大肠杆菌 *pps*A 和 *tkt*A 基因的串联表达

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摘 要 *pps*A 和 *tkt*A 是芳香族氨基酸生物合成中心途径的两个关键酶基因,在大肠杆菌中,*pps*A 基因编码磷酸烯醇式丙酮酸合成酶 A(PpsA),该酶催化丙酮酸合成磷酸烯醇式丙酮酸;*tkt*A 基因编码转酮酶 A,该酶在磷酸戊糖途径中生成 4-磷酸赤藓糖起主要作用。采用 PCR 方法从大肠杆菌 K-12 株中扩增到 *pps*A 和 *tkt*A,并实现了两基因的高效表达,其中 *pps*A 活性提高了 10.8 倍,*tkt*A 活性提高了 3.9 倍,当这两个基因串联在一个质粒上导入大肠杆菌进行表达时,PpsA 的活性变化较大(2.1 ~ 9.1 倍),TktA 的活性相对稳定(3.9 ~ 4.5 倍),且这两个基因单独表达和串联表达都能使芳香族氨基酸生物合成共同途径中关键中间产物 DAHP 的产量提高,且串联表达比单独表达较高。

关键词 芳香族氨基酸,代谢工程,*pps*A 基因,*tkt*A 基因,串联表达

中图分类号 Q786 **文献标识码** A **文章编号** 1000-3061(2003)03-0301-06

芳香族氨基酸包括苯丙氨酸(Phe)、酪氨酸(Tyr)和色氨酸(Trp),是人体和动物的必需氨基酸,在医药、食品和保健品等领域有很大用途。Phe 作为一种新型甜味剂 Aspartame 的合成原料,是近 20 年来市场需求增长最快的氨基酸品种,近来研究发现 Phe 还具有抗高血压的作用[1]。通过导入特异基因等代谢工程手段[2],芳香族氨基酸生物合成途径中间产物分支酸(Chorismate)和终产物 Tyr、Trp 分别可以生成肠菌素、奎宁酸[3]、黑色素和靛蓝[4]。

过去的生物化学家较注重对于分支途径的遗传操作,如解除终产物的反馈抑制和阻遏的调控机制、高表达直接生成终产物的酶[5]以及删除分支途径对终产物的分流等[6]。随着分子生物学技术的发展和代谢工程在天然产品上的应用[7],越来越多的人意识到要想进一步提高芳香族氨基酸的产率和产量,就必需提高宿主菌把碳源导向中心代谢途径的能力[8]。代谢途径分析[9]表明:葡萄糖经糖酵解和磷酸戊糖途径分别生成磷酸烯醇式丙酮酸(Phosphoenolpyrurate,PEP)和 4-磷酸赤藓糖(Erythrose-4-phosphate,E4P),二者在 3-脱氧-α-阿拉伯庚酮糖酸-7 磷酸(3-deoxy-α-arobinoheptuloso nate-7-phosphate,DAHP)合成酶的作用下缩合生成 DAHP,这是中心代谢途径,也是决定芳香族氨基酸生物合成产率和产量的关键步骤。DAHP 是芳香族氨基酸生物合成过程中第一个也是最重要的代谢中间物。然后,DAHP 经莽草酸途径生成分支酸,由分支酸再经不同的途径生成 Try、Phe 或 Trp。代谢流定量分析[10]表明:1mol 葡萄糖进入细胞后经糖酵解可生成 2mol 的 PEP,而葡萄糖要进入细胞需经过磷酸基转移酶系统(Phosphotransferase system,PTS),PEP 是 PTS 中的磷酸基供体[11],1mol 葡萄糖进入细胞要把 1mol 的 PEP 转变成丙酮酸,同时 PEP 又可在丙酮酸激酶和 PEP 羧化酶的作用下分别生成丙酮酸和草酰乙酸。丙酮酸在生物体内因为高能障而不能自发地转变回 PEP,这样大量的碳流就经丙酮酸而生成有机酸、二氧化碳和细胞内容物。Patnaik[12]等通过高表达 PEP 合成酶基因 *pps*A 将丙酮酸重新回流到 PEP,从而使 DAHP 的产量提高了 2 倍,但也对细胞造成生长抑制、糖耗过大和向胞外分泌醋酸盐等副作用[13]。E4P 是糖酵解中的 6-磷酸果糖(6-phosphate fructose,F6P)分流进入磷酸戊糖途径,在非氧化阶段经一步转醛反应和两步转酮反应而得。负责 E4P

