◆特邀专稿◆

木糖产乙醇微生物的育种研究进展*

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摘要:甘蔗渣水解产物中含量第二高的是木糖,利用微生物高效转化木糖产乙醇是目前蔗渣纤维综合利用的关键途径之一,但野生型酿酒酵母(*Saccharomyces cerevisiae*)不能利用木糖发酵产乙醇,这成为当前纤维素乙醇实现产业化生产的主要瓶颈之一。本文从微生物木糖代谢途径、利用木糖产乙醇的微生物、木糖产乙醇基因工程菌的构建、利用传统诱变方法改良木糖乙醇菌种等4个方面综述当前木糖产乙醇微生物的育种研究进展,指出木糖乙醇发酵最为理想的微生物菌种和最有应用前景的微生物分别是树干毕赤酵母(*Pichia stipitis*)和酿酒酵母基因工程菌,为改良木糖乙醇微生物提供理论依据。

关键词:木糖;乙醇;代谢途径;树干毕赤酵母;酿酒酵母

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生物乙醇作为可再生能源是迄今为止最为成功的液体替代燃料,因此受到广泛关注[1-4]。目前生物乙醇的发展存在的主要问题为以传统原料为主,对非粮原料的利用处于工业化初期,面临能耗、物耗高及成本高等难题[5,6]。纤维素乙醇作为新一代先进的非粮生物液体燃料,具备原料丰富、价格低、可再生等优势。因此,开发和利用纤维素乙醇已成为世界各国的发展趋势和必然选择[7-11],我国也不例外。为推动高效、低成本的纤维素乙醇的应用,纤维素乙醇产业已被确立为战略性新兴产业[12-14],且加快纤维素乙醇关键技术研发也被纳入《"十四五"生物经济发展规划》。纤维素乙醇产业发展面临新的机遇。

纤维素乙醇产业的重要原料是甘蔗渣,而甘蔗渣是甘蔗制糖工业重要的副产品,且来源比较集中,便于利用。据统计,2022年我国甘蔗总产量为10 338.13万吨,其中广西甘蔗产量为7 116.54万吨(广西是我国最大的食糖生产基地,多年来每年的甘蔗产量均占全国甘蔗总产量的60%左右,位居全国

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第一),制糖工业产生的甘蔗渣产量超过 800 万吨(广西甘蔗品种一般以 8.5 t 甘蔗产出 1 t 的蔗渣计)[15]。甘蔗渣主要由纤维素、半纤维素和木质素这 3 个成分组成,具有可再生、低成本、环境友好、生物相容、可生物降解等优点。甘蔗渣的水解产物主要是葡萄糖和木糖,其中含量第二高的是木糖。但野生型酿酒酵母(*Saccharomyces cerevisiae*)不能利用木糖发酵产乙醇,这成为当前纤维素乙醇实现产业化生产的主要瓶颈之一。为此,需要选育能高效利用木糖产乙醇的菌株。本文从微生物木糖代谢途径、利用木糖产乙醇的微生物、木糖产乙醇基因工程菌的构建、利用传统诱变方法改良木糖乙醇菌种等 4 个方面综述当前木糖产乙醇微生物的育种研究进展。

1 微生物木糖代谢途径

为了能在生活环境中充分利用木糖,微生物已经进化出独特的木糖利用途径。目前已发现的木糖自然代谢途径有 5 种:木糖还原酶(Xylose Reductase,XR)-木糖醇脱氢酶(Xylitol Dehydrogenase,XDH)途径、木糖异构酶(Xylose Isomerase,XI)途径、α-酮戊二酸(α-Ketoglutarate,α-KG)途径、Dahms 途径和磷酸酮酶(Phosphoketolase,PK)途径(图 1)[16]。上述 5 种途径具体的代谢过程如下。

