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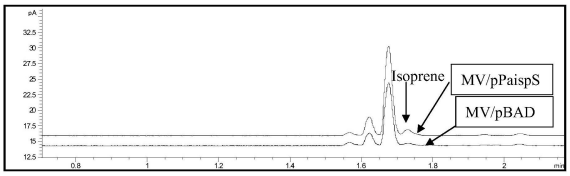
(54)发明名称

五碳平台化合物合成基因及其应用

(57)摘要

本发明涉及一种五碳平台化合物合成基因及其应用,其解决了利用工程菌合成异戊二烯效率不高的技术问题,本发明提供了一种五碳平台化合物合成基因、其表达的蛋白质、含有五碳平台化合物合成基因的原核表达载体及工程菌以及产异戊二烯工程菌的制备方法及应用。本发明可广泛用于异戊二烯制备领域。



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1.一种五碳平台化合物合成基因在制备异戊二烯中的应用,其特征是所述五碳平台化合物合成基因如下(a)或(b)的基因:

(a)所述基因cDNA的核苷酸序列如序列表的序列1所示;

(b)所述基因是编码如下蛋白质的基因:序列表的序列2所示氨基酸序列组成的蛋白质;

使用所述五碳平台化合物合成基因构建产异戊二烯工程菌,使用所述产异戊二烯工程菌制备异戊二烯。

五碳平台化合物合成基因及其应用

技术领域

[0001] 本发明涉及基因工程技术领域，特别涉及一种五碳平台化合物合成基因及其应用。

背景技术

[0002] 自然界中，异戊二烯主要是由某些植物叶片排放至大气中的，而工业生产中，目前异戊二烯主要是由石油裂解物C5馏分萃取蒸馏而来。然而随着石油资源日益枯竭及不可再生，天然植物释放的异戊二烯收集又事倍功半，通过微生物工程菌生产异戊二烯成为一种可持续发展的必然趋势。

[0003] 据报道，每年植物释放到大气中的异戊二烯达到5百万吨，细菌自身又不具有异戊二烯合成酶基因，因此植物是异戊二烯合成酶(ISPS)很好来源。利用基因工程技术开展异戊二烯合成酶基因研究已取得了一些进展，研究人员分离鉴定得到了少量的异戊二烯合成酶基因，但国内尚未有这方面的报道。

[0004] 2000年，Miller B首次在杨树(白杨×颤杨)中获得了全长IspS基因，并且在大肠杆菌中得到了7.7nmol/mgDCW的异戊二烯(Miller B et al.Planta.2001 213(3):483-7)；2005年，Sasaki克隆得到了白杨的IspS基因(Sasaki K et al.FEBS Lett.2005 579(11):2514-8)；Thomas D.Sharkey于2005年克隆得到蒙大拿葛IspS cDNA全长(Sharkey TD et al.Plant Physiol.2005 137(2):700-12.)，随后又扩充了数个杨柳科IspS基因，并且得到了刺槐的IspS cDNA全长序列(Sharkey TD et al,Evolution 2013 67(4):1026-1040)。

[0005] 可见国内外目前获得的异戊二烯合成酶大多限于杨柳科和豆科植物，豆科植物关于五碳平台化合物合成基因的研究主要集中在葛根、刺槐上，杨柳科的相关研究主要集中在杨属，而在禾本科芦苇中并无研究报道，基因库中也没有芦苇五碳平台化合物合成基因。

发明内容

[0006] 本发明就是为了解决利用工程菌合成异戊二烯效率不高的技术问题，提供一种具有较高合成效率的五碳平台化合物合成基因及应用。

[0007] 为达到上述目的，一种五碳平台化合物合成基因，其是如下(a)或(b)的基因：(a)所述基因cDNA的核苷酸序列如序列表的序列1所示；(b)所述基因是编码如下蛋白质的基因：序列表的序列2所示的氨基酸序列组成的蛋白质。

[0008] 本发明同时提供一种五碳平台化合物合成基因表达的蛋白质，其是如下(a)的蛋白质：(a)由序列表的序列2所示的氨基酸序列组成的蛋白质；序列表的序列2所示的氨基酸序列组成的蛋白质是由序列表的序列1所示碱基序列编码。

[0009] 本发明还提供一种五碳平台化合物合成基因的原核表达载体。

[0010] 本发明还提供五碳平台化合物合成基因原核表达载体的产异戊二烯工程菌。

[0011] 本发明同时提供产异戊二烯工程菌在制备异戊二烯中的应用。

[0012] 本发明的有益效果：根据植物在自然界异戊二烯的释放速率，本发明选择了释放

量较高的芦苇五碳平台化合物合成基因进行了分离鉴定和克隆，成功构建异戊二烯生产菌株，为生物法生产异戊二烯寻找到一个高效的异戊二烯合成酶。本发明利用基因工程手段，克隆得到了芦苇基因PaIspS，应用到大肠杆菌中，使用气相色谱进行检测，大肠杆菌具备生产异戊二烯的能力，本发明对于使用微生物进行异戊二烯大规模工业生产提供了一个高效有效的酶。

附图说明

[0013] 图1是芦苇总RNA的琼脂糖电泳结果；

[0014] 图2是芦苇PaIspS基因片段的琼脂糖电泳结果；

[0015] 图3是芦苇PaIspS基因3'-RACE的琼脂糖电泳结果；

[0016] 图4是芦苇PaIspS基因5'-RACE的琼脂糖电泳结果；

[0017] 图5是芦苇PAISPS氨基酸序列BlastP分析结果图；

[0018] 图6是RACE原理图；

[0019] 图7是芦苇PAISPS蛋白在大肠杆菌中表达的SDS-PAGE结果；

[0020] 图8是异戊二烯标准品的气相色谱结果；

[0021] 图9是芦苇PaIspS基因在大肠杆菌中应用的气相检测结果；

[0022] 图10是芦苇PAISPS蛋白经过取代突变之后在大肠杆菌中应用的气相检测结果；

[0023] 图11是芦苇PAISPS蛋白经过添加突变之后在大肠杆菌中应用的气相检测结果；

[0024] 图12是芦苇PAISPS蛋白经过缺失突变之后在大肠杆菌中应用的气相检测结果。

具体实施方式

[0025] 下面结合具体实施方式对本发明进行进一步的详细描述，给出的实施例仅为了阐明本发明，而不是为了限制本发明的范围。下述实施例中的实验方法，如无特殊说明，均为常规方法。下述实施例中所用的材料、试剂等，如无特殊说明，均可从商业途径得到。

[0026] 下述实施例中，大肠杆菌BW25113(Baba T et al.Mol Syst Biol.2006;2:2006.0008.)是一株非病原菌，遗传背景清楚，世代时间短、容易培养且培养基原料低廉。大肠杆菌BW25113公众可从中国科学院微生物研究所获得，以上所述生物材料只为重复本发明的相关实验所用，不可作为其它用途使用。

[0027] 实施例1:基因片段的获得

[0028] 1.提取芦苇叶片总RNA

[0029] 采集芦苇叶片，使用RNeasy Plant Mini Kit(Qiagen公司)提取芦苇叶片总RNA，按照试剂盒说明方法进行，进行电泳(图1)验证RNA提取质量，可见RNA完整性良好，可以进行后续实验。

[0030] 2.RT-PCR

[0031] 以Oligo(dT)$_{20}$做为反转录引物，按照反转录试剂盒SuperScript.III First-Strand Synthesis System for RT-PCR(Invitrogen公司)说明将核酸反转录为cDNA；

[0032] 反应体系如下：

[0033] RNA 1μg

[0034] 10mM dNTP 1μl

[0035] Oligo(dT)20(0.5μg/μl) 1μl

[0036] 65℃5min,置于冰上1min,加入下面的10μl的mix

10×RT buffer	2μl
25mM $MgCl_2$	4μl
0.1M DTT	2μl
RNaseOUT™(40U/μl)	1μl
SuperScript™ III RT	1μl