收稿日期:2002-11-18,修回日期:2003-02-28。

基金项目:军事医学科学院创新基金资助项目(No.9902502)。

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合成的转酮酶有两个：TktA 和 TktB，其中 TktA 起主要作用[14]，高表达 TktA 可以大大提高 Phe 的产量[15]。而在两个质粒同时高表达 PpsA 和 TktA，则可使 DAHP 的产量接近理论计算产量[16]。PEP 和 E4P 是 DAHP 的限制性底物，也是芳香族氨基酸的限制性底物，所以要想提高芳香族氨基酸的产量就必需克服细胞内其它代谢途径对 PEP 的竞争，同时使 E4P 的生成量与 PEP 相平衡，从而提高中心代谢途径中 DAHP 的产量。有关 *pps*A 基因的研究在国内仅见报道，但表达量和酶活性都比较低[17]。本实验在高表达 *pps*A 和 *tkt*A 的基础上，实现了这两个基因的串联表达，为进一步探讨这两个基因的转录调控[18]、PTS 的功能[19]和葡萄糖的转运[20]等理论研究奠定了基础，同时也为进一步构建芳香族氨基酸特别是 Phe 基因工程菌奠定了基础。

1 材料和方法

1.1 材料

1.1.1 菌株和质粒(表 1)

表 1 菌株与质粒

Table 1 Strains and plasmids

Strains and plasmids	Characters	Source and reference
Strains		
Escherichia coli K-12	Donor of genome	ATCC
Escherichia coli DH5α	Recipient strain	ATCC
Plasmids		
p^{GEM-T} Easy	AP^r cloning vector	Promega
pBV220	AP^r expressing vector	Reference [21]
pBV220-*pps*A	AP^r *pps*A	This work
pBV220-*tkt*A	AP^r *tkt*A	This work
PPT-Ⅰ	AP^r *pps*A *tkt*A cis-promoter	This work
PPT-Ⅱ	AP^r *pps*A *tkt*A trans-promoter	This work
PTP-Ⅰ	AP^r *tkt*A *pps*A cis-promoter	This work
PTP-Ⅱ	AP^r *tkt*A *pps*A trans-promoter	This work

1.1.2 酶和试剂

dNTP、TaqDNA 多聚酶及反应缓冲液，限制酶 *Eco*RI、*Bam*HI、*Sph*I、*Bgl*Ⅱ均购自 TaKaRa，TPP(Merck)、6-磷酸葡萄糖脱氢酶(Sigma)、磷酸葡萄糖异构酶(Sigma)、E4P(Sigma)、蛋白 Marker(Sigma)，其他化学试剂均为分析纯。

1.2 方法

1.2.1 PCR

分别根据 Niersbach[22] 和 Blattner[23] 等报道的 *pps*A 和 *tkt*A 的 DNA 序列，设计了两对扩增引物，P1：5′-ccggaattcatgtccaacaatggctcgtc-3′，P2：5′-cgcggatccttatttcttcag ttcagccag-3′，P3：5′-cgcggatccatgtcctcacgtaaagagct-3′，P4：5′-acatgcatgcttacagcagttc ttttgcttt-gc-3′，通过 PCR 方法从 *E. coli* K-12 染色体 DNA 中扩增得到 *pps*A 和 *tkt*A 基因，限制酶酶切分析和测序鉴定其正确性。

1.2.2 基因操作技术和方法见文献[24]。

1.2.3 DNA 序列测定

采用 Sanger 末端终止法，在 ABI100 测序仪上进行。

1.2.4 基因表达

带有 *pps*A 或/和 *tkt*A 的质粒 pBV220-*pps*A、pBV220-*tkt*A、PPT-Ⅰ、PPT-Ⅱ、PTP-Ⅰ和 PTP-Ⅱ分别转化到受体菌 DH5α 后，挑取单克隆，提取质粒，进行限制性酶切分析和 PCR 鉴定其正确性，再划线，挑单菌落接种于含氨苄青霉素的 LB 培养基中，37℃振荡过夜，按 3% ~ 5% 扩大培养到 10mL，30℃培养至 OD_{600} = 0.5 ~ 0.6，迅速置于 42℃诱导培养 4.5h。

