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CN 106148209 A,2016.11.23
CN 106148208 A,2016.11.23
CN 109536398 A,2019.03.29
JingenLi等.Direct production of commodity chemicals from lignocellulose using Myceliophthora thermophila.《Metab Eng》.2019,第416-426页.

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(54)发明名称

一种提高丝状真菌有机酸合成能力的方法

(57)摘要

本发明提供了一种提高丝状真菌重组菌的有机酸合成能力的方法,及其得到和重组菌和生产有机酸的方法。本发明的重组菌株导入有机酸合成正调控基因,和/或下调表达了有机酸合成负调控基因,且所述的重组菌株与其出发菌株相比,有机酸生产能力显著提高。实验证实,经过导入一种或多种所述正调控基因和/或下调所述负调控基因的遗传改造工程菌株,能够有效地利用单糖、多糖、聚糖或混合糖,同时固定$CO_2$来高效合成有机酸。

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1.一种提高丝状真菌重组菌的有机酸合成能力的方法,其特征在于,通过基因工程的方法在产二元有机酸的丝状真菌中导入有机酸合成正调控基因中的5-磷酸核糖激酶以及1,5-双磷酸核糖羧化/加氧酶;所述的二元有机酸选自苹果酸和/或琥珀酸;

所述丝状真菌选自嗜热毁丝霉(*Myceliophthorathermophila*)苹果酸发酵菌株JG207。

2.如权利要求1所述的方法,其特征在于,同时还下调有机酸合成负调控基因,所述有机酸合成负调控基因选自乳酸脱氢酶、丙酮酸脱羧酶、或丙酮酸羧激酶的一种或多种。

3.如权利要求1所述的方法,其特征在于,同时还导入外源性有机酸合成正调控基因糖转运蛋白。

4.如权利要求1至3任一项所述的方法,其特征在于,所述重组菌株与其出发菌株相比,有机酸生产能力增强或提高了至少10%。

5.如权利要求1至3任一项所述的方法,其特征在于,所述5-磷酸核糖激酶的氨基酸序列如SEQ ID NO.3所示、所述1,5-双磷酸核糖羧化/加氧酶的氨基酸序列如SEQ ID NO.1所示、糖转运蛋白的氨基酸序列如SEQ ID NO.56所示;所述的乳酸脱氢酶的氨基酸序列如SEQ ID NO.15所示、丙酮酸脱羧酶的氨基酸序列如SEQ ID NO.13所示、丙酮酸羧激酶的氨基酸序列如SEQ ID NO.17所示。

6.如权利要求1至3任一项所述的方法,其特征在于,所述导入有机酸合成正调控基因的方法是通过导入含有所述正调控基因的表达载体实现。

7.如权利要求2至3任一项所述的方法,其特征在于,所述下调有机酸合成负调控基因是通过基因敲除或基因编辑或抑制剂来实现,所述抑制剂选自所述抗体、抑制性mRNA、反义RNA、microRNA、miRNA、siRNA、shRNA或者活性抑制剂。

8.如权利要求7所述的方法,其特征在于,所述下调有机酸合成负调控基因表达水平是指通过基于CRISPR/Cas9的基因组编辑方法实现。

9.根据权利要求1至8任一项所述的方法制备得到的重组菌。

10.利用权利要求9所述的重组菌生产有机酸的方法,其特征在于,利用所述的重组菌以单糖、聚糖和/或植物生物质为底物发酵生产有机酸。

11.如权利要求10所述的方法,其特征在于,所述的单糖,选自葡萄糖、木糖、阿拉伯糖,或其组合;所述的聚糖,选自晶纤维素、半纤维素,或其组合;所述的植物生物质,选自农作物秸秆、林业废弃物、能源植物或其部分或全部分解产物;其中,所述农作物秸秆选自玉米秸秆,小麦秸秆,水稻秸秆,高粱秸秆,大豆秸秆,棉花秸秆,甘蔗渣,玉米芯;所述林业废弃物选自枝叶,锯末;所述能源植物选自甜高粱,柳枝稷,芒草,芦苇或其组合。

# 一种提高丝状真菌有机酸合成能力的方法

## 技术领域

[0001] 本发明属于基因工程和生物技术领域。具体地，本发明涉及一种提高丝状真菌有机酸合成能力的方法，及其得到和重组菌和生产有机酸的方法。

## 背景技术

[0002] 木质纤维素是自然界中一种丰富的可再生资源，具有成本低、分布广、容易获得、存储量大等优点。全世界每年由光合作用产生的木质纤维素含量高达1550亿吨，被利用的仅为2％。我国的纤维素原料也十分丰富，每年产生的农业废弃生物质资源就高达约7亿吨。木质纤维素的利用受到木质纤维素水解酶(纤维素酶、半纤维素酶等)生产成本过高的限制，已成为制约整个生物炼制产业发展的核心问题。在自然界中，多种微生物都具有降解并快速利用纤维素的能力，特别是子囊菌和担子菌等丝状真菌可以分泌多种木质纤维素水解酶，与其它微生物相比，具有完整的纤维素酶系。通过代谢途径设计与改造，构建一株可以直接利用生物质为原料进行发酵生产大宗有机酸的底盘菌株，可以有效的降低生物质冶炼的成本，具有很广阔的应用前景。

[0003] 另外，$CO_2$是产生“温室效应”主要气体，同时$CO_2$又是地球上最丰富的碳资源之一。利用合成生物学使能技术，改善微生物代谢，实现生物法固定$CO_2$合成大宗化学品，对于解决环境问题和能源危机都具有重要意义。目前，在生物界中发现了6种天然固定$CO_2$途径，其中，卡尔文循环(Calvin-Benson-Bassham cycle，CBB cycle)是生物圈主要的$CO_2$固定途径，据估计每年通过卡尔文循环所固定的$CO_2$达到$5\times10^{14}$千克。在CBB循环中1,5-二磷酸核酮糖羧化酶/加氧酶(Ribulose-1,5-Bisphosphosphate Carboxylase/Oxygenase，RubisCO)是碳同化作用的关键酶。RuBisCO在自然界中含量丰富，能够催化$CO_2$和二磷酸核酮糖(RuBP)转变成两个分子的3-磷酸甘油酸。II-型的RuBisCO由2-8个相同大亚基组成，其异源表达所涉及的基因较少，受到了较多的关注。来源于光能和化能自养细菌(如Rhodospirillum rubrum，Thiobacillus denitrificans)的中II-型RuBisCO已经在大肠杆菌和酿酒酵母中功能表达，用于回收碳代谢所释放的$CO_2$，以提高目标产物的产率。在异养型传统发酵菌种中构建CBB循环对于利用大气中$CO_2$合成大宗化学品和生物燃料具有意义。

[0004] 嗜热毁丝霉是天然纤维素降解高温丝状真菌，是耐高温纤维素酶的优良生产者，天然具有分泌大量生物质降解酶类的能力。嗜热毁丝霉最适生长温度为45-48℃，与纤维素酶最佳酶活温度50℃非常接近，具有非常好的纤维素降解能力，并能充分利用降解产生的纤维二糖、葡萄糖和木糖等多种糖类，因而可以利用生物质为原料进行发酵。同时，嗜热毁丝霉经过一定的代谢工程改造，可以用于发酵生产大宗化学品，像是苹果酸、富马酸等，已被证实可以用来大规模工业发酵。

[0005] 生物质的主要组成单元包括木糖、阿拉伯糖和葡萄糖等。在工业发酵过程中，发酵菌株对三种单糖的快速利用是实现生物质高效率转化的关键。在细胞中，五碳糖(木糖和阿拉伯糖)通过磷酸戊糖途径代谢过程中的中间产物5-磷酸核糖，为CBB循环中5-磷酸核糖激酶(phosphoribulokinase，PRK)的底物，所以在生物质利用中，五碳糖可以作为CBB途径固