[0038] 50℃50min,85℃15min加入1μl RNase H,37℃20min

[0039] 反应完毕,加入100μl水稀释cDNA,得到cDNA第一链。

[0040] 3.简并引物设计

[0041] 根据杨柳科及豆科植物的已知氨基酸序列的保守区,并且参考所有已知IspS的核酸序列及单萜合成酶的核酸序列设计的简并引物PAF1及PAR1:

[0042] PAF1:5'TAYAACACNATCAAYGARAT 3'

[0043] PAR1:5'YTGRTANGTGCARTGNGA 3'

[0044] 4.简并PCR反应

[0045] 反应体系

[0046]

10×pyrobest buffer	1.25μl
dNTP(10mM)	0.25μl
cDNA	0.5μl
PAF1(1mM)	0.2μl
PAR1(1mM)	0.2μl
Pyrobest(TAKARA 公司)	0.06μl
H_2O	to 12.5μl
	12.5μl

[0047] 反应条件:

	94℃	4min	
	94℃	30s	
	65℃	30s	-1℃ 10cycles
	72℃	1min	
[0048]	94℃	30s	
	55℃	30s	30cycles
	72℃	1min	
	72℃	3min	
	10℃	hold	

[0049] 扩增出672bp左右的片段(图2),扩增产物于1.5%琼脂糖凝胶上电泳,产物单一明亮。

[0050] 备注:所示明亮单一条带为PAF1和PAR1引物组扩增产物,Marker为TAKARA 100bp DNA Ladder。

[0051] 5.连接T载体,Sanger测序

[0052] 将单一明亮的条带回收,连接PMD18-T(TAKARA公司)载体,转化至trans5α(TransGen公司)感受态细胞,第二天选择白斑进行验证,选取阳性克隆,送至生工Sanger测序。

[0053] 6.测序及序列分析,

[0054] 测序结果氨基酸序列经过Blast,与高粱(Sorghum bicolor)的假想蛋白有达到62%的同源性,与二穗短柄草(Brachypodium distachyon)的月桂烯合成酶有64%的同源性:可以证明此序列为禾本科IspS基因,此片段命名为PaIspS基因片段,序列如SEQ ID No.3所示。

[0055] 核酸序列进行翻译后氨基酸序列如SEQ ID No.4所示。

[0056] 实施例2:PaIspS基因编码区全长的获得

[0057] 获得cDNA全长的方法为SMARTer-RACE,使用SMARTer® PCR cDNA Synthesis Kit(Clontech公司)进行,以下使用的引物及试剂除GSP均为SMARTer® PCR cDNA Synthesis Kit内提供,按照试剂盒说明方法进行。

[0058] 1.RACE-Ready cDNA的制备

[0059] RACE-Ready cDNA第一链的反转录体系如下:

[0060]

	5’-RACE-Ready cDNA	3’-RACE-Ready cDNA
Mix1	2.75 μl RNA 1.0 μl 5’-CDS Primer A 1.0 μl SMARTer IIA oligo	2.75 μl RNA 1.0 μl 3’-CDS Primer A 1.0 μl H_2O
72℃ 3min，降温至42℃，加入下面的5.25μl的Mix2		
Mix2	2.0 μl 5× First-Strand Buffer 1.0 μl DTT (20 mM) 1.0 μl dNTP Mix (10 mM) 0.25 μl RNase Inhibitor (40 U/μl) 1.0 μl SMARTScribe Reverse Transcriptase (100 U)	
42℃ 90 min，70℃ 10 min 反应完毕，加入100μl水稀释cDNA，得到120μl cDNA第一链		

[0061] 2.基因特异性引物的设计：

[0062] 根据已经获得的PaIspS片段的序列设计基因特异性引物(GSP)，RACE-Ready cDNA做模版，GSP及通用引物(Universal Primer Mix，UPM)做引物进行扩增，可以得到3’-RACE cDNA片段及5’-RACE cDNA片段。引物位置如图6所示，中间黑色部分为简并PCR获得序列，两侧黑色部分为通用引物序列，白色部分为需要得到的未知序列部分。

[0063] 共8个GSP序列，如下表：

[0064]

名称	序列
PA3F1	CGCAAGGTGGGATATTAATTCGAGT
PA3F2	GCAAGGCGTTCCTAGTGGAGGCAA
PA3F3	GGGCTACCCAAGTTTAGTTGAATC
PA3F4	GCATGAGAACGGTGCAAGTGAGAAG
PA5R1	GATTCCTTCTCACTTGCACCGTTC
PA5R2	GGGAAAAGCATGGAGCAACAAAAGT
PA5R3	GCATAGGTCATGCCACCCTTTATG
PA5R4	GGCATCAGTGAAGAGTGTAAGTT

[0065] 3.3’-RACE cDNA末端序列的获得

[0066] 使用芦苇的3’-RACE-Ready cDNA为模版，UPM与GSP为引物进行扩增

[0067] 反应体系：

10×pyrobest buffer		1.0μl
dNTP（10mM）		0.2μl
cDNA		0.4μl
GSP		0.2μl
UPM/OligoT		0.2μl
Pyrobest		0.05μl
H_2O	to	10μl
		10μl

[0068]

[0069] 反应条件：

94℃	4min	
94℃	20s	-1℃ 10cycles
62℃	20s	
72℃	60S	

[0070]

94℃	20s	25cycles
52℃	20s	
72℃	60S	
72℃	3min	
10℃	hold	

[0071] 3'-RACE的琼脂糖凝胶检测结果如图(图3)：

[0072] 得到一个单一明亮的芦苇DNA扩增条带，连接T载体，转化感受态细胞，挑选阳性克隆Sanger测序，得到3'端cDNA序列。

[0073] 4.5'-RACE cDNA末端序列的获得

[0074] 使用芦苇的5'-RACE-Ready cDNA为模版，UPM与GSP为引物进行扩增.

[0075] 反应体系：

[0076]

10×pyrobest buffer		1.0μl
dNTP（10mM）		0.2μl
cDNA		0.4μl
GSP		0.2μl
UPM/OligoT		0.2μl
Pyrobest		0.05μl
H_2O	to	10μl
		10μl

[0077] 反应条件：

94℃ 4min

94℃ 20s
62℃ 20s } -1℃ 10cycles
72℃ 60S

[0078]

94℃ 20s
52℃ 20s } 25cycles
72℃ 60S

72℃ 3min

10℃ hold

[0079] 5'-RACE的琼脂糖凝胶检测结果如图(图4)：

[0080] 由图可见得到单一明亮的扩增条带，选择其一连接T载体，转化感受态细胞，挑选阳性克隆Sanger测序，得到5'端cDNA序列。

[0081] 5.全长序列的获得

[0082] 根据3'-RACE及5'-RACE测序结果，进行序列比对，得到该基因cDNA全长序列(SEQ ID No.1所示序列)，对DNA、氨基酸序列进行分析：该基因有1824bp，编码607个氨基酸，有ATG起始密码子和TGA终止密码子，说明该基因的完整性；其编码的氨基酸含有一个IspS高度保守标签序列DDXXD区域，同时也包含了RRX8W保守区域。利用BLAST软件在NCBI中进行同源比对，结果如图5，显示该基因为Isoprenoid_Biosyn_C1superfamily成员，与二穗短柄草(Brachypodium distachyon])Alpha-terpineol synthase(alpha-松油醇合成酶)同源性为58％，与节节麦(Aegilops tauschii)的月桂烯合成酶有56％同源性，与乌拉图小麦(Triticum urartu)的月桂烯合成酶有53％同源性，说明我们得到的是禾本科异戊二烯合成酶基因，简写为PaIspS基因，得到了编码PAISPS蛋白的氨基酸序列(SEQ ID No.2所示序列)。