1.2.5 SDS-PAGE

12000g 离心 20min 收集菌体，2 倍 SDS 上样缓冲液处理，沸水浴 10min，浓缩胶 5%，分离胶 10%进行电泳，考马斯亮蓝 R250 染色。

1.2.6 PpsA 和 TktA 粗酶液的制备

按 1.2.4 方法进行菌体培养，当诱导结束后，培养液于 4℃ 5000g 离心 20min 收集菌体，分别用 100mmol/L Tris-HCl(pH 7.4)和 50mmol/L Gly-gly (pH 8.5)、5mmol/L $MgCl_2$、0.5mmol/L TPP、1mmol/L DTT 洗涤 2 次并重悬于各自的缓冲液中，在冰水浴中超声波处理，30 × 4s，200W，4℃ 5000g 离心 20min 收集上清。

1.2.7 PpsA 和 TktA 酶活测定

分别按照文献[17]和文献[25]进行 PpsA 和 TktA 的活性测定。

1.2.8 DAHP 含量测定

按文献[26]测定 DAHP 的含量。按 1.2.6 制备 PpsA 和 TktA 粗酶液样品，先在 1mL 样品中加入 0.25mL 0.025mol/L 的高碘酸溶液，室温下放置 45min，加入 0.5mL 0.2%的亚砷酸钠，再放置 2min，然后加入 2mL 0.3%的硫巴比妥酸溶液，并沸水浴 5min，40℃水浴降温，即出现桃红色，迅速在 549nm 比色。同时设立对照，对照加酶液等体积的水。

2 结 果

2.1 *pps*A 和 *tkt*A 基因的 PCR 扩增及其序列分析

按 1.2.1 方法在 *E. coli* K-12 DNA 中进行 *pps*A 和 *tkt*A 基因的 PCR 扩增，分别得到 2.4kb 和 2.0kb 左右的目的片段，经回收与 pGEM-T Easy 载体连接，挑选单克隆，提取质粒，进行限制性酶切分析(图 1)和全序列测定，确定 *pps*A 基因序列全长为 2379bp，

*tkt*A 基因序列全长为 1992bp，*pps*A 用 *Eco*R Ⅰ 和 *Bam*H Ⅰ，*tkt*A 用 *Bam*H Ⅰ 和 *Sph* Ⅰ 分别从 pGEM-T Easy 载体上切下，并克隆入表达载体 pBV220，转化受体菌 DH5α，筛选获得能分别表达 PpsA 和 TktA 的菌株。