定$CO_2$的驱动底物。但目前尚未报道在丝状真菌中导入CBB途径以提高生物质碳源利用，使其能够以生物质和$CO_2$为碳源，进而加强有机酸的合成。

## 发明内容

[0006] 经过广泛而深入的研究，本发明人发现通过基因工程的方法在丝状真菌中导入有机酸合成正调控基因，或/和下调机酸合成负调控基因，所述的重组菌株与其出发菌株相比，二元有机酸生产能力显著提高。经验证，所述的菌株能够显著提高有机酸工程菌株的底物消耗速率，$CO_2$固定效率及其有机酸的生产效率。

[0007] 为达此目的，本发明采用以下技术方案。

[0008] 本发明首先提供一种提高丝状真菌重组菌的有机酸合成能力的方法，其通过基因工程的方法在丝状真菌中导入有机酸合成正调控基因，或/和下调机酸合成负调控基因，所述的重组菌株与其出发菌株相比，二元有机酸生产能力显著提高；其中，所述的有机酸包括苹果酸、琥珀酸、富马酸、草酰乙酸，优选的为苹果酸和/琥珀酸。

[0009] 在具体的实施方式中，所述有机酸合成正调控基因选自5-磷酸核糖激酶、1,5-双磷酸核糖羧化/加氧酶、糖转运蛋白的一种或多种；所述下调机酸合成负调控基因选自乳酸脱氢酶、丙酮酸脱羧酶、或丙酮酸羧激酶的一种或多种。优选地，同时导入外源性有机酸合成正调控基因之一或多种和下调所述负调控基因的一种或多种的表达。

[0010] 具体地，所述丝状真菌细胞，选自脉孢菌(Neurospora)、曲霉(Aspergillus)、木霉(Trichoderma)、青霉(Penicillium)、毁丝霉(Myceliophthora)、侧孢霉(Sporotrichum)、镰孢菌(Fusarium)、根霉(Rhizopus)、毛霉(Mucor)和拟青霉(Paecilomyces)，更优选地，所述毁丝霉菌选自嗜热毁丝霉(Myceliophthora thermophila)、异梭毁丝霉(Myceliophthora heterothallica)。

[0011] 其中，所述重组菌株与其出发菌株相比，有机酸生产能力增强或提高了至少10％；较佳地至少10-50％；更佳地，至少50％-500％。

[0012] 在一个具体实施方式中，所述5-磷酸核糖激酶的氨基酸序列如SEQ ID NO.3所示、所述1,5-双磷酸核糖羧化/加氧酶的氨基酸序列如SEQ ID NO.1所示、糖转运蛋白的氨基酸序列如SEQ ID NO.56所示；所述的乳酸脱氢酶的氨基酸序列如SEQ ID NO.15所示、丙酮酸脱羧酶的氨基酸序列如SEQ ID NO.13所示、丙酮酸羧激酶的氨基酸序列如SEQ ID NO.17所示。

[0013] 在具体实施方式中，所述导入有机酸合成正调控基因的方法是通过导入含有所述正调控基因的表达载体实现；所述下调机酸合成负调控基因是通过基因敲除或基因编辑或抑制剂来实现，所述抑制剂选自所述抗体、抑制性mRNA、反义RNA、microRNA、miRNA、siRNA、shRNA或者活性抑制剂；优选地，下调负调控基因表达水平是指通过基于CRISPR/Cas9的基因组编辑方法实现。

[0014] 本发明还提供根据上述方法制备得到的重组菌。

[0015] 进一步地，本发明提供利用所述的重组菌生产有机酸的方法，其特征在于，利用所述的重组菌以单糖、聚糖和/或植物生物质为底物发酵生产有机酸。

[0016] 在具体实施广度中，所述的单糖，选自葡萄糖、木糖、阿拉伯糖，或其组合；所述的聚糖，包结晶纤维素、半纤维素，或其组合；所述的植物生物质，选自农作物秸秆、林业废弃

物、能源植物或其部分或全部分解产物;其中,所述农作物秸秆包括玉米秸秆,小麦秸秆,水稻秸秆,高粱秸秆,大豆秸秆,棉花秸秆,甘蔗渣,玉米芯;所述林业废弃物包括枝叶,锯末;所述能源植物包括甜高粱,柳枝稷,芒草,芦苇或其组合。

[0017] 优选地,所述重组菌是毁丝霉菌,更优选为嗜热毁丝霉、异梭毁丝霉;所述有机酸是指苹果酸和/或琥珀酸。

[0018] 本发明的重组菌株导入有机酸合成正调控基因,和/或下调表达了有机酸合成负调控基因,且所述的重组菌株与其出发菌株相比,有机酸生产能力显著提高。发明人通过实验证实,经过导入一种或多种所述正调控基因和/或下调所述负调控基因的遗传改造工程菌株,能够有效地利用单糖、多糖、聚糖或混合糖,同时固定$CO_2$来高效合成有机酸。

## 附图说明

[0019] 图1嗜热毁丝霉菌株CP-1表型分析。(A)重组菌株CP-1中RuBisCO酶活测定;(B)有机酸产量分析;(C)菌株CP-1在木糖和阿拉伯糖条件下生物量分析。

[0020] 图2嗜热毁丝霉菌株CP-51固碳效率测定。

[0021] 图3嗜热毁丝霉菌株CP-1发酵性状分析。(A),(C)和(D)分别显示菌株CP-51在木糖,阿拉伯糖和葡萄糖条件下的苹果酸产量;(B)菌株CP-1在木糖条件下生物量分析

[0022] 图4嗜热毁丝霉菌株Gal-1对底物转运效率分析。

[0023] 图5嗜热毁丝霉菌株Gal-1在单糖或混合糖条件下底物利用效率。

[0024] 图6嗜热毁丝霉菌株Gal-1以葡萄糖,木糖和阿拉伯糖所组成的混合糖为碳源时的有机酸产量。

[0025] 图7嗜热毁丝霉菌株Gal-1在多聚糖(木聚糖,结晶纤维素和玉米芯渣)条件下的苹果酸产量。

[0026] 图8嗜热毁丝霉菌株Gal-1在多聚糖(木聚糖,结晶纤维素和玉米芯渣)条件下的琥珀酸产量。

## 具体实施方式

[0027] 为更进一步阐述本发明所采取的技术手段及其效果,以下结合本发明的优选实施例来进一步说明本发明的技术方案,应理解,这些实施例仅用于说明本发明而不用于限制本发明的范围。

[0028] 下述实施例中所用方法如无特别说明均为常规方法,例如Sambrook等人所著的《分子克隆:实验室手册》(New York:Cold SpringHarbor Laboratory Press,1989)中所述的条件,

[0029] 实施例中未注明具体技术或条件者,按照本领域内的文献所描述的技术或条件,或者按照产品说明书进行。所用试剂或仪器未注明生产厂商者,均为可通过正规渠道商购获得的常规产品。其中,所出现的百分比浓度如无特别说明均为质量百分浓度。所用引物和核酸测序均由由苏州金唯智生物科技有限公司GENEWIZ完成。其中,“MYCTH_……”为嗜热毁丝霉的基因位点编号。

[0030] 下面结合具体实施例来进一步描述本发明,本发明的优点和特点将会随着描述而更为清楚。但是应理解所述实施例仅是范例性的,不对本发明的范围构成任何限制。本领域

技术人员应该理解的是,在不偏离本发明的精神和范围下可以对本发明技术方案的细节和形式进行修改或替换,但这些修改或替换均落入本发明的保护范围。

[0031] 实施例1构建有机酸能力显著增强的嗜热毁丝霉重组菌株

[0032] 本实施例主要以前期所获得嗜热毁丝霉苹果酸发酵菌株JG207为出发菌株(Li J,et al.Direct production of commodity chemicals from lignocellulose using Myceliophthora thermophila.Metabolic engineering 2019.DOI:10.1016/j.ymben),导入CBB循坏(Calvin-Benson-Basshamcycle)中的关键酶1,5-双磷酸核糖羧化/加氧酶(Ribulose-1,5-bisphosphate(RuBP)carboxylase-oxygenase,RuBisCO)和5-磷酸核糖激酶(phosphoribulokinase,PRK),以加强苹果酸发酵菌种戊糖利用能力,同时固定$CO_2$,以提高产物产率。