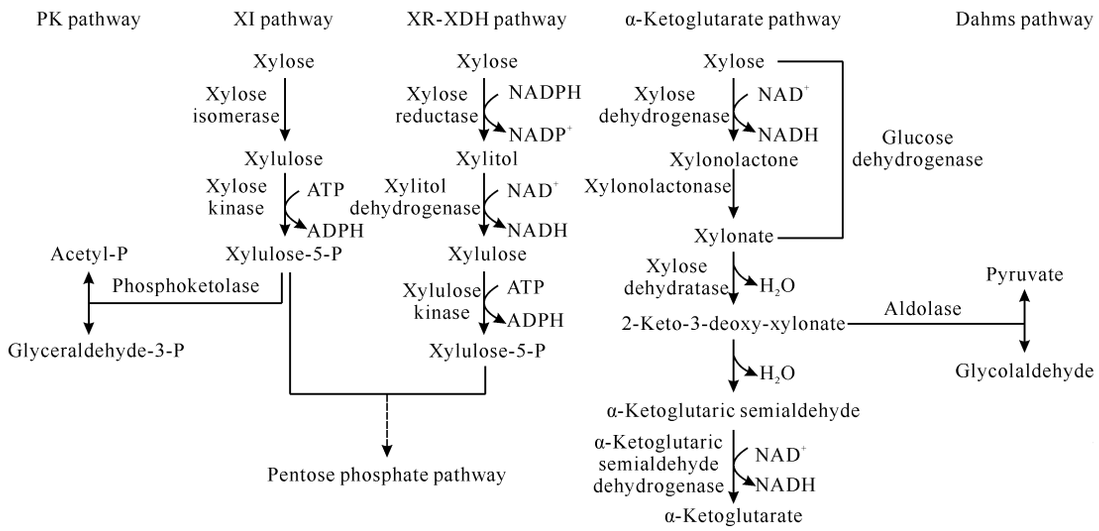

图 1 自然界微生物利用木糖的途径[16]

Fig. 1 Pathways of xylose utilization by microorganisms in nature[16]

(1)途径一(酵母、丝状真菌)

XR-XDH 途径:XR 催化木糖生成木糖醇,后者经 XDH 催化生成木酮糖,即两步法生成木酮糖,XR、XDH 分别需要辅酶 NAD(P)H、NAD^+。木酮糖经过磷酸化后生成 5-磷酸木酮糖,后者再经戊糖磷酸途径(Pentose Phosphate Pathway,PPP)等途径,最后在无氧条件下生成乙醇。该途径存在于酵母、丝状真菌中。

(2)途径二(细菌)

XI 途径:XI 催化木糖生成木酮糖,即一步法生成木酮糖,此反应不需要任何辅酶。此后生成乙醇的途径同途径一。该途径存在于细菌中。

(3)途径三(新月柄杆菌)

α-KG 途径:Stephens 等[17]在新月柄杆菌(*Caulobacter crescentus*)中发现新的木糖代谢途径,即木糖经木糖脱氢酶 xylB(需要辅酶 NAD^+)催化生成 Xylonolactone,后者经 xylC 催化生成 Xylonate,然后经脱水酶 xylD 作用脱水生成 2-Keto-3-deoxy-xylonate,再经脱水、脱氢最后生成 α-Ketoglutarate,进入 TCA 循环,在有氧条件下生成 H_2O 和 CO_2。

(4)途径四(新月柄杆菌)

Dahms 途径:在途径三的基础上,2-Keto-3-deoxy-xylonate 经醛缩酶催化分解为乙醇醛和丙酮酸[16,18]。该途径存在于新月柄杆菌中。

(5)途径五(丙酮丁醇梭菌)

PK 途径:在途径二的基础上,5-磷酸木酮糖经磷酸酮酶催化分解为乙酰磷酸和甘油醛-3-磷酸[19,20]。该途径存在于丙酮丁醇梭菌(*Clostridium acetobutylicum*)中。

上述 5 种木糖自然代谢途径中,代谢路径相对较短的是途径二、途径五,代谢路径相对较长(复杂)的是途径三。而对于微生物利用木糖产乙醇,研究人员

较为关注的是途径一和途径二[16]。这两个途径相比较,途径二代谢路径更简单。

2 利用木糖产乙醇的微生物

目前能利用木糖产乙醇的微生物主要有:(1)酵母,包括管囊酵母(*Pachysolen tannophilus*)[21]、休哈塔假丝酵母(*Candida shehatae*)[22]、树干毕赤酵母(*Scheffersomyces stipitis*,又称 *Pichia stipitis*)[23]、马克斯克鲁维酵母(*Kluyveromyces marxianus*)[24,25]、汉逊酵母(*Ogataea polymorpha*)[26]以及近几年新发现的威克汉姆酵母(*Wickerhamomyces* sp. UFFS-CE-3.1.2)[27]、*Spathaspora hagerdaliae* UFMG-CM-Y303[28]、季也蒙毕赤酵母(*Pichia guilliermondii*)[29]、嗜油脂假丝酵母(*C. oleophila*)[29]、非常规酵母菌株 *S. passalidarum*[30]等;(2)细菌,包括运动发酵单胞菌(*Zymomonas mobilis*)[31]、嗜热厌氧乙醇杆菌(*Thermoanaerobacter ethanolicus*)[32]等。此外,真菌如拟青霉(*Paecilomyces* sp. NF1)[33]也能利用木糖产乙醇。据统计,目前已发现超过100种微生物能利用木糖,其中大部分能产乙醇(表1)。