[0083] 实施例3：大肠杆菌异戊二烯生产菌株的构建

[0084] 全长引物PAFa及PARa序列如下：

[0085] PAFa:5'ATTAACCATGGTGCAGATGACGGCGTCGAAGC 3'

[0086] PARa:5'ATATGGTACCCTAGTTCCGAGGCAGCAAAATGTTT 3'

[0087] 1.大肠杆菌表达载体pBAD-PaIspS构建

[0088] 将使用引物PAFa及PARa获得的PaIspS基因片段进行NcoI和KpnI(TAKARA公司)双酶切,并将pBAD-HisB表达载体(购自Invitrogen公司)进行NcoI和KpnI双酶切,PaIspS基因连接至pBAD-HisB载体后转化至trans5α感受态细胞,选取阳性克隆进行测序,pBAD-PaIspS的核苷酸序列是SEQ ID No.5。

[0089] 2.异戊二烯生产菌株MV/pPaIspS的构建

[0090] 将构建好的pBAD-PaIspS与质粒p1及p2共转化至BW25113宿主得到异戊二烯生产菌株MV/pPaIspS。

[0091] 并且构建对照菌株MV/pBAD,方法为将pBAD-HisB与质粒p1及p2共转至BW25113宿主,得到无异戊二烯合成酶基因的对照菌株MV/pBAD。

[0092] 上述异戊二烯生产菌的构建方法中,p1,p2包含异戊二烯合成途径-甲羟戊酸(MVA)途径基因。其中p1由MvaE(乙酰辅酶A乙酰转移酶)基因、MvaS(HMG-乙酰辅酶A合成酶)基因及MVK(甲羟戊酸激酶)基因组成,所述MvaE基因编码由SEQ ID No.8所示的氨基酸序列组成的蛋白质;所述MvaS基因编码由SEQ ID No.9所示的氨基酸序列组成的蛋白质;所述MVK基因编码由SEQ ID No.10所示的氨基酸序列组成的蛋白质。p2由PMK(磷酸甲羟戊酸激酶)基因、MVD(焦磷酸甲羟戊酸脱羧酶)基因及idi(异戊二烯焦磷酸异构酶)基因组成,所述PMK基因编码由SEQ ID No.11所示的氨基酸序列组成的蛋白质;所述MVD基因编码由SEQ ID No.12所示的氨基酸序列组成的蛋白质;所述idi基因编码由SEQ ID No.13所示的氨基酸序列组成的蛋白质。

[0093] 其中,p1为链霉素抗性阿拉伯糖诱导型表达载体,p1的核苷酸序列是SEQ ID No.6,包含MVA上游途径基因表达盒,MVA上游途径基因表达盒的核苷酸序列是SEQ ID No.6的第1307-5821位,SEQ ID No.6的第89-964位为阿拉伯糖启动子,SEQ ID No.6的第5930-6087位为TrrnB终止子,SEQ ID No.6的第1307-3729位是MvaE基因的编码序列,SEQ ID No.6的第3730-4904位是MvaS基因的编码序列,SEQ ID No.6的第4905-5821位是MVK基因的编码序列。

[0094] P2为氯霉素抗性阿拉伯糖诱导型表达载体,p2的核苷酸序列是SEQ ID No.7,包含MVA下游途径基因表达盒,MVA下游途径基因表达盒的核苷酸序列是SEQ ID No.7的第1309-4442位,SEQ ID No.6的第89-964位为阿拉伯糖启动子,SEQ ID No.6的第4569-4726位为TrrnB终止子,SEQ ID No.6的第1309-2661位是PMK基因的编码序列,SEQ ID No.6的第2677-3864位是MVD基因的编码序列,SEQ ID No.6的第3894-4442位是idi基因的编码序列。

[0095] 实施例4:PaIspS基因在大肠杆菌中的应用

[0096] 1、ISPS蛋白表达

[0097] 上述大肠杆菌工程菌MV/pPaIspS,使用L-arab诱导之后,蛋白表达结果SDS-PAGE如图所示(图7),可见不含异戊二烯合成酶的大肠杆菌宿主本身不表达异戊二烯合成酶ISPS,PaIspS基因的转入使宿主细胞表达ISPS蛋白。

[0098] 备注:图为PAISPS蛋白在大肠杆菌工程菌中表达情况。

[0099] 2、大肠杆菌发酵产物的检测

[0100] 将上述2个菌株进行发酵，方法如下：将工程菌以百分之一的接种量转接到30mL(500mL三角瓶)含有链霉素、氯霉素及氨苄抗性的阿拉伯糖自诱导培养基(ZYM)中，30℃，280rpm培养20h后。4℃，4000rpm离心收集菌液，用含有4％葡萄糖的M9培养基重悬至600D菌体浓度，取1mL重悬菌液置于20mL顶空瓶内，37℃，280rpm震荡培养30h。

[0101] 含有链霉素、氯霉素及氨苄的自诱导培养基自诱导培养基ZYM配方如下：100mL A+2mL B+2mL C+200μL D+100μL E(以下均为质量百分比浓度)；

[0102] A.ZY：1％胰蛋白胨，0.5％酵母粉；

[0103] B.50×M：1.25M Na2HPO4，1.25M KH2PO4，2.5M NH4Cl和0.25M Na2SO4；

[0104] C.50×5052：25％甘油，2.5％葡萄糖，10％乳糖；

[0105] D.1M MgSO4；

[0106] E.1000×微量元素：50Mm FeCl3，20mM CaCl2，10mM MnCl2，10mM ZnSO4，CoCl2、NiCl2、Na2Mo4、Na2SeO3和H3BO3各2mM；

[0107] 链霉素：终浓度50mg/L、氯霉素终浓度34mg/L、氨苄终浓度100mg/L。

[0108] M9培养基配方如分子克隆实验指南(科学出版社)第三版1595页所示。

[0109] 反应结束后，进行气相色谱(GC)分析，使用的气相色谱分析仪为Agilent 7890A GC Sysytem及Agilent7697A headspace Sampler顶空进样器，气相分离柱为HP-5。顶空取样方法如下，Time：GC cycle time 20min，Vial equib time 6min；Temperature(℃)：Oven 51，Loop/Valve 55，Transfer line 60。GC方法如下：流速：2mL/min，0min～4min 50℃，4min～8.5min 50～280℃，8.5min～10.6min 280℃。

[0110] 此方法下异戊二烯标准品(Sigma公司)出峰时间为1.75min(图8)，大肠杆菌工程菌MV/pPaIspS及阴性对照菌株MV/pBAD的GC色谱图(图9)，可见MV/pPaIspS在1.75min的保留峰，而对照没有。可见未加入PaIspS基因的菌株不具备异戊二烯生产能力，转入PaIspS基因后使得大肠杆菌具备了生产异戊二烯的能力，大肠杆菌中产量可达860mg/L。

[0111] 实施例5：经过氨基酸突变的PAISPS蛋白在大肠杆菌中的应用

[0112] 对PAISPS蛋白进行取代、添加和缺失突变，使用实施例3构建的pBAD-PaIspS为模版，按照Fast Mutagenesis System(TransGen公司)试剂盒说明进行突变。

[0113] 1、PAISPS蛋白的氨基酸突变

[0114] 氨基酸的取代突变：将53位氨基酸T突变为A，即将核酸序列157-159位的ACG突变为GCG，使用突变引物为1F和1R；

[0115] 氨基酸的添加突变：53位氨基酸T后面添加一个氨基酸A，即将核酸序列159位之后添加GCG碱基，使用的突变引物为2F和2R；

[0116] 氨基酸的缺失突变：将53位氨基酸T去掉，即将核酸序列157-159位碱基ACG去掉，使用的突变引物为3F和3R。

[0117] 突变引物序列如下：

[0118]