[0033] 1.RuBisCO及PRK表达载体构建

[0034] 以质粒pAN52-bar(Gu SY,Li JG,Chen BC,Sun T,Liu Q,Xiao DG,Tian CG.Metabolic engineering of the thermophilic filamentous fungus Myceliophthora thermophila to produce fumaric acid.Biotechnology for Biofuels.2018,11:323.)为骨架构建分别构建prk和cbbM(RuBisCO)的表达载体,所选的组成型强启动子分别为嗜热毁丝霉丙酮酸脱酸酶基因pdc(Mycth_112121)的启动子Ppdc和甘油醛-3-磷酸脱氢酶编码基因gpdA(Mycth_2311855)启动子pgpdA。

[0035] RuBisCO氨基酸序列如SEQ ID No.1所示;RuBisCO编码核苷酸序列(cbbM)如SEQ ID No.2所示;PRK氨基酸序列如SEQ ID No.3所示;PRK编码核苷酸序列(cbbM)如SEQ ID No.4所示;启动子Ppdc核苷酸序列如SEQ ID No.5所示;启动子pgpdA核苷酸序列如SEQ ID No.6所示。

[0036] 经PCR扩增后,获得所需的DNA片段,所用引物如表1所示,采用Gibson Assembly技术体系对上述多个PCR片段进行快速组装到由限制性内切酶Bgl II和BamH I双酶切的骨架质粒pAN52-bar上,从而构建prk和cbbM重组表达载体pAN52-prk和pAN52-cbbM,

[0037] PCR反应体系为:5×phusion HF buffer 10μL,10mM dNTPs 1μL,10mM引物-F和引物-R各2.0μL,模板DNA 1μL,Phusion DNA polymerase 0.5μL,水33.5μL。PCR反应条件为:先98℃ 30s;然后98℃ 10s,65℃ 30s,72℃ 1.5min,35个循环;最后72℃ 10min,4℃ 10min。

[0038] 2.CBB关键基因prk和cbbm在嗜热毁丝霉中重组表达

[0039] 将10μg由限制性内切酶Hind III线型化重组表达载体pAN52-prk和pAN52-cbbM同时转化导入嗜热毁丝霉菌株JG207原生质体细胞后中,通过在平板中加入草丁膦(PPT)筛选出转化子,具体步骤如下:

[0040] A.嗜热毁丝霉菌株的培养

[0041] 将嗜热毁丝霉苹果酸菌株JG207在MM培养基上45℃培养10天后待用。

[0042] MM培养基:50×Vogel's盐20mL,蔗糖20g,琼脂15g,定容体积到1L,高压灭菌。

[0043] 所用试剂配方:

[0044] 50×Vogel's盐(1L):柠檬酸三钠(1/2$H_2O$)150g,无水$KH_2PO_4$ 250g,无水$NH_4NO_3$100g,$MgSO_4 \cdot 7H_2O$ 10g,$CaCl_2 \cdot 2H_2O$ 5g,微量元素盐溶液5mL,生物素(0.1mg/mL)2.5mL,定容体积到1L。

[0045] 微量元素盐溶液:$C_6H_8O \cdot 7H_2$ 0.5g/L,$ZnSO_4 \cdot 7H_2O$ 0.5g/L,$Fe(NH_4)_2(SO_4) \cdot 6H_2O$ 0.1g/L,$CuSO_4 \cdot 5H_2O$ 0.025g/L,$MnSO_4 \cdot H_2O$ 0.005g/L,$H_3BO_3$ 0.005g/L,$NaMoO_4 \cdot 2H_2O$ 0.005g/L

[0046] B.嗜热毁丝霉原生质体转化

[0047] a.菌丝体准备:将成熟的毁丝霉孢子,用0.05%吐温-80灭菌水收集,经擦镜纸过滤出去菌丝后,涂布于铺有玻璃纸的MM平板,45℃培养16h。

[0048] b.原生质体制备:将带有菌丝的玻璃纸放置于30mL裂解液(配方:0.15g裂解酶,无菌操作加入30mL溶液A,过滤除菌;溶液A:1.0361g磷酸二氢钾,21.864g山梨醇,溶于90mL去离子水,氢氧化钾调pH到5.6,定量至100mL,高温灭菌)中,30℃裂解2h,每隔20min轻轻摇动。而后经过玻璃纸过滤后,2000rpm 4℃离心10min,弃上清,加入4mL溶液B(0.735g氯化钙,18.22g山梨醇,1mL Tris-HCl 1M pH 7.5,溶于90mL去离子水,盐酸调pH到7.6,定量至100mL,高温灭菌),2000rpm 4℃离心10min;弃上清,按200μL/质粒加入一定体积溶液B。

[0049] c.原生质体转化:预冷的15mL离心管,依次加入50μL预冷PEG(12.5g PEG 6000,0.368g氯化钙,500μL Tris HCl 1M pH 7.5),将转化的DNA片段加入200μL原生质体。放置冰上20min后加入2mL预冷PEG,室温5min,加入4mL溶液B,轻轻混匀。取3mL上述溶液加入12mL融化的含相应抗生素MM培养基中,置于平板中,35℃培养,3d后于挑取单个菌丝体于相应抗性平板生长。

[0050] C.嗜热毁丝霉转化子验证

[0051] a.基因组提取:

[0052] 采用酚氯仿法从上述转化中挑选的转化子提取基因组DNA,具体包括以下操作:

[0053] 1)在2.0mL无菌的DNA提取管中加入200mg的锆珠及1mL的裂解液(lysis buffer,配方:0.2M Tris·HCl(pH7.5),0.5M NaCl,10mM EDTA,1%SDS(w/v)),挑取平板中生长的嗜热毁丝霉菌丝于DNA提取管中;

[0054] 2)将所有DNA提取管置于助磨器上,最大转速振荡30s,重复两次;

[0055] 3)65℃水浴30分,在水浴过程中每个几分钟取出漩涡振荡;

[0056] 4)水浴结束后取出,每管加入80μL pH 7.5的1M的Tris·HCl中和;

[0057] 5)加入400μL的酚:氯仿(1:1),13000rpm离心5分钟;

[0058] 6)取300μL上清液于新的1.5mL EP管中,加入600μL 95%的乙醇(DNA级);

[0059] 7)冰上孵育一小时,随后4℃、13000rpm离心,可看到白色的DNA沉淀到EP管底部;

[0060] 8)用75%的酒精(DNA级)400μL清洗,4℃13000 rpm离心,轻轻取出上清液;

[0061] 9)将EP管置于真空浓缩仪中,真空干燥酒精;

[0062] 10)加入50μL $ddH_2O$溶解DNA,用NanoDrop测DNA浓度,测完浓度后将提取的DNA置于-20℃冰箱保存,以备下一步进行PCR验证。

[0063] b.PCR验证嗜热毁丝霉转化子:

[0064] 以提取的基因组DNA为模版,分别用引物Ppdc_prk-F/OEprk-R和OEcbbM-F/R验证(表1)对转化子进行基因PCR验证。

[0065] PCR反应体系为:5×phusion GC buffer 4μL,10mM dNTPs 0.2μL,引物各1μL,基因组1μL,DMSO 0.6μL,Phusion DNA polymerase 0.1μL,水12.1μL。PCR反应条件为:先98℃ 30s;然后98℃ 10s,62℃ 30s,72℃ 1.5min,30个循环;最后72℃ 10min,4℃10min。

[0066] 对PCR扩增产物进行1％琼脂糖凝胶电泳(120V电压,30分钟),在凝胶成像系统下看基因扩增条带,结果表明prk和cbbM都成功导入嗜热毁丝霉苹果酸发酵菌株JG207基因组中,进而得到重组菌株CP-1。