表1 自然界中利用木糖的微生物

Table 1 Microorganisms using xylose in nature

菌种名称 Name of strain	木糖/(g/L) Xylose/(g/L)	乙醇/(%,V/V) Ethanol/(%,V/V)	糖醇转化率/(g/g) Conversation of xylose to ethanol/(g/g)	文献 Reference
Paecilomyces sp. NF1	50	2.50	0.39	[33]
P. sp. NF1	80	3.99	0.39	[33]
P. sp. NF1	100	5.04	0.39	[33]
P. sp. NF1	150	7.56	0.39	[33]
P. sp. NF1	200	9.31	0.38	[33]
Pachysolen tannophilus RL-171	80	2.66	0.26	[34]
P. tannophilus RL-171	100	2.98	0.24	[34]
P. tannophilus RL-171	120	2.53	0.17	[34]
P. tannophilus NRRL-Y2460	20	0.67	0.26	[35]
P. tannophilus NRRL-Y2460	50	1.90	0.30	[36]
Candida XF217	50	2.66	0.42	[37]
C. shehatae CBIR-Y492	50	2.28	0.36	[38]
C. shehatae CBIR-Y492	90	3.32	0.29	[39]
C. tropicalis ATCC 1369	75	1.05	0.11	[40]
Pichia stipitis CBS 5576	50	2.83	0.45	[41]
P. segobiensis CBS 6857	20	0.63	0.25	[42]
P. stipitis CBS 5573(5)	20	0.75	0.30	[42]
P. stipitis NRRL Y-7124	30		0.28	[43]
Spathaspora passalidarum NRRL Y-27907	30		0.35	[30]
Pestalotiopsis sp. XE-1	97.71	0.54	0.34	[44]
Zymomonas mobilis A3	100	5.46	0.45	[45]
Spathaspora hagerdaliae UFMG-CM-Y303	28.2		0.28-0.36	[28]
S. hagerdaliae UFMG-CM-Y303	20.6	0.43	0.33	[46]
C. maltosa UFMG-CM-Y2131	30	0.44	0.12	[47]
Scheffersomyces sp. UFMG-CM-Y365	30	1.01	0.25	[47]
S. stipitis UFMG-CM-Y2303	30	1.78	0.42	[47]
S. stipitis UFMG-CM-Y2108	30	1.78	0.42	[47]
Spathaspora boniae UFMG-CM-Y306	30	0.78	0.19	[47]
S. boniae UFMG-CM-Y363	30	0.91	0.22	[47]
Sugiyamaella xylolytica UFMG-CM-Y348	30	0.61	0.14	[47]
Wickerhamomyces sp.1 UFMG-CM-Y6241	30	0.43	0.14	[47]

目前文献报道木糖产乙醇浓度最高的微生物是拟青霉,其产乙醇浓度为 9.31%(*V*/*V*)[33],但发酵时间为 12 d,周期太长,发酵效率低。酵母菌种中研究较多的是休哈塔假丝酵母、管囊酵母和树干毕赤酵母[48,49],三者的木糖乙醇发酵性能(糖醇转化率,g/g)相比较,管囊酵母相对较弱,其糖醇转换率为 0.17 - 0.30 g/g[34-36],而休哈塔假丝酵母[38,39]次之,最强的是树干毕赤酵母,糖醇转化率为 0.42 - 0.45 g/g[41,47]。非常规酵母菌株方面,比如 *Spathaspora passalidarum* NRRL Y-27907,其糖醇转化率为 0.35 g/g[30],与休哈塔假丝酵母接近。细菌方面,目前较为优良的是运动发酵单胞菌,其糖醇转化率达到 0.45 g/g[41]。此外,酵母菌种在酒精耐受度等方面比细菌有优势。而理想菌种具备的特征主要包括发酵周期短、糖醇转化率高等。因此,对于木糖乙醇发酵,目前最为理想的菌种是树干毕赤酵母[50-52]。

3 木糖产乙醇基因工程菌的构建

目前,利用基因工程技术等手段对木糖产乙醇微生物进行改造,构建的基因工程菌的种类主要包括酿酒酵母、大肠杆菌(*Escherichia coli*)、运动发酵单胞菌等。