编号	序列
1F	CCGGCAATGCGGGTGCAGATGGCGGCGTCGAAGCAGTTTCAGTCTC
1R	GAGACTGAAACTGCTTCGACGCCGCCATCTGCACCCGCATTGCCGG
2F	CCGGCAATGCGGGTGCAGATGACGGCGGCGTCGAAGCAGTTTCAGTCTC

2R	GAGACTGAAACTGCTTCGACGCCGCCGTCATCTGCACCCGCATTGCCGG
3F	CCGGCAATGCGGGTGCAGATGGCGTCGAAGCAGTTTCAGTCTC
3R	GAGACTGAAACTGCTTCGACGCCATCTGCACCCGCATTGCCGG

[0119] 备注:有下划线碱基为突变碱基

[0120] PCR体系

[0121]

Control Plasmid	1-5 ng
Control Primers	1μl
5×TransStart FastPfu buffer	10 μl
10 mM dNTPs	1μl
TransStart FastPfu DNA Polymerase	1μl
ddH20	to 50 μl

[0122] PCR条件

[0123]

94℃	2-5 min	
94℃	30 sec	
55℃	30 sec	20-25 cycles
72℃	15-30 sec/kb	
72℃	10 min	

[0124] 电泳检测

[0125] 取10μl PCR产物,1%琼脂糖凝胶电泳检测。

[0126] 观察到目的条带大小正确,可用DMT酶消化及转化反应。

[0127] PCR产物的消化

[0128] 加1μl DMT酶于PCR产物中,混匀,37℃孵育1h。

[0129] 转化

[0130] a.加入2-5μl DMT酶消化产物于50μl DMT感受态细胞中(在感受态细胞刚刚解冻时加入产物),轻弹混匀,冰浴30分钟。

[0131] b.42℃准确热激45秒,立即置于冰上2min。

[0132] c.加250μl平衡至室温的SOC,225转,37℃培养1小时。

[0133] d.取200μl菌液铺板,培养过夜(为得到较多的克隆,4000rpm离心1min,弃掉部分上清,保留100-150μl,轻弹悬浮菌体,取全部菌液涂板,培养过夜)

[0134] 用对照质粒模板(4.5Kb)检验突变效率,在含氨苄的平板上涂8μl500mM IPTG,40μl 40mg/mlX-gal,突变成功的菌落呈蓝色。

[0135] 挑选蓝色菌落进行质粒抽提(Plasmid Mini Kit 1,OMEGA公司),Sanger测序。得到正确突变克隆,取代突变株命名为pBAD-PaIspSc1,添加突变株命名为pBAD-PaIspSc2,取代突变株命名为pBAD-PaIspSc3。

[0136] 2、异戊二烯生产菌株MV/pPaIspSc的构建

[0137] 将构建好的pBAD-PaIspSc1与质粒p1及p2共转化至BW25113宿主得到异戊二烯生

产菌株MV/pPaIspSc1；

[0138] 将构建好的pBAD-PaIspSc2与质粒p1及p2共转化至BW25113宿主得到异戊二烯生产菌株MV/pPaIspSc2；

[0139] 将构建好的pBAD-PaIspSc3与质粒p1及p2共转化至BW25113宿主得到异戊二烯生产菌株MV/pPaIspSc3。

[0140] 3、大肠杆菌发酵产物的检测

[0141] 具体检测方法同实施例4所述方法。MV/pPaIspSc1得到的气相检测结果如图10，MV/pPaIspSc2得到的气相检测结果如图11，MV/pPaIspSc3得到的气相检测结果如图12所示，可见MV/pPaIspSc1、MV/pPaIspSc2及MV/pPaIspSc3菌株同样具备生产异戊二烯的能力，产量分别达到480mg/L，360mg/L及260mg/L。

序列表

<110> 山东常青藤生物科技有限公司

<120> 五碳平台化合物合成基因及其应用

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Arg Val Gln Met Thr Ala Ser Lys Gln Phe Gln Ser Pro Glu Arg Arg
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Ser Ala Asn Tyr Gln Pro Asn Ser Trp Asp Tyr Asp Ser Leu Gln Ser
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Leu Arg Gly Gly Arg Ala Ala Gln Asp Met Asp His Pro Tyr Val Phe
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Asp Lys Leu Lys Ala Ser Val Arg Asn Leu Met Ile Asn Lys Pro Glu
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[0003]

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Ser Tyr His Phe Glu Glu Glu Ile Asn Asp Ser Leu Ser Ser Ile Ser
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Arg Pro Ile Tyr Cys Thr Ile Ile Ser Thr Ser Asn Glu Met Ala Glu
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Gly Trp His Asp Leu Cys Lys Ala Phe Leu Val Glu Ala Lys Trp His
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Tyr Gly Asn His Arg Pro Ser Leu Asn Glu Tyr Leu Asn Asn Gly Trp
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Leu Pro Asp Tyr Met Arg Pro Ile Tyr Cys Thr Ile Ile Ser Thr Ser
35 40 45

Asn Glu Met Ala Glu His Val Leu Arg Glu His Gly Leu Ser Thr His
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His Leu Phe His Lys Gly Trp His Asp Leu Cys Lys Ala Phe Leu Val
65 70 75 80

Glu Ala Lys Trp His Tyr Gly Asn His Arg Pro Ser Leu Asn Glu Tyr
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Leu Asn Asn Gly Trp Met Ser Ser Ser Gly Pro Leu Leu Leu Leu His
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[0007]

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Lys Glu Ser Arg Glu Ala Met His Glu His Thr Ile Glu Thr Trp Lys
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[0022]

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<210> 8
<211> 806
<212> PRT
<213> 粪肠球菌（Enterococcus faecalis）

<400> 8

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Ile Gly Lys Tyr Lys Gly Ser Leu Ser Gln Val Ser Ala Val Asp Leu
            20                  25                  30

Gly Thr His Val Thr Thr Gln Leu Leu Lys Arg His Ser Thr Ile Ser
        35                  40                  45

Glu Glu Ile Asp Gln Val Ile Phe Gly Asn Val Leu Gln Ala Gly Asn
    50                  55                  60

Gly Gln Asn Pro Ala Arg Gln Ile Ala Ile Asn Ser Gly Leu Ser His
65                  70                  75                  80

Glu Ile Pro Ala Met Thr Val Asn Glu Val Cys Gly Ser Gly Met Lys
                85                  90                  95
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[0024]

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Ala Val Ile Leu Ala Lys Gln Leu Ile Gln Leu Gly Glu Ala Glu Val
            100                 105                 110

Leu Ile Ala Gly Gly Ile Glu Asn Met Ser Gln Ala Pro Lys Leu Gln
        115                 120                 125

Arg Phe Asn Tyr Glu Thr Glu Ser Tyr Asp Ala Pro Phe Ser Ser Met
    130                 135                 140

Met Tyr Asp Gly Leu Thr Asp Ala Phe Ser Gly Gln Ala Met Gly Leu
145                 150                 155                 160

Thr Ala Glu Asn Val Ala Glu Lys Tyr His Val Thr Arg Glu Glu Gln
                165                 170                 175

Asp Gln Phe Ser Val His Ser Gln Leu Lys Ala Ala Gln Ala Gln Ala
            180                 185                 190
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Glu Gly Ile Phe Ala Asp Glu Ile Ala Pro Leu Glu Val Ser Gly Thr
195 200 205

Leu Val Glu Lys Asp Glu Gly Ile Arg Pro Asn Ser Ser Val Glu Lys
210 215 220

Leu Gly Thr Leu Lys Thr Val Phe Lys Glu Asp Gly Thr Val Thr Ala
225 230 235 240

Gly Asn Ala Ser Thr Ile Asn Asp Gly Ala Ser Ala Leu Ile Ile Ala
245 250 255

Ser Gln Glu Tyr Ala Glu Ala His Gly Leu Pro Tyr Leu Ala Ile Ile
260 265 270

Arg Asp Ser Val Glu Val Gly Ile Asp Pro Ala Tyr Met Gly Ile Ser
275 280 285

[0025]

