.036	Methylthioribose kinase
	HD73_4343	0.16	0.045	Sensory box/GGDEF family protein
	HD73_5707	0.17	0.028	Sensory box/GGDEF

参考文献

[1] Fedhila S, Buisson C, Dussurget O, Serror P, Glomski IJ, Liehl P, Lereclus D, Nielsen-LeRoux C. Comparative analysis of the virulence of invertebrate and mammalian pathogenic bacteria in the oral insect infection model *Galleria mellonella*. *Journal of Invertebrate Pathology*, 2010, 103(1): 24–29.

[2] Berenbaum M. Adaptive significance of midgut pH in larval Lepidoptera. *American Naturalist*, 1980: 138–146.

[3] Knowles BH. Mechanism of action of *Bacillus thuringiensis*

insecticidal d-endotoxins. *Advances in Insect Physiology*, 1994, 24: 275–308.

[4] Cao M, Kobel PA, Morshedi MM, Wu MFW, Paddon C, Helmann JD. Defining the *Bacillus subtilis* δ^W regulon: a comparative analysis of promoter consensus search, run-off transcription/macroarray analysis (ROMA), and transcriptional profiling approaches. *Journal of Molecular Biology*, 2002, 316(3): 443–457.

[5] Flahaut S, Hartke A, Giard J-C, Auffray Y. Alkaline stress response in *Enterococcus faecalis*: adaptation, cross-protection, and changes in protein synthesis. *Applied and Environmental Microbiology*, 1997, 63(2): 812–814.

[6] Kleerebezem M, Crielaard W, Tommassen J. Involvement of stress protein PspA (phage shock protein A) of *Escherichia coli* in maintenance of the protonmotive force under stress conditions. *The EMBO Journal*, 1996, 15(1): 162.

[7] Wiegert T, Homuth G, Versteeg S, Schumann W. Alkaline shock induces the *Bacillus subtilis* δ^W regulon. *Molecular Microbiology*, 2001, 41(1): 59–71.

[8] Stancik LM, Stancik DM, Schmidt B, Barnhart DM, Yoncheva YN, Slonczewski JL. pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*. *Journal of Bacteriology*, 2002, 184(15): 4246–4258.

[9] Kawano M, Igarashi K, Kakinuma Y. Isolation of *Enterococcus hirae* mutant deficient in low-affinity potassium uptake at alkaline pH. *Bioscience, Biotechnology, and Biochemistry*, 2002, 66(7): 1597–1600.

[10] Trchounian A, Kobayashi H. Relationship of K^+-uptaking system with H^+-translocating ATPase in *Enterococcus hirae*, grown at a high or low alkaline pH. *Current Microbiology*, 1998, 36(2): 114–118.

[11] Kakinuma Y, Igarashi K. Mutants of *Streptococcus faecalis* sensitive to alkaline pH lack Na (+)-ATPase. *Journal of Bacteriology*, 1990, 172(4): 1732–1735.

[12] Fujisawa M, Wada Y, Ito M. Modulation of the K^+ efflux activity of *Bacillus subtilis* YhaU by YhaT and the C-terminal region of YhaS. *FEMS Microbiology Letters*, 2004, 231(2): 211–217.

[13] Hamamoto T, Hashimoto M, Hino M, Kitada M, Seto Y, Kudo T, Horikoshi K. Characterization of a gene responsible for the Na^+/H^+ antiporter system of alkalophilic *Bacillus* species strain C–125. *Molecular Microbiology*, 1994, 14(5): 939–946.

[14] Qiu LL, Peng Q, Qu N, Liu CX, Li J, Zhang J, Song FP. Effect of *clpP* disruption on alkaline sensitivity of *Bacillus thuringiensis*. *Acta Microbiologica Sinica*, 2013, 53(6): 615–622. (in Chinese)
邱丽丽, 彭琦, 曲宁, 刘春霞, 李杰, 张杰, 宋福平. 苏云金芽胞杆菌*clpP*基因缺失对碱刺激敏感性的影响. 微生物学报, 2013, 53(6): 615–622.