3.1 产木糖乙醇的酿酒酵母基因工程菌

(1)酿酒酵母木糖乙醇发酵途径的改造

酿酒酵母具有高产乙醇、较高乙醇耐受性等优点,但野生型酿酒酵母不能利用木糖发酵生产乙醇。为此,通过基因工程和代谢工程等方法或策略来构建高效利用多种糖类(尤其是木糖)产乙醇的酿酒酵母工程菌已成为全球各国研究人员关注的焦点和努力解决的热点问题。

第一条改造思路:主要借鉴 XR-XDH 途径,通过在酿酒酵母中引入 XR-XDH 途径相关的异源基因来构建。Amore 等[53]较早开展了相关的探索,将来自树干毕赤酵母 CBS5773 的 2 个基因(木糖还原酶基因 *XYL1* 以及木糖醇脱氢酶基因 *XYL2*)导入宿主酿酒酵母 JD2-9A 中进行异源表达,宿主能检测到木糖还原酶和木糖醇脱氢酶的活性。Watanabe 等[54]将毕赤酵母木糖醇脱氢酶基因和定点突变的木糖还原酶基因导入酿酒酵母中表达,重组菌乙醇产量比原始菌提高 5.1%。Petschacher 等[55]将 *C. tenuis* 木糖还原酶基因和 *Galactocandida mastotermitis* 木糖醇脱氢酶基因连接在酿酒酵母强启动子 TDH3 下游后整合至酿酒酵母基因组,重组子木糖乙醇产量提高 42%。而 Ho 等[56]作了更进一步的研究,在酿酒酵母中同时异源表达 3 个基因(来自树干毕赤酵母的 *XYL1*、*XYL2* 基因以及木酮糖激酶基因),重组菌所产的酒精高于 3 个基因来源的树干毕赤酵母菌株。然而研究发现在酿酒酵母中异源表达 *XYL1*、*XYL2* 基因以及木酮糖激酶基因,重组菌株在木糖醇积累和乙醇产量方面表现出较大的变化,主要原因是编码木糖醇脱氢酶的 *XYL2* 基因低表达水平[57],这也是木糖高效发酵的瓶颈之一。为解决此问题,Kim 等[57]在酿酒酵母工程菌株 YSX3 能异源表达 *XYL1*、*XYL2* 基因以及木酮糖激酶基因的基础上,构建过量表达树干毕赤酵母 *XYL2* 基因(利用组成型强启动子整合至核基因组上表达)的重组工程菌株 YSX3-pX2,使其木糖醇积累减少,乙醇产量提高,即木糖醇产率从 0.4 g/g 下降至 0.1 g/g,乙醇产率从 0.1 g/g 提高至 0.3 g/g。此外,所有乙醇脱氢酶(Alcohol Dehydrogenase,ADH)基因适合于优化酵母代谢途径,而 *SFA1* 基因是 ADH 基因中的一种重要基因[58]。鉴于此,曹利民团队在酿酒酵母工程菌株 WXY70 能异源表达 *XYL1*、*XYL2* 基因以及木酮糖激酶基因的基础上,构建过量表达 *SFA1* 基因的重组酿酒酵母 $SFA1^{OE}$,该菌株发酵 48 h 的醪液中残留木糖含量和乙醇产量分别为 1.2 g/L 和 53.20 g/L,而原始菌株 TSH-01 中分别为 4.03 g/L 和 51.36 g/L[59],木糖利用率和乙醇产量均得到提高。

第二条改造思路:在酿酒酵母中异源表达木糖异构酶基因。由于木糖经木糖异构酶催化生成木酮糖无需任何辅酶,因此不存在胞内还原力不平衡的问题[60]。Lönn 等[61]将来自嗜热栖热菌(*Thermus thermophilus*)的木糖异构酶基因导入酿酒酵母中表达,酶活提高 8 倍。王青艳等[62]将来自嗜热放线细菌(*Thermobifida fusca*)的木糖异构酶基因 *xylA* 连接于酵母表达载体 pYES2 的半乳糖诱导启动子 PGAL 下,得到重组质粒 pYES2-xylA,用其转化酵母菌 INVSc1,构建出重组菌株 INVSc1-xylA,其在木糖葡萄糖共发酵实验中消耗的木糖和产生的乙醇分别比对照菌提高 53.8% 和 36.0%。巴西盛产乙醇,其乙醇发酵水平处于国际领先水平(包括利用甘蔗糖蜜等原料),最佳的工业酿酒酵母菌种为 CAT-1[63]和 PE-2[64],其中 CAT-1 菌株是来自 Fermentec 公司的商业化菌种,被广泛应用于南美酒精生产企业。Coimbra 等[65]将来自腔色链霉菌(*Streptomyces coelicolor*)的木糖异构酶基因在 CAT-1 菌株中进行