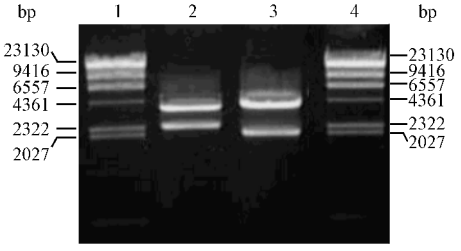

图 1　*pps*A 和 *tkt*A 与 pGEME-T Easy 载体连接的酶切鉴定

Fig.1　Digestion of pGEME-T Easy vector with *pps*A or *tkt*A

1、4. λ*Hin*dⅢ marker(23130,9416,6557,4361,2322,2027bp)；

2. Double digestion of pGEM-T Easy vector with *pps*A by *Eco*R Ⅰ and *Bam*H Ⅰ；

3. Double digestion of pGEM-T Easy vector with *tkt*A by *Sph* Ⅰ and *Bam*H Ⅰ

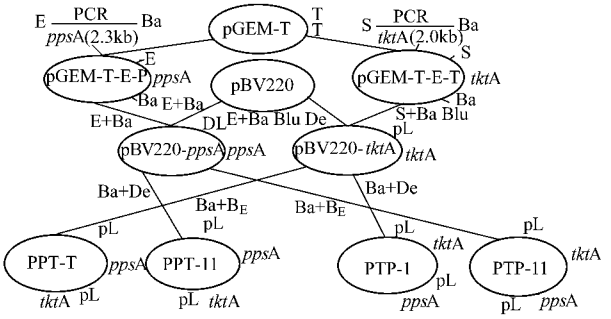

图 2　*pps*A 和 *tkt*A 串联表达质粒的构建

Fig.2　Construction of recombinant plasmids of *pps*A and *tkt*A co-expression

PCR：polymerase chain reaction；pGEM-T-E-P：pGEM-T Easy vector with *pps*A；pGEM-T-E-T：pGEM-T Easy vector with *tkt*A；E：*Eco*R Ⅰ；Ba：*Bam*H Ⅰ；Blu：Blunting；de：dephosphorylation；S：*Sph* Ⅰ；Bg：*Bgl* Ⅱ；pBV220-*pps*A：pBV220 vector with *pps*A；pBV220-*tkt*A：pBV220 vector with *tkt*A；PPT-Ⅰ：pBV220 vector with *pps*A and *tkt*A，cis-promoter；PPT-Ⅱ：pBV220 vector with *pps*A and *tkt*A，trans-promoter；PTP-Ⅰ：pBV220 vector with *tkt*A and *pps*A，cis-promoter；PTP-Ⅱ：pBV220 vector with *tkt*A and *pps*A，trans-promoter

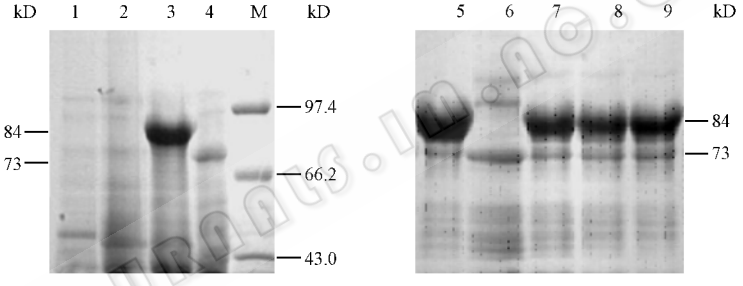

图 3　*pps*A 和 *tkt*A 单独和串联表达的聚丙烯酰胺电泳分析

Fig.3　SDS-PAGE analysis for the single-and co-expression of *pps*A and/or *tkt*A in *E. coli*

M. Protein marker；1. host DH5α；2. DH5α/pbv220；3. DH5α/pbv220-*pps*A；4 and 6. DH5α/pbv220-*tkt*A；5. DH5α/ PPT-Ⅰ；7. DH5α/ PPT-Ⅱ；8. DH5α/ PTP-Ⅰ；9. DH5α/ PTP-Ⅱ

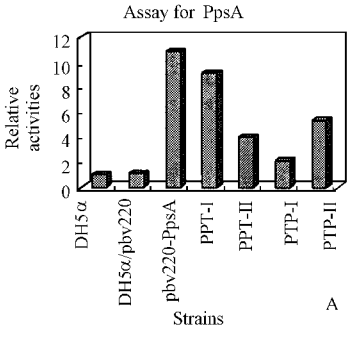

图 4A　PpsA 活性测定

Fig.4A　Assay for PpsA

Standard curve：slope = 0.2887；R^2 = 0.9963

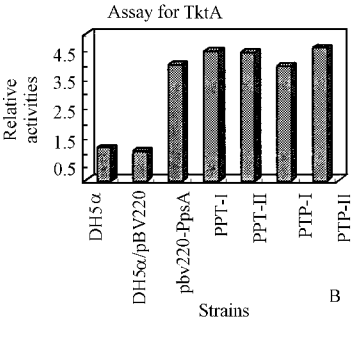

图 4B　TktA 活性测定

Fig.4B　Assay for TktA

Standard curve：slope = 0.0045；R^2 = 0.9948

2.2　*pps*A 和 *tkt*A 串联表达重组子的构建

构建过程如图 2 所示。

2.3　SDS-PAGE 检测 *pps*A 和 *tkt*A 的蛋白表达

按 1.2.4 和 1.2.5 方法培养并诱导含 pBV220-*pps*A、pBV220-*tkt*A、PPT-Ⅰ、PPT-Ⅱ、PTP-Ⅰ、PTP-Ⅱ和 pBV220 质粒的菌株及 DH5α 空宿主菌，SDS-PAGE 结果如图 3 所示，含 pBV220-*pps*A、pBV220-*tkt*A 的宿主菌分别有 84kD 和 73kD 的蛋白表达带产生，而