Pro Ile Lys Ala Ile Gln Lys Leu Leu Ala Arg Asn Gln Leu Thr Thr
290 295 300

Glu Glu Ile Asp Leu Tyr Glu Ile Asn Glu Ala Phe Ala Ala Thr Ser
305 310 315 320

Ile Val Val Gln Arg Glu Leu Ala Leu Pro Glu Glu Lys Val Asn Ile
325 330 335

Tyr Gly Gly Gly Ile Ser Leu Gly His Ala Ile Gly Ala Thr Gly Ala
340 345 350

Arg Leu Leu Thr Ser Leu Ser Tyr Gln Leu Asn Gln Lys Glu Lys Lys
355 360 365

Tyr Gly Val Ala Ser Leu Cys Ile Gly Gly Gly Leu Gly Leu Ala Met
370 375 380

Leu Leu Glu Arg Pro Gln Gln Lys Lys Asn Ser Arg Phe Tyr Gln Met
385 390 395 400

Ser Pro Glu Glu Arg Leu Ala Ser Leu Leu Asn Glu Gly Gln Ile Ser
405 410 415

Ala Asp Thr Lys Lys Glu Phe Glu Asn Thr Ala Leu Ser Ser Gln Ile
420 425 430

Ala Asn His Met Ile Glu Asn Gln Ile Ser Glu Thr Glu Val Pro Met
435 440 445

Gly Val Gly Leu His Leu Thr Val Asp Glu Thr Asp Tyr Leu Val Pro
450 455 460

Met Ala Thr Glu Glu Pro Ser Val Ile Ala Ala Leu Ser Asn Gly Ala
465 470 475 480

[0026]

Lys Ile Ala Gln Gly Phe Lys Thr Val Asn Gln Gln Arg Leu Met Arg
485 490 495

Gly Gln Ile Val Phe Tyr Asp Val Ala Asp Pro Glu Ser Leu Ile Asp
500 505 510

Lys Leu Gln Val Arg Glu Ala Glu Val Phe Gln Gln Ala Glu Leu Ser
515 520 525

Tyr Pro Ser Ile Val Lys Arg Gly Gly Gly Leu Arg Asp Leu Gln Tyr
530 535 540

Arg Thr Phe Asp Glu Ser Phe Val Ser Val Asp Phe Leu Val Asp Val
545 550 555 560

Lys Asp Ala Met Gly Ala Asn Ile Val Asn Ala Met Leu Glu Gly Val

565 570 575

Ala Glu Leu Phe Arg Glu Trp Phe Ala Glu Gln Lys Ile Leu Phe Ser
580 585 590

Ile Leu Ser Asn Tyr Ala Thr Glu Ser Val Val Thr Met Lys Thr Ala
595 600 605

Ile Pro Val Ser Arg Leu Ser Lys Gly Ser Asn Gly Arg Glu Ile Ala
610 615 620

Glu Lys Ile Val Leu Ala Ser Arg Tyr Ala Ser Leu Asp Pro Tyr Arg
625 630 635 640

Ala Val Thr His Asn Lys Gly Ile Met Asn Gly Ile Glu Ala Val Val
645 650 655

[0027]

Leu Ala Thr Gly Asn Asp Thr Arg Ala Val Ser Ala Ser Cys His Ala
660 665 670

Phe Ala Val Lys Glu Gly Arg Tyr Gln Gly Leu Thr Ser Trp Thr Leu
675 680 685

Asp Gly Glu Gln Leu Ile Gly Glu Ile Ser Val Pro Leu Ala Leu Ala
690 695 700

Thr Val Gly Gly Ala Thr Lys Val Leu Pro Lys Ser Gln Ala Ala Ala
705 710 715 720

Asp Leu Leu Ala Val Thr Asp Ala Lys Glu Leu Ser Arg Val Val Ala
725 730 735

Ala Val Gly Leu Ala Gln Asn Leu Ala Ala Leu Arg Ala Leu Val Ser
740 745 750

Glu Gly Ile Gln Lys Gly His Met Ala Leu Gln Ala Arg Ser Leu Ala
755 760 765

Met Thr Val Gly Ala Thr Gly Lys Glu Val Glu Ala Val Ala Gln Gln
770 775 780

Leu Lys Arg Gln Lys Thr Met Asn Gln Asp Arg Ala Met Ala Ile Leu
785 790 795 800

Asn Asp Leu Arg Lys Gln
805

<210> 9
<211> 383
<212> PRT
<213> 粪肠球菌（Enterococcus faecalis）

<400> 9

[0028]

Met Thr Ile Gly Ile Asp Lys Ile Ser Phe Phe Val Pro Pro Tyr Tyr
1 5 10 15

Ile Asp Met Thr Ala Leu Ala Glu Ala Arg Asn Val Asp Pro Gly Lys
20 25 30

Phe His Ile Gly Ile Gly Gln Asp Gln Met Ala Val Asn Pro Ile Ser
35 40 45

Gln Asp Ile Val Thr Phe Ala Ala Asn Ala Ala Glu Ala Ile Leu Thr
50 55 60

Lys Glu Asp Lys Glu Ala Ile Asp Met Val Ile Val Gly Thr Glu Ser
65 70 75 80

Ser Ile Asp Glu Ser Lys Ala Ala Ala Val Val Leu His Arg Leu Met
85 90 95

Gly Ile Gln Pro Phe Ala Arg Ser Phe Glu Ile Lys Glu Ala Cys Tyr
100 105 110

Gly Ala Thr Ala Gly Leu Gln Leu Ala Lys Asn His Val Ala Leu His
115 120 125

Pro Asp Lys Lys Val Leu Val Val Ala Ala Asp Ile Ala Lys Tyr Gly
130 135 140

Leu Asn Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Leu Val Ala Ser Glu Pro Arg Ile Leu Ala Leu Lys Glu Asp Asn Val
165 170 175

Met Leu Thr Gln Asp Ile Tyr Asp Phe Trp Arg Pro Thr Gly His Pro
180 185 190

[0029]

Tyr Pro Met Val Asp Gly Pro Leu Ser Asn Glu Thr Tyr Ile Gln Ser
195 200 205

Phe Ala Gln Val Trp Asp Glu His Lys Lys Arg Thr Gly Leu Asp Phe
210 215 220

Ala Asp Tyr Asp Ala Leu Ala Phe His Ile Pro Tyr Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Leu Ala Lys Ile Ser Asp Gln Thr Glu Ala Glu Gln
245 250 255

Glu Arg Ile Leu Ala Arg Tyr Glu Glu Ser Ile Val Tyr Ser Arg Arg
260 265 270

Val Gly Asn Leu Tyr Thr Gly Ser Leu Tyr Leu Gly Leu Ile Ser Leu
275 280 285

Leu Glu Asn Ala Thr Thr Leu Thr Ala Gly Asn Gln Ile Gly Leu Phe
290 295 300

Ser Tyr Gly Ser Gly Ala Val Ala Glu Phe Phe Thr Gly Glu Leu Val
305 310 315 320

Ala Gly Tyr Gln Asn His Leu Gln Lys Glu Thr His Leu Ala Leu Leu
325 330 335

Asp Asn Arg Thr Glu Leu Ser Ile Ala Glu Tyr Glu Ala Met Phe Ala
340 345 350

Glu Thr Leu Asp Thr Asp Ile Asp Gln Thr Leu Glu Asp Glu Leu Lys
355 360 365

Tyr Ser Ile Ser Ala Ile Asn Asn Thr Val Arg Ser Tyr Arg Asn
370 375 380

[0030]