异源表达,最后构建出重组菌株 CAT-1-XIT(pRS42K::XI)。木糖乙醇发酵 132 h,重组菌株 CAT-1-XIT(pRS42K::XI)消耗 74%的 D-木糖,产生 12.6 g/L 乙醇(0.31 g/g 木糖),其乙醇产率接近 0.10 g/(L·h)。但异源表达的木糖异构酶的最适温度一般较高,在酵母生长所需的常温条件(如 30 ℃)下酶活很低,因此,木糖乙醇发酵效率不高,需要对菌种作进一步改良。近期,南京理工大学金明杰团队通过大数据挖掘、理性改造和祖先序列重构获得了 13 个在酿酒酵母中有活性的木糖异构酶,并以此构建出具有高效利用木糖能力的重组酿酒酵母菌株 CRD5HS,其以玉米秸秆和玉米芯为原料进行发酵,生产的燃料乙醇分别高达 85.95 g/L 和 94.76 g/L[66]。这为今后以酿酒酵母为底盘细胞的木质纤维素生物炼制提供了思路。

第三条改造思路:在酿酒酵母中异源表达木糖异构酶基因和 XR-XDH 途径相关的基因。沈煜等[67]将来自嗜热栖热菌的木糖异构酶基因、酿酒酵母木酮糖激酶基因整合至酿酒酵母工业菌株 NAN-227 染色体中表达;Ha 等[68]将粗糙脉孢菌(*Neurospora crassa*)的纤维糊精运输因子基因和 β-半乳糖苷酶基因,树干毕赤酵母的木糖异构酶基因(原始 XR 及突变 XR^{F276-1})、木糖醇脱氢酶基因和木酮糖激酶基因导入酿酒酵母,重组酵母能同步利用纤维二糖和木糖共发酵生产乙醇。

(2)木糖运输途径的改造

酿酒酵母木糖跨膜运输主要依靠细胞膜上的 HXT4、Gal2 等跨膜运输因子来完成,这些因子是高亲和力葡萄糖运输因子,但此运输过程会受到葡萄糖的强烈抑制[69]。Saloheimo 等[70]从能高效代谢戊糖的里氏木霉 cDNA 文库中,筛选到 *hxt1*、*trxlt1* 等 5 种戊糖的运输因子基因,这 5 种基因共表达能促进重组子利用木糖。Leandro 等[71]将中间假丝酵母同向转运因子(木糖/葡萄糖)基因导入酿酒酵母,提高木糖的跨膜运输能力。Madhavan 等[72]在酵母中超表达毕赤酵母糖转运蛋白(SUT1)基因、木酮糖激酶基因以及真菌 *Orpinomyces* 木糖异构酶基因,促进木糖转运和乙醇生成。

(3)通过其他方式的改造

Mig1 和 Snf1 是酿酒酵母葡萄糖阻遏效应的两个关键调控因子,分别由 *MIG1*、*SNF1* 基因编码。王正祥团队为提高酿酒酵母同时利用葡萄糖和木糖的能力,分别对 *MIG1* 和 *SNF1* 基因进行单敲除和双敲除,发现单敲除 *MIG1* 对共利用混合糖无影响,单敲除 *SNF1* 能加快混合糖中木糖的利用且两种糖能同时被利用;而双敲除能加快利用葡萄糖却使木糖的利用速度变慢,但由于两种糖能同时被利用,最终加快了乙醇的生成[73]。Tran 等[74]利用 DNA 组装方法对戊糖磷酸途径的靶点基因进行过量表达,构建酿酒酵母重组菌株 XUSEA,该菌株木糖消耗量和乙醇产量增加两倍,并且共发酵时间缩短一半。本团队也开展了木糖产乙醇的工业酿酒酵母菌株(野生型工业酿酒酵母菌株 MF01)构建等相关工作,主要针对一些转录因子,目前取得了一定的进展。