PPT-Ⅰ、PPT-Ⅱ、PTP-Ⅰ、PTP-Ⅱ则同时表达 84kD 和 73kD 的蛋白。

2.4 *pps*A 和 *tkt*A 的酶活性测定

按 1.2.6 和 1.2.7 方法分别进行 PpsA 和 TktA 的酶活性测定,以空宿主菌的粗提物酶活性比为 1,结果发现,携带 pBV220-*pps*A 质粒的宿主菌,其 PpsA 酶活性提高了 10.8 倍,在串联质粒中,其活性分别提高了 9.1、4.0、2.1 和 5.3 倍(图 4A);携带 pBV220-*tkt*A 质粒的宿主菌,其 TktA 酶活性提高了 3.9 倍,在串联质粒中,其活性分别提高了 4.4、4.4、3.9 和 4.5 倍(图 4B)。

2.5 DAHP 含量的测定

按 1.2.8 方法进行 DAHP 含量的测定,因无标准 DAHP,故只对 *OD* 值进行比较(图 5),结果表明含 pBV220-*pps*A 质粒的宿主菌和含 pBV220-*tkt*A 质粒的宿主菌均比对照菌株高,且前者比后者为高;PpsA 和 TktA 串联表达质粒比单独表达为高;在串联表达质粒中 PPT-Ⅱ、PTP-Ⅰ又比 PPT-Ⅰ、PTP-Ⅱ为高。

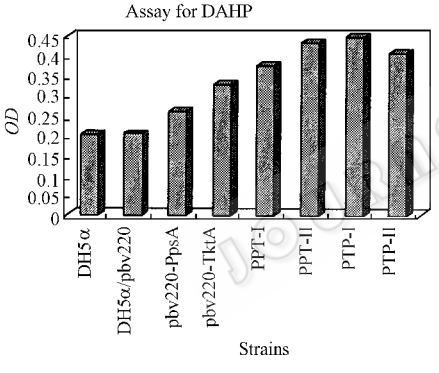

图 5 DAHP 含量的测定

Fig.5 Assay for DAHP

All the hosts are DH5α, DH5α、DH5α/pbv220 as controls.

3 讨 论

从图 4 可以看到,就 *pps*A 来说单基因比两基因串联表达的酶活性高许多,这可能与基因自身结构、串联位置及翻译起始方向有关;而对于 *tkt*A 来说,无论串联位置如何,其活性变化不是很明显。然而图 5 可以看到并非是基因表达的蛋白活性愈高,DAHP 的产量愈高(PPT-Ⅰ、PTP-Ⅱ),而是两基因表达蛋白活性相对协调一致的其 DAHP 的产量较高(PPT-Ⅱ、PTP-Ⅰ)。这也进一步说明有时过高地表达代谢途径中的某种蛋白质(酶),反而给细胞带来代谢负担而无益于整体基因工程菌的构建[27]。从图 3 可以看到,*pps*A 和 *tkt*A 表达水平很大(图 3)且其表达产物为可溶性蛋白,本研究的目的旨在于这两个基因在过表达时对 DAHP 生成的影响,在实际应用时应该控制它们的表达,使其既能最大限度的合成 DAHP 又不对菌体自身产生负担为宜。由图 5 也可以看到单一表达一个中心途径的关键酶也可以使关键性中间产物 DAHP 的含量有很大的增加[28]。

国外有将 *pps*A 和 *tkt*A 在两个不同质粒上进行表达使 DAHP 产量升高的例子[29],考虑到多质粒对细胞生理、生化及代谢的影响[30],本文第一次实现了这两个基因的串联表达,并使 DAHP 的含量得到了提高,为以后在芳香族氨基酸生产上实现中心代谢途径和分支代谢途径关键酶的结合从而构建基因工程菌奠定了基础。

REFERENCES(参考文献)

[1] Li Z B, Zhao G S. Phenylalanine effect on blood pressure and cardiovascular tissue pathological changes in spontaneously hypertension rats. *Acta Uni Med Sec Shanghai*, 2000, **20**(2): 115 – 117

[2] Frost J, Lievense J. Prospects for biocatlytic synthesis of aromatics in the 21st century. *New J Chem*, 1994, **18**: 341 – 348

[3] Draths K M, Ward T L, Frost J W. Biocatalysis and nineteenth century organic chemistry: conversione of D-glucose into quinoid organics. *J Am Chem Soc*, 1992, **114**: 9725 – 9726