[0067] 表1本实施例中载体构建所用引物

[0068]

| SEQ ID NO. | 引物 | 序列（5'-3'） |
|---|---|---|
| 7 | Ppdc_prk-F | TTTGCAGTTGGCTGACTTGAAGTAATCTCTGCACATTCGATATGCAACCCGATC |
| 8 | Ppdc_prk-R | TCGTGGGGATGGTGTACACGGTGCAGACCGCCATGTCTGTTGTTGGCGATGTTTG |
| 9 | OEprk-F | CTTGTGACCACAAACATCGCCAACAACAGACATGGCGGTCTGCACCGTGTAC |

[0069]

| | | |
|---|---|---|
| 10 | OEprk-R: | GTTTGATGATTTCAGTAACGTTAAGTGGATCTCAGGCCTTGGCGGCGGTGGC |
| 11 | OEcbbM-F | TCACTCACCTCCCCACATCACAGAAATCAAAAATGGACCAGTCGAGCCGGTAC |
| 12 | OEcbbM-R | CTGTTTGATGATTTCAGTAACGTTAAGTGGATCTCAGGCCGGCAGCGCCGAGCG |

[0070] 实施例2嗜热毁丝霉重组菌株CP-1表型分析

[0071] 1.重组菌株CP-1中RuBisCO酶活测定

[0072] 将重组菌株CP-1菌丝培养后,用液氮冷冻并研磨,而后加入100mM Tris(pH 7.4)溶液,4℃条件离心,测定上清液中蛋白浓度和RuBisCO活力。

[0073] 使用伯乐Bradford蛋白快速测试试剂盒检测上清中的蛋白浓度。

[0074] RuBisCO活性测定方法如下:将适量粗酶液体加入将反应液(100mM Tris,(pH 7.4),10mM MgCl2,20mM NaHCO3,10mM KCl,1mM DTT,2mM oxaloacetate,5mM creatine phosphate,10U 3-phosphoglycerate kinase,10U glyceraldehyde 3-phosphate dehydrogenase,10U creatine phosphokinase,0.2mM NADH)在30℃条件下放置15min,而后添加RuBP(终浓度为0.5mM)开始反应,测定起始5min内吸光值在340nm下的变化。

[0075] 结果显示(图1A)重组菌株RuBisCO活力达到22.3U/mg蛋白,而在对照菌株JG207中测到相应酶活,说明重组菌株RuBisCO在嗜热毁丝霉酶能够正确表达。

[0076] 2.重组菌株CP-1有机酸产量分析

[0077] 将所获得的重组菌株CP-1与对照菌株嗜热毁丝霉JG207菌株接种于50mL苹果酸发酵培养基中(磷酸二氢钾150mg/L,磷酸氢二钾150mg/L,硫酸镁100mg/L,氯化钙100mg/L,生物素1mL/L,微量元素液1mL/L,碳酸钙80g/L),其碳源分别为75g/L木糖和75g/L阿拉伯糖,,接种量均为$2.5*10^5$个/mL,培养基体积为50mL/瓶,45℃条件下培养,摇床转速150rpm。发酵8天后,取出1mL样品测定发酵液中有机酸产量。

[0078] 样品处理方法:取1mL发酵液于15mL离心管中,并添加1mL 1M $H_2SO_4$,而后80℃下放置30min,每个隔0min进行充分震荡。之后将2mL双蒸水添加至离心管中,充分震荡后,取1mL液体于1.5mL离心管中,12000rpm离心10min,取上清液测定C4-二羧酸含量。

[0079] C4-二羧酸含量测定:处理后的样品用高效液相色谱测定苹果酸和琥珀酸含量,其中检测器为紫外检测器,5mM $H_2SO_4$为流动相,流速为0.5mL/min。

[0080] 结果如图1B所示,导入CBB途径后,重组菌株CP-1的苹果酸和琥珀酸产量得到显著提高。在木糖条件下,重组菌株CP-1菌株苹果酸产量达到41.4g/L,比菌株JG207发酵8天苹果酸产量30g/L提高了38％;同时,在阿拉伯糖条件下,重组菌株CP-1菌株苹果酸产量达到

60.2g/L,比菌株JG207发酵8天苹果酸产量52.3g/L提高了15.1%。

[0081] 在木糖和阿拉伯糖条件下,CP-1菌株发酵液中琥珀酸含量分提高15%和7%。

[0082] 3.重组菌株CP-1生物量测定

[0083] 在重组菌株CP-1发酵第4天时,测定发酵液中生物量,其方法为:取2mL发酵液于已称重的15mL离心管中(M1),并加入2mL稀盐酸(浓盐酸与水以体积比1:5配制而成),混匀后离心至上清液呈澄清状,弃去上清,重复2-3次,而后用2mL水洗涤3次,将离心管放置于80℃烘箱中至恒重,称重M2,菌丝干重为M2-M1。

[0084] 结果如图1C所示,在木糖和阿拉伯糖条件下,重组菌株CP-1的生物量分别提高1.38倍和1.18倍。

[0085] 实施例3通过代谢工程手段进一步改造有机酸发酵菌株

[0086] 在本实施例中,采用基于CRISPR/Cas9的基因组编辑技术(Liu Q,Gao RR,Li JG,Lin LC,Zhao JQ,Sun WL,Tian CG.Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal Myceliophthora species and its application to hyper-cellulase production strain engineering.Biotechnology for Biofuels.2017,10:1.)将CBB途径基因prk,RuBisCO(cbbM)和筛选基因neo分别插入至菌株JG207基因中乳酸脱氢酶ldh,丙酮酸脱羧酶基因pdc和丙酮酸羧激酶pck位点,以达到过表达目标基因的同时,敲除代谢支路,提高菌株有机酸合成性能。其中,Cas9蛋白表达质粒也上述文章所描述方法构建的。

[0087] PDC氨基酸序列如SEQ ID No.13所示;PDC编码核苷酸序列如SEQ ID No.14所示;LDH氨基酸序列如SEQ ID No.15所示;LDH编码核苷酸序列如SEQ ID No.16所示;PCK氨基酸序列如SEQ ID No.17所示;PCK编码核苷酸序列如SEQ ID No.18所示。

[0088] 1.sgRNA转录框载体的构建

[0089] 通过软件sgRNACas9 tool分别设计目标基因pdc(Mycth_112121),ldh(Mycth_110317)和pck(Mycth_2315623)的靶标位点。采用融合PCR的方法将序列U6p启动子、protospacer及sgRNA连接在一起,采用基因重叠延伸(SOE)方法构建sgRNA表达框载体。

[0090] PCR反应体系为:2×Phanta®Max Buffer 25μL,10mM dNTPs 1μL,上游/下游引物各1.5μL,模板1μL,Phanta®Max Super-Fidelity DNA Polymerase 1μl,水19μl。

[0091] PCR反应条件为:先95℃30s;然后98℃15s,58℃15s,72℃45s,32个循环;最后72℃5min,4℃10min。

[0092] 通过SOE-PCR的扩增形成sgRNA表达质粒U6p-pdc-sgRNA、U6p-ldh-sgRNA和U6p-pck-sgRNA,其序列分别为SEQ ID No.19,SEQ ID No.20和SEQ ID No.21所示。

[0093] 2.供体DNA载体构建

[0094] 本发明中供体DNA片段分别由目标基因上/下游约1000bp同源片段,目标基因(cbbM和prk)或遗传霉素(G418)抗性基因表达框PtrpC-neo片段,通过Gibson Assembly的方法连接到由限制性内切酶XbaI和EcoRV线性化的质粒PPk2BarGFP中,最终构建供体DNA片段donor-ldh-prk、donor-pdc-cbbM、和donor-pck-neo,其核酸序列分别如SEQ ID No.22,SEQ ID No.23和SEQ ID No.24所示。