<210> 10
<211> 301
<212> PRT
<213> 马氏甲烷八叠球菌（Methanosarcina mazei Go1）

<400> 10

Met Val Ser Cys Ser Ala Pro Gly Lys Ile Tyr Leu Phe Gly Glu His
1 5 10 15

Ala Val Val Tyr Gly Glu Thr Ala Ile Ala Cys Ala Val Glu Leu Arg
20 25 30

Thr Arg Val Arg Ala Glu Leu Asn Asp Ser Ile Thr Ile Gln Ser Gln
35 40 45

Ile Gly Arg Thr Gly Leu Asp Phe Glu Lys His Pro Tyr Val Ser Ala
50 55 60

Val Ile Glu Lys Met Arg Lys Ser Ile Pro Ile Asn Gly Val Phe Leu
65 70 75 80

Thr Val Asp Ser Asp Ile Pro Val Gly Ser Gly Leu Gly Ser Ser Ala
85 90 95

Ala Val Thr Ile Ala Ser Ile Gly Ala Leu Asn Glu Leu Phe Gly Phe
100 105 110

Gly Leu Ser Leu Gln Glu Ile Ala Lys Leu Gly His Glu Ile Glu Ile
115 120 125

Lys Val Gln Gly Ala Ala Ser Pro Thr Asp Thr Tyr Val Ser Thr Phe
130 135 140

Gly Gly Val Val Thr Ile Pro Glu Arg Arg Lys Leu Lys Thr Pro Asp
145 150 155 160

[0031]

Cys Gly Ile Val Ile Gly Asp Thr Gly Val Phe Ser Ser Thr Lys Glu
165 170 175

Leu Val Ala Asn Val Arg Gln Leu Arg Glu Ser Tyr Pro Asp Leu Ile
180 185 190

Glu Pro Leu Met Thr Ser Ile Gly Lys Ile Ser Arg Ile Gly Glu Gln
195 200 205

Leu Val Leu Ser Gly Asp Tyr Ala Ser Ile Gly Arg Leu Met Asn Val
210 215 220

Asn Gln Gly Leu Leu Asp Ala Leu Gly Val Asn Ile Leu Glu Leu Ser
225 230 235 240

Gln Leu Ile Tyr Ser Ala Arg Ala Ala Gly Ala Phe Gly Ala Lys Ile
245 250 255

Thr Gly Ala Gly Gly Gly Gly Cys Met Val Ala Leu Thr Ala Pro Glu
260 265 270

Lys Cys Asn Gln Val Ala Glu Ala Val Ala Gly Ala Gly Gly Lys Val
275 280 285

Thr Ile Thr Lys Pro Thr Glu Gln Gly Leu Lys Val Asp
290 295 300

<210> 11
<211> 451
<212> PRT
<213> 酿酒酵母 (Saccharomyces cerevisiae S288c)

<400> 11

Met Ser Glu Leu Arg Ala Phe Ser Ala Pro Gly Lys Ala Leu Leu Ala
1 5 10 15

[0032]

Gly Gly Tyr Leu Val Leu Asp Thr Lys Tyr Glu Ala Phe Val Val Gly
20 25 30

Leu Ser Ala Arg Met His Ala Val Ala His Pro Tyr Gly Ser Leu Gln
35 40 45

Gly Ser Asp Lys Phe Glu Val Arg Val Lys Ser Lys Gln Phe Lys Asp
50 55 60

Gly Glu Trp Leu Tyr His Ile Ser Pro Lys Ser Gly Phe Ile Pro Val
65 70 75 80

Ser Ile Gly Gly Ser Lys Asn Pro Phe Ile Glu Lys Val Ile Ala Asn
85 90 95

Val Phe Ser Tyr Phe Lys Pro Asn Met Asp Asp Tyr Cys Asn Arg Asn
100 105 110

Leu Phe Val Ile Asp Ile Phe Ser Asp Asp Ala Tyr His Ser Gln Glu
115 120 125

Asp Ser Val Thr Glu His Arg Gly Asn Arg Arg Leu Ser Phe His Ser
130 135 140

His Arg Ile Glu Glu Val Pro Lys Thr Gly Leu Gly Ser Ser Ala Gly
145 150 155 160

Leu Val Thr Val Leu Thr Thr Ala Leu Ala Ser Phe Phe Val Ser Asp
165 170 175

Leu Glu Asn Asn Val Asp Lys Tyr Arg Glu Val Ile His Asn Leu Ala
180 185 190

Gln Val Ala His Cys Gln Ala Gln Gly Lys Ile Gly Ser Gly Phe Asp
195 200 205

[0033]

Val Ala Ala Ala Ala Tyr Gly Ser Ile Arg Tyr Arg Arg Phe Pro Pro
210 215 220

Ala Leu Ile Ser Asn Leu Pro Asp Ile Gly Ser Ala Thr Tyr Gly Ser
225 230 235 240

Lys Leu Ala His Leu Val Asp Glu Glu Asp Trp Asn Ile Thr Ile Lys
245 250 255

Ser Asn His Leu Pro Ser Gly Leu Thr Leu Trp Met Gly Asp Ile Lys
260 265 270

Asn Gly Ser Glu Thr Val Lys Leu Val Gln Lys Val Lys Asn Trp Tyr
275 280 285

Asp Ser His Met Pro Glu Ser Leu Lys Ile Tyr Thr Glu Leu Asp His

290 295 300

Ala Asn Ser Arg Phe Met Asp Gly Leu Ser Lys Leu Asp Arg Leu His
305 310 315 320

Glu Thr His Asp Asp Tyr Ser Asp Gln Ile Phe Glu Ser Leu Glu Arg
325 330 335

Asn Asp Cys Thr Cys Gln Lys Tyr Pro Glu Ile Thr Glu Val Arg Asp
340 345 350

Ala Val Ala Thr Ile Arg Arg Ser Phe Arg Lys Ile Thr Lys Glu Ser
355 360 365

Gly Ala Asp Ile Glu Pro Pro Val Gln Thr Ser Leu Leu Asp Asp Cys
370 375 380

[0034] Gln Thr Leu Lys Gly Val Leu Thr Cys Leu Ile Pro Gly Ala Gly Gly
385 390 395 400

Tyr Asp Ala Ile Ala Val Ile Thr Lys Gln Asp Val Asp Leu Arg Ala
405 410 415

Gln Thr Ala Asn Asp Lys Arg Phe Ser Lys Val Gln Trp Leu Asp Val
420 425 430

Thr Gln Ala Asp Trp Gly Val Arg Lys Glu Lys Asp Pro Glu Thr Tyr
435 440 445

Leu Asp Lys
450

<210> 12
<211> 411
<212> PRT
<213> 酿酒酵母（Saccharomyces cerevisiae S288c）

〈400〉 12

Met Thr Val Tyr Thr Ala Ser Val Thr Ala Pro Val Asn Ile Ala Thr
1 5 10 15

Leu Lys Tyr Trp Gly Lys Arg Asp Thr Lys Leu Asn Leu Pro Thr Asn
20 25 30

Ser Ser Ile Ser Val Thr Leu Ser Gln Asp Asp Leu Arg Thr Leu Thr
35 40 45

Ser Ala Ala Thr Ala Pro Glu Phe Glu Arg Asp Thr Leu Trp Leu Asn
50 55 60

Gly Glu Pro His Ser Ile Asp Asn Glu Arg Thr Gln Asn Cys Leu Arg
65 70 75 80

[0035]

Asp Leu Arg Gln Leu Arg Lys Glu Met Glu Ser Lys Asp Ala Ser Leu
85 90 95

Pro Thr Leu Ser Gln Trp Lys Leu His Ile Val Ser Glu Asn Asn Phe
100 105 110

Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ala Gly Phe Ala Ala Leu
115 120 125