[4] Murdock D, Ensley B D, Serdar C. Construction od metabolic oprons catalyzing in de novo biosynthesis of indigo in *Escherichia coli*. *Bio/Technology*, 1993, **11**: 381 – 385

[5] Ikeda M, Katsumata R. Metabolic engineering to produce tyrosine or phenylalanine in a tryptophen-producing *Corynebacterium glutamicum* strain. *Appl Env Microbiol*, 1992, **58**(3): 781 – 785

[6] Aiba S, Tsunekawa H, Imanaka T. New approach to tryptophan production by *Escherichia coli*: Genetic manipulation of composite plasmids *in vitro*. *Appl Env Microbiol*, 1982, **43**: 289 – 297

[7] Strohl W R. Biochemical engineering of nature product biosynthesis pathway. *Metab Eng*, 2001, **3**(1): 4 – 14

[8] Stephanopoulos G. Metabolic fluxes and metabolic engineering. *Metab Eng*, 1999, **1**(1): 1 – 11

[9] Liao J C, Hou S Y, Chao Y P. Pathway analysis, engineering, and physiological considerations for redirecting central metabolism. *Biotech Bioeng*, 1996, **52**(1): 129 – 140

[10] Liao J C, Oh M K. Toward predicting metabolic fluxes in metabolically engineering strains. *Metab Eng*, 1999, **1**(3): 214 – 223

[11] Postma P W. Phosphotransferase system for glucose and other sugers, pp. 127 – 141 in *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. Neidhardt, F. C. (ed). American society for microbiology, Washington, D.C.

[12] Patnaik R, Liao J C. Engineering of *Escherichia* central metabolism for aromatic metabolite production with near theoretical yield. *Appl Env Microbiology*, 1994, **60**(11): 3903 – 3908

[13] Oh M K, Rohlin L, Kao K C, Global expression profiling of acetate-

grown *Escherichia coli*. *J Biol Chem*, 2002, **277**(15): 13175 – 13183

[14] Iida A, Teshiba S, Mizobuchi K. Identification and characterization of the *tkt*A gene encoding a second transketolase in *Escherichia coli* K-12. *J Bacteriol*, 1993, **175**(17): 5375 – 5383

[15] Tatarko M, Romeo T. Disruption of a global regulatory gene to enhance central carbon flux into phenylalanine biosynthesis in *Escherichia coli*. *Curr Microbiol*, 2001, **43**(1): 26 – 32

[16] Patnaik R, Roof W D, Young R F *et al*. Stimulation of glucose catabolism in *Escherichia coli* by a potential futile cycle. *J Bacteriol*, 1992, **174**: 7527 – 7532

[17] CHAI H M(柴红梅), ZHAO Y C(赵永昌), SONG L R(宋令荣) *et al*. Cloning and expression of phosphoenolpyruvate synthetase (PpsA). *Chin J Biochem Mol Biol*(中国生物化学与分子生物学报), 2000, **16**(4): 559 – 561

[18] Negre D, Oudot C, Prost J F *et al*. FruR-mediated transcriptional activation at the *pps*A promoter of *Escherichia coli*. *J Mol Biol*, 1998, **276**(2): 355 – 365

[19] Hesterkamp T, Erni B. A reporter gene assay for inhibitors of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. *J Mol Microbiol Biotechnol*, 1999, **1**(2): 309 – 317

[20] Dwyer DS. Model of the 3-D structure of the GLUT3 glucose transporter and molecular dynamics simulation of glucose transporter. *Protains*, 2001, **42**(4): 531 – 541

[21] ZHANG Z Q(张智清), YAO L H(姚立红), HOU Y D(候云德). Construction and application of a high level expression vector containing P_RP_L promoter. *Chin J Virology*(病毒学报), 1990, **6**(2): 111 – 116

[22] Niersbach M, Kreuzaler F, Geerse RH *et al*. Cloning and nucleotide sequence of the *Escherischia coli* K-12 *pps*A gene, encoding PEP synthetase. *Mol Gen Genet*, 1992, **231**: 332 – 336

[23] Blattner F R, Plunkett GIII, Bloch C A *et al*. The complete genome sequence of *Escherichia coli* K-12, *Science*, 1997, **277**(5): 1453 – 1474

[24] Sambrook J, Fritsch E F, Maniatis T *et al*. Molecular cloning: a laboratory manual, 2nd ed. The Science Press, 2002