[0095] 构建供体DNA片段其所需的PCR引物序列如表1所示,

[0096] PCR反应体系与PCR反应条件均与上述sgRNA表达框载体的构建中所述条件相同。

[0097] 3.嗜热毁丝霉原生质体转化

[0098] 如实施例1中的操作方法，将Cas9蛋白表达质粒p0380-Ptef1-Cas9、sgRNA转录框(U6p-pdc-sgRNA、U6p-ldh-sgRNA和U6p-pck-sgRNA)供体DNA(donor-pdc-prk、donor-ldh-cbbM、和donor-pck-neo)以等比例混合后共转化进入嗜热毁丝霉酶菌株JG207原生质体细胞后，Cas9在gRNA介导下，通过靶标序列与宿主细胞基因组上的目标基因的DNA链配对来识别靶标位点进行切割，随后供体DNA片段与靶标位点两侧序列发生同源重组，从而达到在基因组中定点插入目标基因的目的，通过在平板中加入遗传霉素G418的平板中筛选转化子。

[0099] 4.嗜热毁丝霉转化子验证

[0100] 基因组提取方法与上述一致，然后进行PCR验证转化子。

[0101] 以上述提取的基因组DNA为模版，分别用引物pdc-out-F/R，ldh-out-F/R和pck-out-F/R对转化子进行基因PCR验证。PCR反应试剂购于南京诺唯赞生物科技有限公司。

[0102] PCR反应体系为：10×Taq Buffer 2μL，10mM dNTP Mix 0.2μL，上游/下游引物各0.4μL，DNA模板1μL，Taq DNA Polymerase 0.2μL，水15.8μL。

[0103] PCR反应条件为：先94℃5min；然后94℃30s，55℃30s，72℃1.2min，30个循环；最后72℃7min，4℃10min。

[0104] 对PCR扩增产物进行1％琼脂糖凝胶电泳(110V电压，30分钟)，在凝胶成像系统下显示出明显的基因扩增条带，结果如图5所示，表明供体DNA片段与靶标位点两侧序列发生同源重组，进而得到重组菌株CP-51。

[0105] 表2本实施例中载体构建所用引物

[0106]

| SEQ ID NO. | 引物 | 序列（5’-3’） |
|---|---|---|
| 25 | U6p-F | AGGATCGGTGGAGTGAAGTTCGGAA |
| 26 | U6p-pck-R | CTAGCTCTAAAACGTCTCGTATACGAGTGCATCGAGGAAAGAAAGAAAAGAAG |
| 27 | U6p-ldh-R | TATTTCTAGCTCTAAAACTGACATCTGCTGCGTTTGGCGAGGAAAGAAAGAAAAGAAG |
| 28 | U6p-pdc-R | CTCTAAAACTTTGTGCGGGCATACCCGTCGAGGAAAGAAAGAAAAGAAG |
| 29 | g-pck-F | TCTTTCTTTCCTCGATGCACTCGTATACGAGACGTTTTAGAGCTAGAAATAG |
| 30 | g-ldh-F | TTTTCTTTCTTTCCTCGCCAAACGCAGCAGATGTCAGTTTTAGAGCTAGAAATAGC |
| 31 | gRNA-R | AAAAAGCACCGACTCGGTGCCACTT |
| 32 | Dpck1-5’-F | GTGGAGATGTGGAGTGGGCGCTTACACAGTACACGAGGACTTGCTCGAGTTTTGTCGCATTCG |
| 33 | Dpck1-5’-R | CAAAAAATGCTCCTTCAATATCAGTTAACGTCGTCAGTAGAGCGCGGCGACTGAAG |
| 34 | Neo-F | CGACGTTAACTGATATTGAAGGA |
| 35 | Neo-R | TCAGAAGAACTCGTCAAGAA |
| 36 | Dpck1-3’-F | GCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGTCAGGTCGACTATATGCCGTC |
| 37 | Dpck1-3’-R | CAAGTCATGTGATTGTAATCGACCGACGGAATTGAGGATGACGTTCTCCAGAACGGAGC |
| 38 | Dpdc-5’-F | TGTGGAGTGGGCGCTTACACAGTACACGAGGACTTGGTAAACCGAAAGCTGGGGAA |
| 39 | Dpdc-5’-R | GTCTGTTGTTGGCGATGTTTG |
| 40 | DcbbM-F | CTCCTTGTGACCACAAACATCGCCAACAACAGACATGGACCAGTCGAGCCGGTAC |
| 41 | DcbbM-R | AGTCGATCGTGTTGAGTTGGGACGTCCGGTACTATCATCAGGCCGGCAGCGCCGAGC |
| 42 | Dpdc-3’-F | GTACCGGACGTCCCAACTCAAC |
| 43 | Dpdc-3’-R | CATGTGATTGTAATCGACCGACGGAATTGAGGATAAGAGCCGCTGCAGATGGACTC |
| 44 | Dldh-5’-F | AGATGTGGAGTGGGCGCTTACACAGTACACGAGGACTTGTGACAAATACCAGAGTCTAG |
| 45 | Dldh-5’-R | CTTGAATCCGTAAAGGTAATAAA |
| 46 | Dprk-F | CGAGTTATTTATTACCTTTACGGATTCAAGATGGCGGTCTGCACCGTGTAC |
| 47 | Dprk-R | GATGGCGTCGCTGTCGGTGACTCAGGCCTTGGCGGCGGTGGC |
| 48 | Dldh-3’-F | GTCACCGACAGCGACGCCATC |
| 49 | Dldh-3’-R | AAGTCATGTGATTGTAATCGACCGACGGAATTGAGGATGTCGCCCGCTCCAGACCCAG |
| 50 | pck-out-F | ACACGTGACGCAGAGGAGGAA |
| 51 | pck-out-R | GTACTCGGTACCCAAGATGACCAT |
| 52 | pdc-out-F | GTGACCACAAACATCGCCAACAA |

[0107]

| | | |
|---|---|---|
| 53 | pdc-out-R | ATCGTGTTGAGTTGGGACGT |
| 54 | ldh-out-F | GTGACGGATTCGATCATTCTA |
| 55 | ldh-out-R | GCCGACCCTCGAGACCGTCAT |

[0108] 实施例4嗜热毁丝霉重组菌株CP-51在有机酸合成中的生物学表型分析

[0109] 1.重组菌株CP-51固碳效率分析

[0110] 为了菌株CP-51的固碳效率，将CP-51与对照菌株嗜热毁丝霉JG207菌株接种于50mL苹果酸发酵培养基中(磷酸二氢钾150mg/L，磷酸氢二钾150mg/L，硫酸镁100mg/L，氯化钙100mg/L，生物素1mL/L，微量元素液1mL/L)，其碳源分别为75g/L木糖，其中和剂为80g/L $Ca^{13}CO_3$，接种量均为2.5*$10^5$个/mL，培养基体积为50mL/瓶，45℃条件下培养，摇床转速150rpm。发酵结束通过LC LC-MS/MS测定含有$^{13}C$的苹果酸比例。结果如图2所示，在菌株CP-51发酵液中含有1个$^{13}C$原子的苹果酸在总苹果酸中的比例为50.9％，含有2个$^{13}C$原子的苹果酸所占比例为8.8％，均高于对照菌株JG207(分别为23.1％和7.2％)。重组菌种CP-51合成苹果酸中17.1％的碳原子来源所固定外界的$CO_2$，显著高于出发菌株JG207(9.4％)。

[0111] 2.重组菌株CP-51有机酸合成分析

[0112] 将所获得的重组菌株CP-51与对照菌株嗜热毁丝霉JG207菌株接种于50mL苹果酸发酵培养基中(磷酸二氢钾150mg/L，磷酸氢二钾150mg/L，硫酸镁100mg/L，氯化钙100mg/L，生物素1mL/L，微量元素液1mL/L，碳酸钙80g/L)，其碳源分别为75g/L木糖、75g/L阿拉伯糖和75g/L葡萄糖，接种量均为2.5*$10^5$个/mL，培养基体积为50mL/瓶，45℃条件下培养，摇床转速150rpm。发酵8天后，取出1mL样品测定发酵液中有机酸产量。