[15] Liu G, Song L, Shu C, Wang P, Deng C, Peng Q, Lereclus D, Wang X, Huang D, Zhang J. Complete genome sequence of *Bacillus thuringiensis* subsp. *kurstaki* strain HD73. *Genome Announcements*, 2013, 1(2): e00080–00013.

[16] Lemos JA, Abranches J, Burne RA. Responses of *cariogenic streptococci* to environmental stresses. *Current Issues in Molecular Biology*, 2005, 7(1): 95–108.

[17] Rodrigues TE, Gerhardt E, Oliveira MA, Chubatsu LS, Pedrosa FO, Souza EM, Souza GA, MüllerSantos M, Huergo LF. Search for novel targets of the PII signal transduction protein in Bacteria identifies the BCCP component of acetylCoA carboxylase as a PII binding partner. *Molecular Microbiology*, 2014, 91(4): 751–761.

[18] Fernández M, Zúñiga M. Amino acid catabolic pathways of lactic acid bacteria. *Critical Reviews in Microbiology*, 2006, 32(3): 155–183.

[19] Slonczewski JL, Rosen BP, Alger JR, Macnab RM. pH homeostasis in *Escherichia coli*: measurement by 31P nuclear magnetic resonance of methylphosphonate and phosphate. *Proceedings of the National Academy of Sciences*, 1981, 78(10): 6271–6275.

[20] Mugikura S, Nishikawa M, Igarashi K, Kobayashi H. Maintenance of a neutral cytoplasmic pH is not obligatory for growth of *Escherichia coli* and *Streptococcus faecalis* at an alkaline pH. *Journal of Biochemistry*, 1990, 108(1): 86–91.

[21] Žárský V. Signal transduction: GABA receptor found in plants. *Nature Plants*, 2015, 1(8).

[22] Yohannes E, Barnhart DM, Slonczewski JL. pH-dependent catabolic protein expression during anaerobic growth of *Escherichia coli* K-12. *Journal of Bacteriology*, 2004, 186(1): 192–199.

The analysis of major metabolic pathways in *Bacillus thuringiensis* under alkaline stress

Guiwei Kao[1], Lili Qiu[1,2], Qi Peng[1], Jie Zhang[1], Jie Li[2], Fuping Song[1 *]

[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[2] College of Life Sciences, Northeast Agricultural University, Harbin 150030, Heilongjiang Province, China

Abstract: [**Objective**] The study aimed to determine the appropriate stage for exploring the response of *Bacillus thuringiensis* to the alkaline stress, to profile the metabolic pathways under this stress. [**Methods**] Using semi-quantitative RT-PCR and qRT-PCR, the proper stage was defined by monitoring the transcriptional changes of marker gene *pspA*, which was known as a responsive gene under the alkaline stress. The total RNA was then extracted to perform the microarray hybridizations for samples under stress and control, respectively. Gene Ontology and pathway enrichments were conducted to analyze the global changes of carbon metabolism, metabolism of fatty acid synthesis and amino acid. [**Results**] For *B. thuringiensis* in the mid-log growth phase, treatment of 28 mmol/L NaOH for 10 mins is the feasible approach to analyze the response of *B. thuringiensis* to this stress. More than twenty genes encoding important enzymes in glycolytic pathway were up-regulated and majority of genes involved in catalyzing alpha-ketoglutarate into malic acid were also found to up-regulated more than two folds. [**Conclusion**] By analyzing the gene expression profile, the major metabolisms of *B. thuringiensis* were found to be clearly enhanced under alkaline stress. Large quantities of acid including malic acid and lactic acid may contribute a lot to the adaptation of alkaline condition.

Keywords: *Bacillus thuringiensis*, alkaline resistance, major metabolic pathways, malic acid

(本文责编：李磊)

Supported by the National Natural Science Foundation of China (31530095)

*Corresponding author. Tel: +86-10-62896634; E-mail: fpsong@ippcaas.cn

Received: 25 December 2015; Revised: 21 January 2016; Published online: 2 February 2016