[25] Sprenger G A. Transketolase A of *E. coli* K-12, purification and properties of the enzyme from recombinent strains. *Eur J Biochem*, 1995, **230**: 525 – 532

[26] Spinivasan P R, Sprinson D B. 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate synthetase. *J Biol Chem*, 1959, **234**: 716 – 722

[27] Kholodenko B N, Westerhoff H V, Schwaber J *et al*. Engineering a living cell to desired metabolite concentrations and fluxes: pathways with multifunction enzymes. *Metab Eng*, 2000, **2**(1): 1 – 13

[28] Emmerling M, Bailey J E, Sauer U. Glucose catabolism of *Escherichia coli* strain with increased activities and altered regulation of key glycolytic enzymes. *Metab Eng*, 1999, **1**(2): 117 – 127

[29] Patnaik R, Spitzer R G, Liao J C. Pathway engineering for production of aromatics in *Escherichia coli*: confirmation of stoichiometric analysis by independent modulation of AroG, TktA and PpsA activities. *Biotechnol Bioeng*, 1995, **46**: 361 – 370

[30] Juan CDR, Hernandez ME. Plasmid effects on *Escheichia coli* metabolism. *Curr Rev in Biotech*, 2000, **20**(2): 79 – 108

Co-expressions of Phosphoenolpyruvate Synthetase A(*ppsA*) and Transketolase A (*tktA*) Genes of *Escherichia coli*

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Abstract　Metabolic engineering is the analysis of metabolic pathway and designing rational genetic modification to optimize cellular properties by using principle of molecular biology. Aromatic metabolites such as tryptophan, phenylalanine, and tyrosine are essential amino acids for human and animals. In addition, phenylalanine is used in aspartame production. *Escherichia coli* and many other microoganism synthesize aromatic amino acids through the condensation reaction between phospho-enolpyruvate(PEP) and erythrose-4-phosphate(E4P) to form 3-deoxy-D-arabinoheptulosonate 7-phosphate(DAHP). But many enzymes compete for intracellular PEP, especially the phosphotransferase system which is responsible for glucose transport in *E. coli*. This system uses PEP as a phosphate donor and converts it to pyruvate, which is less likely to recycle back to PEP. To channel more carbon flux into the aromatic pathway, one has to overcome pathways competing for PEP. *pps*A and *tkt*A are the key genes in central metabolism of aromatic amino acids biosynthesis. *pps*A encoding phosphoenolpyrucate synthetase A(PpsA) which catalyzes pyruvate into PEP; *tkt*A encoding transketolase A which plays a major role in erythrose-4-phosphate (E4P) production of pentose pathway. We amplified *pps*A and *tkt*A from *E. coli* K-12 by PCR and constructed recombinant plasmids of them in pBV220 vector containing P_RP_L promoter. Because of each gene carrying P_L promoter, four productions of ligation were obtained. The monoclonal host containing recombinant plasmids was routinely grown in Luria-Bertani(LB) medium added Ampicillin at 37℃ overnight, and then inoculated in LB(Ap^r) medium by 3% ~ 5% in flasks on a rotary shaker at 30℃, induced at 42℃ for 4.5

hours when $OD_{600} \approx 0.4$, cells were obtained by centrifugation at 10000 r/min at 4℃. The results of SDS-PAGE demonstrated that the bands at 84kD and 73kD were more intensive than the same ones of the controls. The specific activity of PpsA in crude extracts was increased by 10.8-fold, and TktA, by 3.9-fold. When both genes were co-expressed in *E. coli*, the activity of PpsA varied from 2.1 ~ 9.1 fold comparing to control, but the activity of TktA was relatively stable(3.9 ~ 4.5 fold). Whatever the two genes were expressed respectively or cooperatively, both could promote the production of DAHP, the first intermediate of the common aromatic pathway, but co-expression was more effective on forming DAHP. The results demonstrate that co-expression of *pps*A and *tkt*A can improve the production of DAHP to near theoretical yield. This report details a different strategy based on co-expression of two genes in one vector *in vivo* to release the burden and paves the way for construction of genetic engineering bacteria for further research.

Key words aromatic amino acids, *pps*A, *tkt*A, metabolic engineering, co-expression

Received: 11-18-2002

This work was supported by Foundation for Scientific and Technological Innovation of Academy of Military Medical Sciences(No.9902502).

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