[0113] 样品的处理方法与检测方法与实施例2中所述相同。

[0114] 结果如图3所示，发酵结束时，重组菌株CP-51在木糖、阿拉伯糖及葡萄糖条件下的苹果酸产量分别达到46.7g/L，64.5g/L and 74.3g/L，显著高于出发菌株JG207以及实施例1中所构菌株CP-1。同时，重组菌株CP-51发酵液中琥珀酸含量也得到显著提高。

[0115] 3.重组菌株CP-51有机酸合成中细胞干重分析

[0116] 在木糖条件下，在重组菌株CP-51发酵第4天时，测定发酵液中生物量，其方法为：取2mL发酵液于已称重的15mL离心管中(M1)，并加入2mL稀盐酸(浓盐酸与水以体积比1:5配制而成)，混匀后离心至上清液呈澄清状，弃去上清，重复2-3次，而后用2mL水洗涤3次，将离心管放置于80℃烘箱中至恒重，称重M2，菌丝干重为M2-M1。结果如图3B所示，在木糖条件下，重组菌株CP-51的生物量提高1.54倍。

[0117] 实施例5底物转运显著增强的有机酸发酵菌株构建

[0118] 本实施例通过基因手段在实施例3中所构的重组菌种CP-51中导入转运蛋白Gal-2M，具体如下：

[0119] 1.gla2M过表达载体构建

[0120] 以质粒pAN52-bar(Gu SY,Li JG,Chen BC,Sun T,Liu Q,Xiao DG,Tian CG.Metabolic engineering of the thermophilic filamentous fungus Myceliophthora thermophila to produce fumaric acid.Biotechnology for Biofuels.2018,11:323.)为骨架构建基因构建gal-2M的表达载体，所选的组成型强启动子为嗜热毁丝霉丙酮酸脱酸酶转录起始因子eif(Mycth_2297659)的启动子Peif。转运蛋白Gal2M通过突变来自酿酒酵母转运蛋白Gal-2(N376F)的氨基酸序列所构建。

[0121] Gal-2M氨基酸序列如SEQ ID No.56所示；Gal-2M编码核苷酸序列(cbbM)如SEQ ID No.57所示；启动子Peif核苷酸序列如SEQ ID No.58所示。

[0122] 通过SOE-PCR方式将点突变(N376F)引入gal-1()编码核苷酸酸中，进而获得gal2M核苷酸。随后经PCR扩增后，获得所需的DNA片段，所用引物如表3所示，采用Gibson Assembly技术体系对上述多个PCR片段进行快速组装到由限制性内切酶Bgl II和BamH I双

酶切的骨架质粒pAN52-bar上，从而构建gla2M重组表达载体pAN52-gla2M。PCR反应体系为：5×phusion HF buffer 10μL，10mM dNTPs 1μL，10mM引物-F和引物-R各2.0μL，模板DNA 1μL，Phusion DNA polymerase 0.5μL，水33.5μL。PCR反应条件为：先98℃ 30s；然后98℃ 10s，65℃ 30s，72℃ 1.5min，35个循环；最后72℃ 10min，4℃ 10min。

[0123] 2.gla-2M在嗜热毁丝霉中重组表达

[0124] 将10μg由限制性内切酶Hind III线型化重组表达载体pAN52-gal2M转化导入嗜热毁丝霉菌株CP-51原生质体细胞后中，通过在平板中加入草丁膦(PPT)筛选出转化子。提取转化子基因组DNA后，用引物Gal1-F和Gal2-R对转化子进行基因PCR验证。PCR反应体系为：10×Taq Buffer 2μL，10mM dNTP Mix 0.2μL，上游/下游引物各0.4μL，DNA模板1μL，Taq DNA Polymerase 0.2μL，水15.8μL。PCR反应条件为：先94℃5min；然后94℃30s，55℃30s，72℃1.2min，30个循环；最后72℃7min，4℃10min.

[0125] 对PCR扩增产物进行1％琼脂糖凝胶电泳(110V电压，30分钟)，在凝胶成像系统下显示出明显的基因扩增条带，结果如图5所示，表明供体DNA片段与靶标位点两侧序列发生同源重组，进而得到重组菌株Gal-1。

[0126] 本实施例中嗜热毁丝霉原生质制备及转化、转化子基因组提取均按照实施例1中所述方法进行。

[0127] 表2本实施例中载体构建所用引物

[0128]

| SEQ ID NO. | 引物 | 序列（5'-3'） |
|---|---|---|
| 59 | Peif-gal-F | TTGGCTGACTTGAAGTAATCTCTGCAGATCTTTAATTAACACAGCAGTTCGCACGCTCC |
| 60 | Peif-gal-R | GTGAAACAACAGGCATATTGTTCTCCTCAACTGCCATCTTGTTGTTGTTGTTGTGTTG |
| 61 | Gal1-F | CGCTCACCGTTACGCTCCTCAACACAACAACAACAACAAGATGGCAGTTGAGGAGAACAA |
| 62 | Gal1-R | CAAACTAAAGAAAGTGGAGGCAAAGAAGACTACACCAATGACAATGG |
| 63 | Gal2-F | TCCATTGTCATTGGTGTAGTCTTCTTTGCCTCCACTTTCTTTAGTT |
| 64 | Gal2-R | TCAAGCTGTTTGATGATTTCAGTAACGTTAAGTGGATCTTATTCTAGCATGGCCTTGT |

[0129] 实施例6重组菌株Gal-1在有机酸合成中的生物学表型分析

[0130] 1.重组菌株Gal-1底物转运效率分析

[0131] 将重组菌株Gal-1及其对照菌株CP-51先在100mL葡萄糖培养基中培养18h，收集菌丝，用1×Vogel's盐溶液(配方见实施例1)洗涤3次，而后分别转接到0.5％葡萄糖培养基、0.5％木糖培养基或0.5％阿拉伯糖培养基中培养4h；从中取10mL培养液离心，用1×Vogel's盐溶液洗涤后，重悬在1mL加有放线菌酮(cycloheximide)(100μg/mL)的无菌水中，向分别其中加入100μL葡萄糖(10mM)、100μL木糖(10mM)或100μL阿拉伯糖(10mM)；反应20min后离心去除测定菌丝干重，并测定上清液中残糖浓度。

[0132] 实验结果显示(图4)，重组菌株Gal-1对葡萄糖的转运速率与对照菌株CP-51相似，而重组菌株Gal-1对戊糖(木糖和阿拉伯糖)的转运速率显著快提高，说明糖转运蛋白Gal-2M在嗜热毁丝霉中的过表达，能够显著提高重组菌株对底物的转运效率。

[0133] 2.重组菌株Gal-1对混合底物利用效率分析

[0134] 将重组菌株Gal-1及其对照菌株CP-51分别接种至1×Vogel's盐溶液培养基中，其碳源为一种，两种或三种单糖(20g/L木糖、20g/L阿拉伯糖和40g/L葡萄糖)所组成，接种量

均为2.5*10$^5$个/mL，培养基体积为100mL/瓶45℃条件下培养，摇床转速150rpm。每隔一段时间天取样测定发酵液中的残糖含量。

[0135] 结果显示，gal-2M的过表达显著提高了重组菌株Gal-1的在单糖及其混合物中底物效率速率。菌株Gal-1对木糖和阿拉伯糖的消耗速率显著高于对照菌株CP-51。同时，在两种或三种单糖的混合物中，菌株Gal-1的底物利用效率也明显优于对照菌株CP-51。