3.2 产木糖乙醇的运动发酵单胞菌基因工程菌

运动发酵单胞菌是目前唯一利用 ED 途径(Entner-Doudoroff pathway,ED pathway)在厌氧条件下生成乙醇的微生物,其能高效转化己糖产乙醇,是当前乙醇发酵能力较强的细菌之一,但不能利用戊糖生产乙醇。为此,Seo 等[75]和 Kouvelis 等[76]分别对 ZM4、NCIMB 11163 菌株进行全基因组测序,并公布相应的基因组序列,为后续开展相关的研究打下基础。Zhang 等[77]将来自大肠杆菌的 *xylA*、*xylB*、*talB* 和 *tktA* 基因导入运动发酵单胞菌,重组菌 CP4 能利用木糖生产乙醇,产率为 0.44 g/(L·h),发酵效率为 84%,同时将上述基因整合至染色体,结果发现重组菌的乳酸产量减少,乙醇产率无变化。张颖等[78]将大肠杆菌木糖代谢途径的关键酶基因导入运动发酵单胞菌构建重组子,进行木糖和葡萄糖共发酵,发酵效率分别为 63.1%和 81.2%。Sarkar 等[79]采用系统适应性实验室进化策略,在严格的木糖浓度增加的选择压力下对重组菌 ATCC ZW658 进行驯化(200 d),选育出优良菌株,该菌株的木糖利用率比原始菌株提高 1.65 倍。

3.3 产木糖乙醇的大肠杆菌基因工程菌

大肠杆菌是一种理想的模式生物,在基因工程中的研究最广泛、最深入,具有基因组小、生长迅速等特点。孙金凤等[80]和谢丽萍等[81]将运动发酵单胞菌的乙醇脱氢酶基因、丙酮酸脱羧酶基因在不同启动子控制下导入大肠杆菌,成功构建能利用木糖产乙醇的重组子。Ingram 等[82]首次将含有 *pet* 操纵子的质粒转化大肠杆菌,重组子能使己糖、戊糖代谢生成的丙酮酸流向乙醇生成方向。Dien 等[83]和 Hespell 等[84]构建含 *pet* 操纵子质粒的重组菌,其在不含抗生素的培养基中发酵能维持遗传稳定性,乙醇浓度为 3.9%-4.2%(*W*/*V*)。另外,表达 *isc* 基因簇能使大

肠杆菌 KO11 菌株的乙醇产量提高、乙醇耐受性增强[85]。此外,大肠杆菌 SSK101 菌株缺失 *pgi* 基因,对糠醛和 5-羟甲基糠醛(5-HMF)的耐受性增强,且葡萄糖和木糖共发酵的特性也得到改善[86]。在大肠杆菌 RM10 菌株中过量表达乙醛脱氢酶基因 *aldB*,重组子在生长速度和木糖乙醇产量方面均比原始菌株有优势[87]。

正如前文所述,对于木糖乙醇发酵,目前最为理想的菌种是树干毕赤酵母[50-52],但其与酿酒酵母基因工程菌相比,后者在乙醇耐受性等方面具有显著的优势。因此,鉴于上述的研究及总结,笔者认为木糖乙醇发酵最有应用前景的微生物是酿酒酵母基因工程菌。

4 利用传统诱变方法改良木糖乙醇菌种

菌种诱变改良方法有物理诱变、化学诱变、物理诱变结合化学诱变等方法。物理诱变常用紫外线(UV)、放射性元素如 Co^{60} 等,化学诱变常用硫酸二乙酯(DES)、亚硝基胍(NTG)等。这些传统的诱变方法的优点是能够提高突变率,在较短的时间内获得更多的优良变异类型,但其缺点是有益突变频率仍然较低,诱发突变的方向难以控制,突变体难以集中多个理想性状。

任佳等[88]利用 Co^{60} 诱变管囊酵母,获得一株优良菌株 800-3,其发酵 50 g/L 木糖 72 h,木糖消耗率和乙醇浓度分别比原始菌株高 52.2%和 120%,但其糖醇转化率为 0.14 g/g,发酵效率为 30.43%,乙醇产率为 0.08 g/(L·h)。Zhao 等[89,90]和赵磊[91]利用紫外线、微波和硫酸二乙酯诱变管囊酵母,结果发现紫外线和硫酸二乙酯复合诱变效果最佳,选育出两株糖醇转化率提高(分别为 0.17 g/g 和 0.18 g/g,分别比原始菌株提高 23.9%和 28.7%)的菌株。Watanabe 等[92]利用紫外线诱变树干毕赤酵母菌株 NBRC1687,选育出一株优良突变菌株 PXF-58,该突变株的乙醇浓度为 5.45%(糖醇转化率为 0.38 g/g),比原始菌株提高 38.68%(表 2)。