Val Ser Ala Ile Ala Lys Leu Tyr Gln Leu Pro Gln Ser Thr Ser Glu
130 135 140

Ile Ser Arg Ile Ala Arg Lys Gly Ser Gly Ser Ala Cys Arg Ser Leu
145 150 155 160

Phe Gly Gly Tyr Val Ala Trp Glu Met Gly Lys Ala Glu Asp Gly His
165 170 175

Asp Ser Met Ala Val Gln Ile Ala Asp Ser Ser Asp Trp Pro Gln Met
180 185 190

Lys Ala Cys Val Leu Val Val Ser Asp Ile Lys Lys Asp Val Ser Ser
195 200 205

Thr Gln Gly Met Gln Leu Thr Val Ala Thr Ser Glu Leu Phe Lys Glu
210 215 220

Arg Ile Glu His Val Val Pro Lys Arg Phe Glu Val Met Arg Lys Ala
225 230 235 240

Ile Val Glu Lys Asp Phe Ala Thr Phe Ala Lys Glu Thr Met Met Asp
245 250 255

Ser Asn Ser Phe His Ala Thr Cys Leu Asp Ser Phe Pro Pro Ile Phe
260 265 270

[0036]

Tyr Met Asn Asp Thr Ser Lys Arg Ile Ile Ser Trp Cys His Thr Ile
275 280 285

Asn Gln Phe Tyr Gly Glu Thr Ile Val Ala Tyr Thr Phe Asp Ala Gly
290 295 300

Pro Asn Ala Val Leu Tyr Tyr Leu Ala Glu Asn Glu Ser Lys Leu Phe
305 310 315 320

Ala Phe Ile Tyr Lys Leu Phe Gly Ser Val Pro Gly Trp Asp Lys Lys
325 330 335

Phe Thr Thr Glu Gln Leu Glu Ala Phe Asn His Gln Phe Glu Ser Ser
340 345 350

Asn Phe Thr Ala Arg Glu Leu Asp Leu Glu Leu Gln Lys Asp Val Ala
355 360 365

Arg Val Ile Leu Thr Gln Val Gly Ser Gly Pro Gln Glu Thr Asn Glu
370 375 380

Ser Leu Ile Asp Ala Lys Thr Gly Leu Pro Lys Glu Leu Gln Arg Arg
385 390 395 400

Ile Thr Ile Cys Lys Arg Asn Thr Ser Phe Tyr
405 410

<210> 13
<211> 182
<212> PRT
<213> 大肠杆菌 (Escherichia coli str. K-12 substr. MG1655)

<400> 13

Met Gln Thr Glu His Val Ile Leu Leu Asn Ala Gln Gly Val Pro Thr
1 5 10 15

[0037]

Gly Thr Leu Glu Lys Tyr Ala Ala His Thr Ala Asp Thr Arg Leu His
20 25 30

Leu Ala Phe Ser Ser Trp Leu Phe Asn Ala Lys Gly Gln Leu Leu Val
35 40 45

Thr Arg Arg Ala Leu Ser Lys Lys Ala Trp Pro Gly Val Trp Thr Asn
50 55 60

Ser Val Cys Gly His Pro Gln Leu Gly Glu Ser Asn Glu Asp Ala Val
65 70 75 80

Ile Arg Arg Cys Arg Tyr Glu Leu Gly Val Glu Ile Thr Pro Pro Glu
85 90 95

Ser Ile Tyr Pro Asp Phe Arg Tyr Arg Ala Thr Asp Pro Ser Gly Ile
100 105 110

Val Glu Asn Glu Val Cys Pro Val Phe Ala Ala Arg Thr Thr Ser Ala
115 120 125

Leu Gln Ile Asn Asp Asp Glu Val Met Asp Tyr Gln Trp Cys Asp Leu
130 135 140

[0038]

Ala Asp Val Leu His Gly Ile Asp Ala Thr Pro Trp Ala Phe Ser Pro
145 150 155 160

Trp Met Val Met Gln Ala Thr Asn Arg Glu Ala Arg Lys Arg Leu Ser
165 170 175

Ala Phe Thr Gln Leu Lys
180

图1

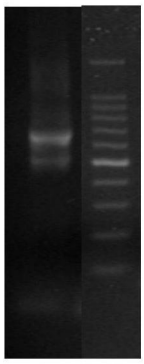


图2

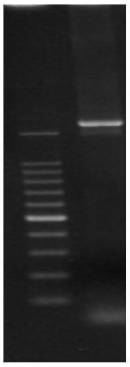


图3

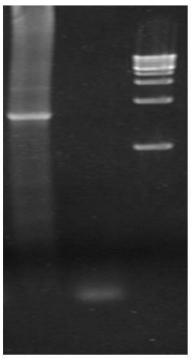
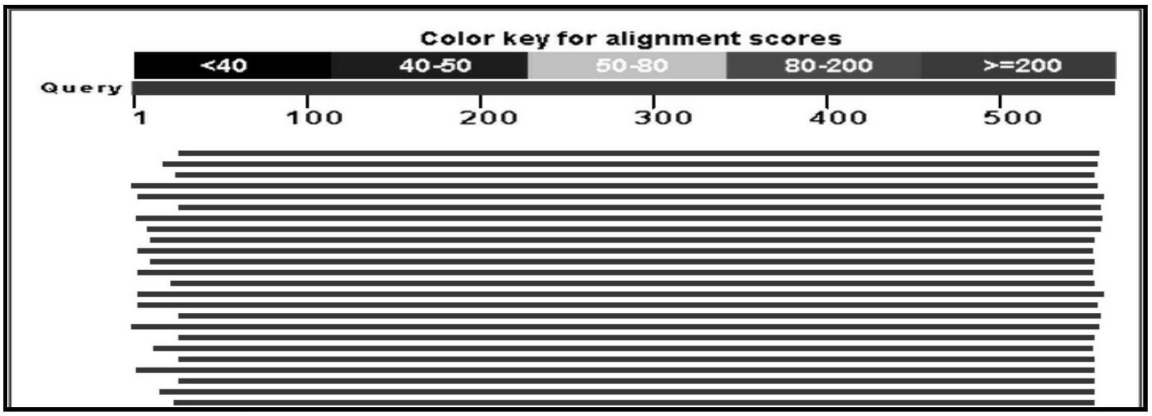


图4



Alignments Download GenPept Graphics Distance tree of results Multiple alignment

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	hypothetical protein SORBIDRAFT_01g039090 [Sorghum bicolor]	652	652	93%	0.0	58%	XP_002468073.1
☐	hypothetical protein SORBIDRAFT_06g002820 [Sorghum bicolor]	638	638	95%	0.0	56%	XP_002447528.1
☐	PREDICTED: alpha-terpineol synthase, chloroplastic-like [Brachypodium distachyon]	629	629	93%	0.0	58%	XP_003580946.1
☐	hypothetical protein [Zea mays]	627	627	98%	0.0	54%	NP_001183298.1
☐	predicted protein [Hordeum vulgare subsp. vulgare]	602	602	98%	0.0	54%	BAJ98202.1
☐	Myrcene synthase, chloroplastic [Aegilops tauschii]	578	578	93%	0.0	56%	EMT09332.1
☐	Myrcene synthase, chloroplastic [Triticum urartu]	558	558	98%	0.0	53%	EMS46876.1
☐	Myrcene synthase, chloroplastic [Triticum urartu]	533	533	97%	0.0	52%	EMS48565.1

图5

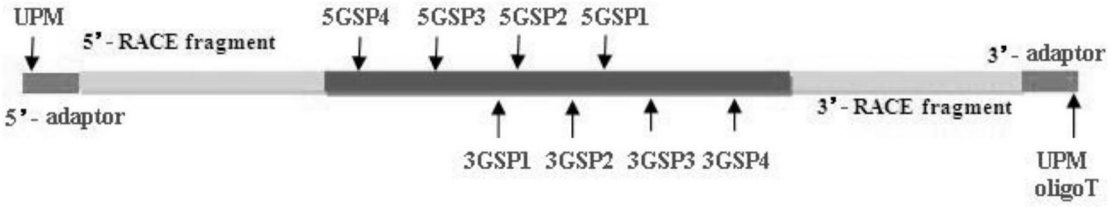

UPM
5'-RACE fragment
5GSP4
5GSP3
5GSP2
5GSP1
3'-adaptor
5'-adaptor
3'-RACE fragment
3GSP1
3GSP2
3GSP3
3GSP4
UPM
oligoT