[0136] 3.利用重组菌株Gal-1转化生物质单糖混合物合成有机酸

[0137] 将所获得的重组菌株Gal-1和对照菌株CP-51接种于50mL苹果酸发酵培养基中(磷酸二氢钾150mg/L，磷酸氢二钾150mg/L，硫酸镁100mg/L，氯化钙100mg/L，生物素1mL/L，微量元素液1mL/L，碳酸钙80g/L)，其碳源为单糖混合物，包含20g/L木糖、20g/L阿拉伯糖和40g/L葡萄糖，接种量均为2.5*10$^5$个/mL，培养基体积为50mL/瓶，45℃条件下培养，摇床转速150rpm。每2天取出1mL样品测定发酵液中的残糖含量及其有机酸含量。

[0138] 样品的处理方法与检测方法与实施例2中所述相同。

[0139] 结果如图6所示，发酵结束时，重组菌株Gal-1对木糖、阿拉伯糖和葡萄糖所组成的混合物的利用速率显著快于对照菌株CP-51，其发酵液中有机酸的含量也得到了显著提高。发酵结束时，菌株Gal-1发酵液中的苹果酸浓度达到84.6g/L，相比对照菌株CP-51(69.5g/L)提高22％。

[0140] 实施例7有机酸高产菌株Gal-1在转化生物质合成化学品中应用

[0141] 将所获得的重组菌株Gal-1和原始菌株JG207接种于50mL苹果酸发酵培养基中(磷酸二氢钾150mg/L，磷酸氢二钾150mg/L，硫酸镁100mg/L，氯化钙100mg/L，生物素1mL/L，微量元素液1mL/L，碳酸钙80g/L)，其碳源为多聚糖类(75g/L结晶纤维素，75g/L木聚糖和75g/L玉米芯渣)接种量均为2.5*10$^5$个/mL，培养基体积为50mL/瓶，45℃条件下培养，摇床转速150rpm，发酵8天后，取出1mL样品测定发酵液中的残糖含量及其有机酸含量。

[0142] 结果显示(图7)，在木聚糖，结晶纤维素和玉米芯渣条件下，重组菌株Gal-1的苹果酸含量分别达到了56.8g/L，69.9g/L和44.4g/L，相比菌株JG207分别提高了17％，7％和23.2％。同时，菌株Gal-1发酵液中琥珀酸含量分别达到12.6g/L，11.4g/L和3.7g/L。

[0001] 序列表

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[0004] <120> 一种提高丝状真菌有机酸合成能力的方法

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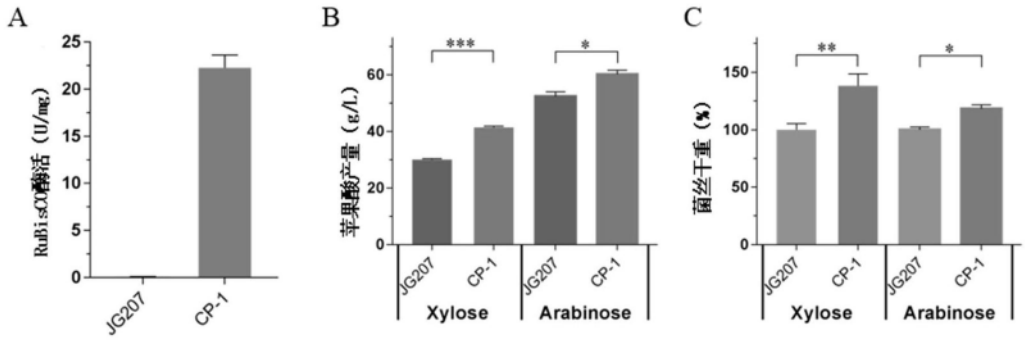
A
B
C
25
20
15
10
5
0
JG207
CP-1
60
40
20
0
***
*
Xylose
Arabinose
150
100
50
**

图1

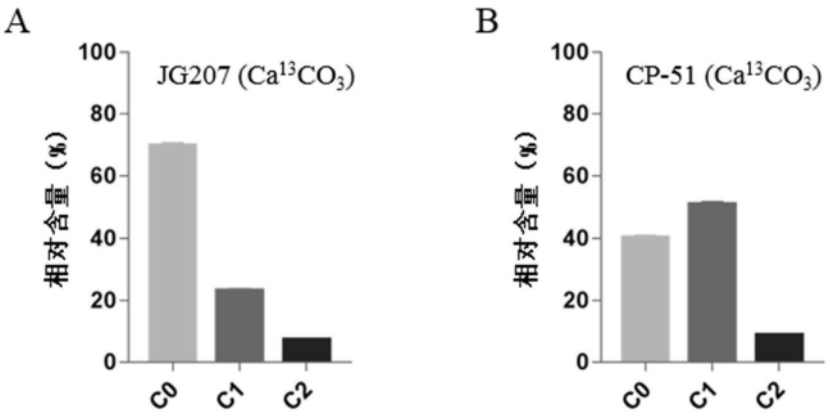
A
B
JG207 (Ca13CO3)
CP-51 (Ca13CO3)
相对含量（%）
100
80
60
40
20
0
C0
C1
C2

图2

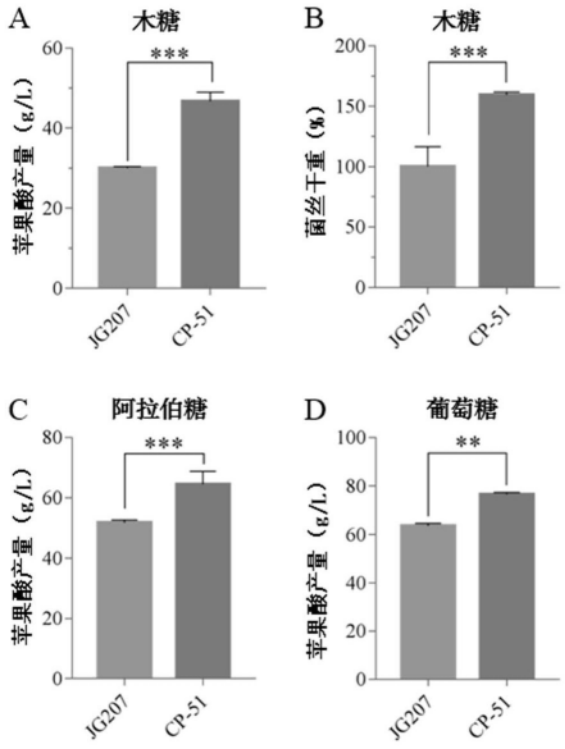
A
木糖
苹果酸产量（g/L）
60
40
20
0
***
JG207
CP-51
B
木糖
菌丝干重（%）
200
150
100
50
0
***
JG207
CP-51
C
阿拉伯糖
苹果酸产量（g/L）
80
60
40
20
0
***
JG207
CP-51
D
葡萄糖
苹果酸产量（g/L）
100
80
60
40
20
0
**
JG207
CP-51

图3

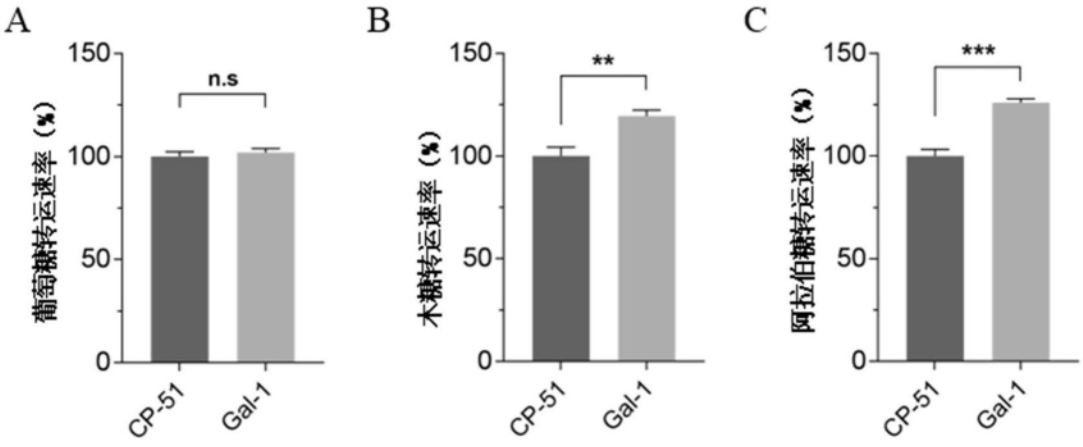
A
葡萄糖转运速率（%）
150
100
50
0
n.s
CP-51
Gal-1
B
木糖转运速率（%）
150
100
50
0
**
CP-51
Gal-1
C
阿拉伯糖转运速率（%）
150
100
50
0
***
CP-51
Gal-1