Morais 等[47]选育的木糖乙醇菌株中(包括一些新种),发酵性能较为优良的是树干毕赤酵母,发酵效率超过 82%;休哈塔假丝酵母的发酵效率能达到 78.26%,但比树干毕赤酵母略低;运动发酵单胞菌 A3 的木糖乙醇发酵效率相对较高,达到 88.20%[45]。目前,以木糖为原料发酵,乙醇含量较高的菌种是拟青霉。利用该菌种分别进行 150、200 g/L 木糖发酵,乙醇浓度相应为 7.56%和 9.31%,糖醇转化率分别为 0.39、0.38 g/g,发酵效率均为 75%,但发酵周期过长(分别需要 12 d 和 13 d),乙醇产率相应为 0.21、0.24 g/(L·h)[33]。本研究团队在 Co^{60} 诱变选育高产菌株的基础上,通过长期驯化途径,最终选育构建出高产菌株 31.1(专利菌株保藏在中国典型培养物保藏中心,保藏号为 M20211067),但 31.1 菌株的生长速率比原始低产菌株慢。高产菌株 31.1 的木糖乙醇发酵效率为 82.61%,乙醇产率为 0.78 g/(L·h),均比拟青霉高。尽管高产菌株 31.1 的发酵效率低于树干毕赤酵母 CBS 5576 菌株(97.83%),但其乙醇产率比 CBS 5576 菌株[0.34 g/(L·h)]高。此外,高产菌株 31.1 的发酵效率与最近报道的两株树干毕赤酵母 UFMG-CM-Y2303、UFMG-CM-Y2108 菌株相差不大,但这两株菌株的木糖乙醇产率分别为 0.39、0.40 g/(L·h),明显比高产菌株 31.1 低(表 2)。因此,高产菌株 31.1 的木糖乙醇发酵性能在国内同类研究中处于前列水平(数据未发表)。综上所述,采用传统诱变育种方法具有一定的成效。

表 2 木糖乙醇发酵性能的比较

Table 2 Comparison of xylose ethanol fermentation performance

菌种名称 Name of strain	木糖/(g/L) Xylose/(g/L)	糖醇转化率/(g/g) Conversation of xylose to ethanol/(g/g)	发酵效率/% Fermentation efficiency/%	乙醇产率/[g/(L·h)] Ethanol yield/[g/(L·h)]	文献 Reference
Paecilomyces sp. NF1	150	0.39	75.00	0.21	[33]
P. sp. NF1	200	0.38	75.00	0.24	[33]
Pachysolen tannophilus RL-171	160	0.10	21.74		[34]
P. tannophilus RL-171	200	0.06	13.04		[34]
P. tannophilus NRRL-Y2460	115	0.24	52.17		[37]
P. tannophilus NRRL-Y2460	158	0.21	45.65		[37]
Candida shehatae CBIR-Y492	50	0.36	78.26		[39]

续表

Continued table

菌种名称 Name of strain	木糖/(g/L) Xylose/(g/L)	糖醇转化率/(g/g) Conversation of xylose to ethanol/(g/g)	发酵效率/% Fermentation efficiency/%	乙醇产率/[g/(L·h)] Ethanol yield/[g/(L·h)]	文献 Reference
C. shehatae CBIR-Y492	90	0.29	63.04		[39]
C. tropicalis ATCC 1369	75	0.11	23.91		[40]
Pichia stipitis CBS 5576	50	0.45	97.83	0.34	[41]
P. segobiensis CBS 6857	20	0.25	54.35	0.02	[42]
P. stipitis NRRL Y-7124	30	0.28	60.87		[43]
Spathaspora passalidarum NRRL Y-27907	30	0.35	76.09		[30]
Pestalotiopsis sp. XE-1	97.71	0.34	73.91	0.06	[44]
Zymomonas mobilis A3	100	0.45	88.20	0.94	[45]
S. hagerdaliae UFMG-CM-Y303	28.2	0.28－0.36	60.87－78.26	0.06－0.13	[46]
C. maltosa UFMG-CM-Y2131	30	0.12	23.97	0.09	[47]
Scheffersomyces sp. UFMG-CM-Y365	30	0.25	49.81	0.22	[47]
S. stipitis UFMG-CM-Y2303	30	0.42	82.00	0.39	[47]
S. stipitis UFMG-CM-Y2108	30	0.42	83.00	0.40	[47]
Spathaspora boniae UFMG-CM-Y306	30	0.19	38.02	0.25	[47]
S. boniae UFMG-CM-Y363	30	0.22	43.87	0.29	[47]
Sugiyamaella xylolytica UFMG-CM-Y348	30	0.14	28.00	0.08	[47]
Wickerhamomyces sp.1 UFMG-CM-Y6241	30	0.14	28.54	0.05	[47]
Saccharomyces cerevisiae CAT-1-XIT(pRS42K::XI)	30	0.31	67.39	0.10	[69]
Pachysolen tannophilus 800-3	50	0.14	30.43	0.08	[88]
P. tannophilus 1770-7	20	0.17	36.96		[91]
P. tannophilus 1770-11	20	0.18	39.13		[91]
Pichia stipitis NBRC 1678	114	0.27	59.12	0.50	[92]
P. stipitis PXF58	114	0.38	82.00	0.60	[92]
P. stipitis CICC1960[a]	150	0.31	67.39	0.55	Our team
P. stipitis 1K-9[a]	150	0.34	73.91	0.61	Our team
P. stipitis 12.1[b]	150	0.29	63.04	0.43	Our team
P. stipitis CICC1960[b]	150	0.33	71.74	0.66	Our team
P. stipitis 31.1[b]	150	0.38	82.61	0.78	Our team