图6

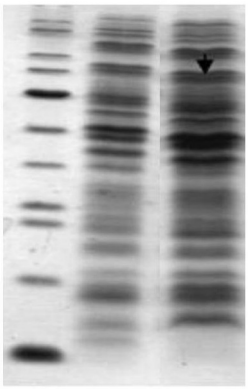


图7

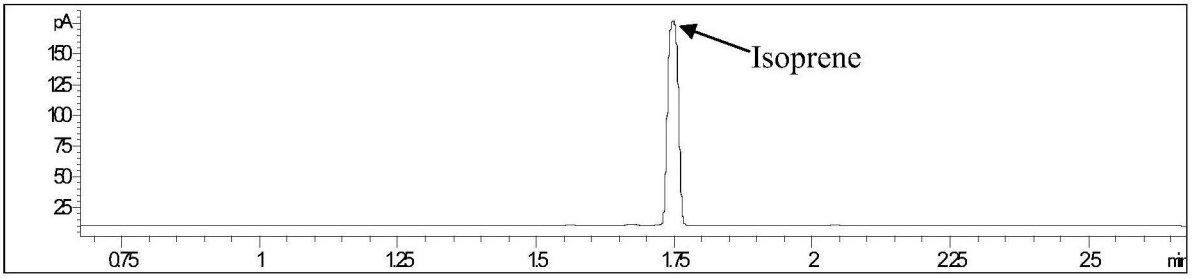

pA
150
125
100
75
50
25
Isoprene
0.75
1
1.25
1.5
1.75
2
2.25
2.5
min

图8

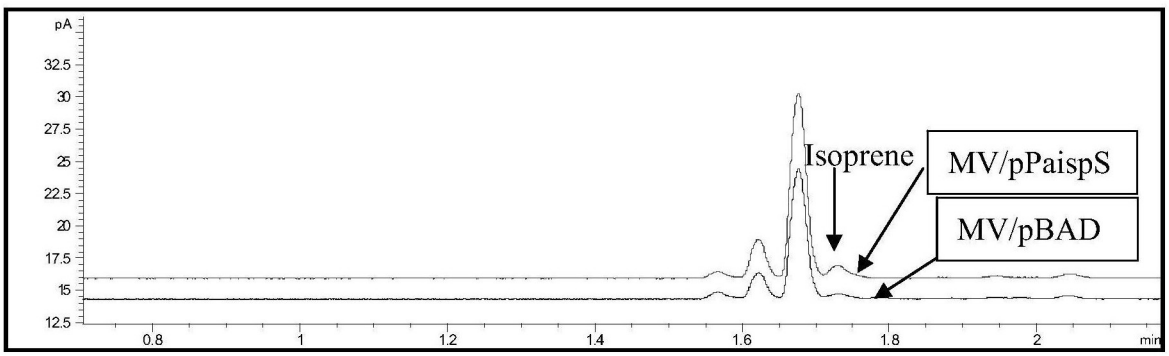

pA
32.5
30
27.5
25
22.5
20
17.5
15
12.5
Isoprene
MV/pPaispS
MV/pBAD
0.8
1
1.2
1.4
1.6
1.8
2
min

图9

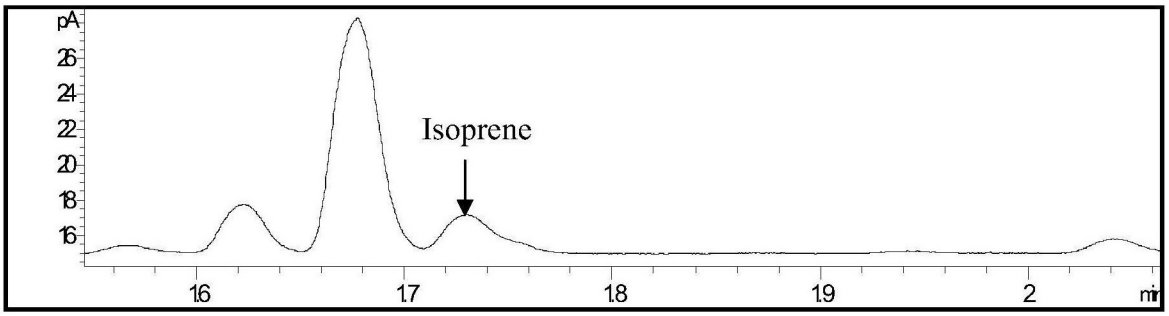

pA
26
24
22
20
18
16
Isoprene
1.6
1.7
1.8
1.9
2
min

图10

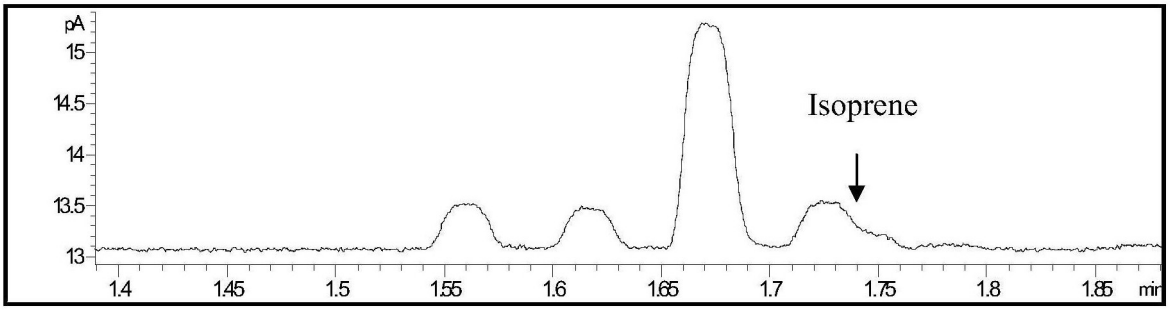

pA
15
14.5
14
13.5
13
Isoprene
1.4
1.45
1.5
1.55
1.6
1.65
1.7
1.75
1.8
1.85
min

图11

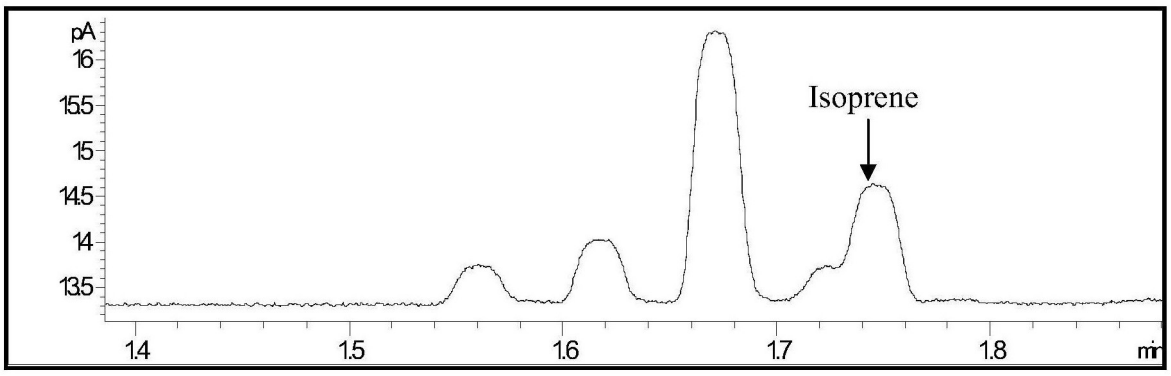

pA
16
15.5
15
14.5
14
13.5
Isoprene
1.4
1.5
1.6
1.7
1.8
min

图12