图4

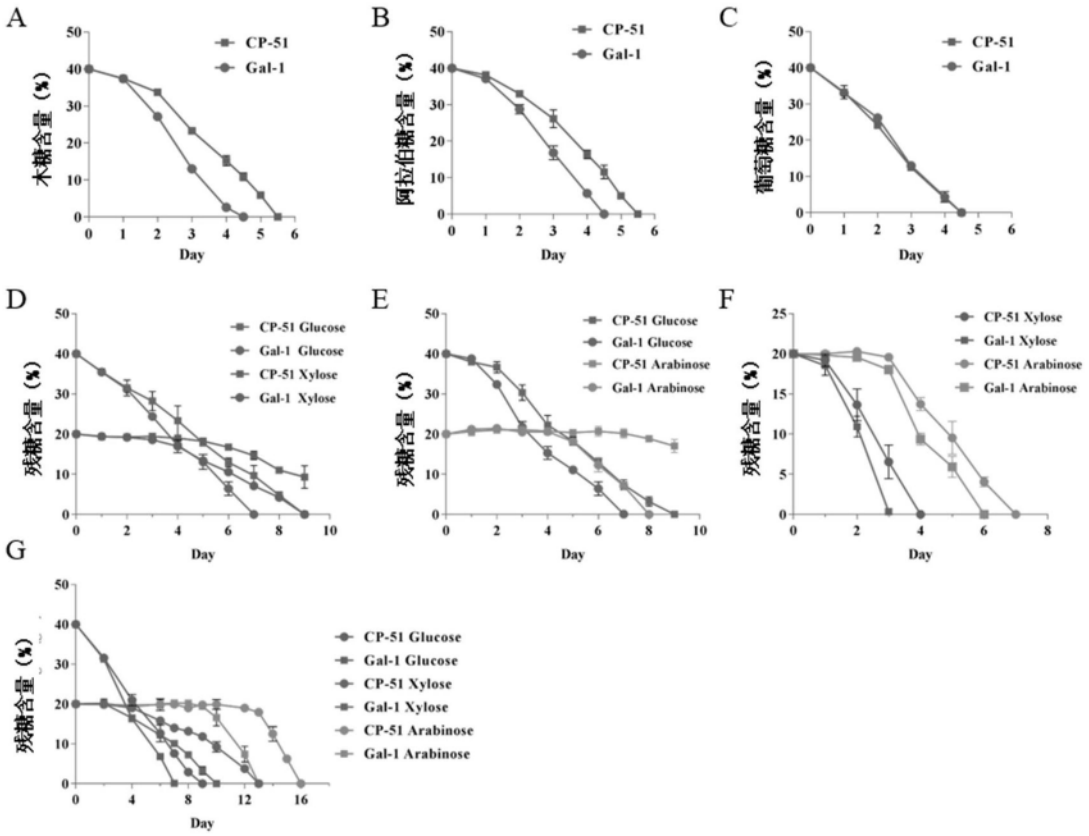
A
木糖含量（%）
CP-51
Gal-1
Day
B
阿拉伯糖含量（%）
CP-51
Gal-1
Day
C
葡萄糖含量（%）
CP-51
Gal-1
Day
D
残糖含量（%）
CP-51 Glucose
Gal-1 Glucose
CP-51 Xylose
Gal-1 Xylose
Day
E
残糖含量（%）
CP-51 Glucose
Gal-1 Glucose
CP-51 Arabinose
Gal-1 Arabinose
Day
F
残糖含量（%）
CP-51 Xylose
Gal-1 Xylose
CP-51 Arabinose
Gal-1 Arabinose
Day
G
残糖含量（%）
CP-51 Glucose
Gal-1 Glucose
CP-51 Xylose
Gal-1 Xylose
CP-51 Arabinose
Gal-1 Arabinose
Day

图5

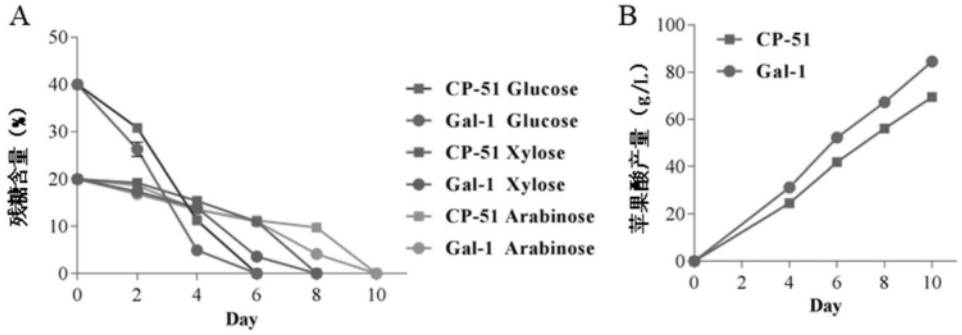
A
残糖含量（%）
CP-51 Glucose
Gal-1 Glucose
CP-51 Xylose
Gal-1 Xylose
CP-51 Arabinose
Gal-1 Arabinose
Day
B
苹果酸产量（g/L）
CP-51
Gal-1
Day

图6

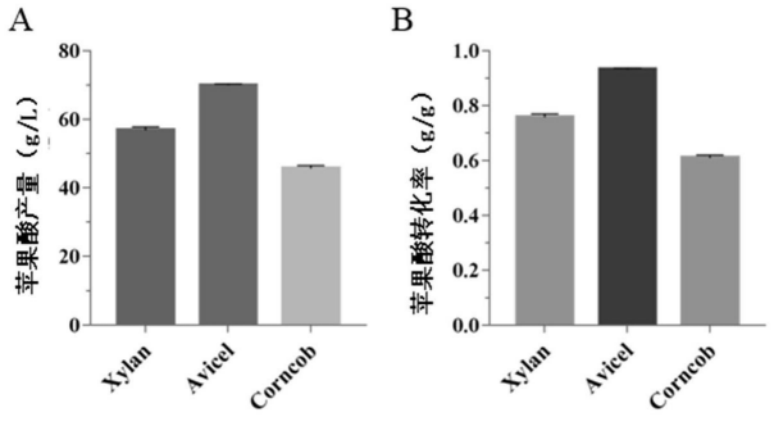
A
苹果酸产量（g/L）
80
60
40
20
0
Xylan
Avicel
Corncob
B
苹果酸转化率（g/g）
1.0
0.8
0.6
0.4
0.2
0.0
Xylan
Avicel
Corncob

图7

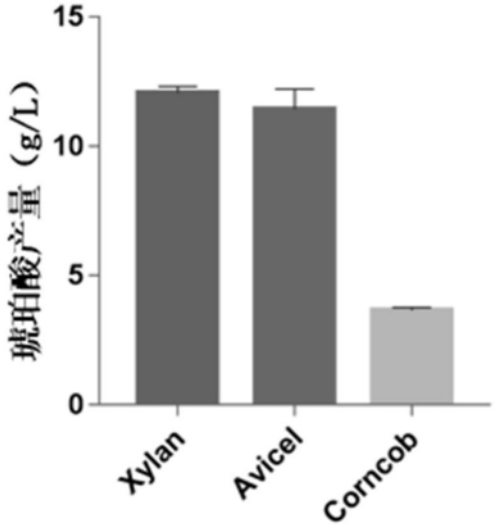
琥珀酸产量（g/L）
15
10
5
0
Xylan
Avicel
Corncob

图8