Note:[a] means value of fermentation for 84 h,[b] means value of fermentation for 60 h.

5 展望

尽管运动发酵单胞菌具有很强的木糖乙醇发酵性能,但其为细菌,在工业应用上具有一定的局限性。酵母为工业上应用较为广泛的微生物之一,在工业应用上具有一系列优势。而对于纤维素乙醇高效低成本生产的技术研发,关键因素之一在于选育能高效发酵木糖产乙醇的酵母菌种。为此,笔者认为未来的研究应着眼于以下两点。

(1)选育及改良非传统酵母

随着世界各国研究人员的努力,从自然界选育构建的非传统酵母会愈来愈多。未来期望能从自然界

选育出新的、能高效利用木糖的非传统酵母菌株,并采用传统诱变手段或结合现代分子生物学技术等手段对其进行改良。

(2)持续改良酿酒酵母或选育新的酿酒酵母

作为传统的、常用的酒精发酵生产菌种,酿酒酵母具有一系列显著的优势。因此,笔者认为应继续选择酿酒酵母作为木糖乙醇发酵的改良对象,其将是最有应用前景的微生物。其一,随着人工智能、合成生物学等技术的快速发展,尤其是人工智能软件 AlphaFold2 在蛋白质结构预测领域取得巨大进展,采用人工智能技术等手段会助力木糖产乙醇的酿酒酵母微生物菌种的选育构建;其二,随着太空育种技术的进步,未来将采用新的诱变育种技术,选育木糖产乙醇的酿酒酵母微生物菌种;其三,未来期望能选育出新的、能直接利用甚至高效发酵木糖的酿酒酵母菌株,并采用传统诱变手段或结合现代分子育种技术等手段对其进行改良。随着国家大力支持种业的发展,包括一系列的政策扶持和研发投入的加大等,相信在不久的将来,适用于纤维素乙醇高效低成本产业化生产的良种酵母将会出现在市场上并得以推广应用。

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Research Progress on Breeding of Microorganisms for Producing Ethanol from Xylose

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Abstract:The second highest content in sugarcane bagasse hydrolysis products is xylose. The efficient conversion of xylose to ethanol by microorganisms is currently one of the key pathways in the comprehensive utilization of bagasse fibers. However,wild-type *Saccharomyces cerevisiae* could not produce ethanol by xylose fermentation,which has become one of the main bottlenecks in the industrialization of cellulosic ethanol. In

this article, the research progress on the breeding of xylose ethanol producing microorganisms was reviewed from four aspects: microbial xylose metabolic pathway, microorganisms using xylose to produce ethanol, construction of xylose ethanol-producing genetic engineering bacteria, and improvement of xylose ethanol-producing strains by traditional mutation methods. One of the most ideal microbial strains and the most promising microorganisms for xylose ethanol fermentation are *Pichia stipitis* and *S. cerevisiae* genetically engineering bacteria were pointed out, respectively, which provides a theoretical basis for improving xylose ethanol microorganisms.

Key words: xylose; ethanol; metabolic pathways; *Pichia stipitis*; *Saccharomyces cerevisiae*

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