Disrupting sfa1 Gene to Enhance Biosynthesis of Ethanol in \textit{Saccharomyces cerevisiae} \textsuperscript{*}

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Abstract: The sfa1 gene encoded a bifunctional enzyme with the activities of both alcohol dehydrogenase and glutathione-dependent formaldehyde dehydrogenase in \textit{Saccharomyces cerevisiae}. The gene disruption cassette produced by PCR using the same long oligonucleotides which comprise 19 or 22 nucleotides complementary to sequences in the templates (pUG6 and pUG66 marker plasmid) at 3' end and 45 nucleotides at 5' end that annealed to sites upstream or downstream of the genomic target sequence to be deleted. After two linear disruption cassettes with a Cre/loxP mediated marker were transformed into the cells of \textit{Saccharomyces cerevisiae} YS9, the positive transformants were checked by PCR to correct the integration of the cassette and concurrent deletion of the chromosomal target sequence. Once correctly integrated into the genome, the selectable marker can be efficiently rescued by transforming the plasmid pSH47 into YS9 and inducing the Cre expression with a Cre/loxP-mediated marker removal procedure. The expression of the Cre recombinase finally resulted in the removal of the marker gene, leaving behind a single loxP site at the chromosomal locus. The diploid mutant YS9- sfa1 was generated, which could enhance the output of ethanol with 8.0% by shaking culture in flask compared with the original strain YS9.

Key words: \textit{Saccharomyces cerevisiae}; Gene disruption; sfa1; PCR

材料与方法

1.1 材料

1.1.1 原种及质粒：酿酒酵母（\textit{Saccharomyces cerevisiae}）
是扩增筛选标记的正反向引物。草霉素、分子生物学试剂等分别购于

引物(表

菌

引物

带有

扩增方法

测序验证后共转化

培养基:酵母提取物

、蛋白胨

氯化铵

、磷酸二氢钾

酵母,随后将

灭菌

培养基中连续传代丢失质粒

质粒为模板,用

酶切除

酶,带

扩增筛选标记的反向引物

序列

扩增筛选标记的正向引物

系统:依据

图

得到基因敲除组件

基因敲除组件的验证:将目的产物连接到

基因敲除组件的构建:以质粒

酵母

感受态细胞,醋酸锂转化

感受态细胞,分别转化质粒

平板上,

进行

进行

目的产物

DNA

条件:

pMD-18T

pMD-18T

DH5α

pUG6/pUG66

pSH47

sfa1

克隆子的筛选:菌液涂在含

条件

克隆子

pSH47

sfa1

sfa1

sfa1

sfa1

sfa1

sfa1

sfa1

sfa1

sfa1

sfa1

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sfa1
克隆子的验证:挑取性克隆子,液体培养提取基因组作为模板,进行两次验证。用引物,进行初次验证,条件:

随后用引物进行二次验证,条件:

筛选标记的切除:将质粒转入阳性克隆子,在乳糖培养基中摇床培养,C,取适量涂布平板,获得单菌落,分别影印含或腐草霉素的不同平板,筛选和抗性标记丢失的菌株。提取基因组,用引物进行验证。

突变株发酵产乙醇分析:将菌株和突变株分别接种发酵培养基进行摇瓶发酵。气相色谱分析发酵液中乙醇含量。色谱分析条件为:

结果酵母菌基因验证以B@基因组为模板,用引物进行产物大小与预计条带大小相符,表明B"基因存在。图B@基因验证J .55K? &'( J9LM<L,产物:$.07K?,产物:$.7#K?,与预计片段大小相符。

敲除组件基因测序验证对敲除组件进行测序,测序结果如下(图!,图#),与报道序列进行比对,完全一致。
图1

验证克隆子

挑取平板上典型克隆子单菌落（图1），液体培养后提取基因组DNA作为模板，用引物#%$进行初次验证，扩增产物大小与预期结果相符。其他引物/#-%、/-%0123、0124#-二次验证也分别得到预计目的条带（图6）。表明!#$和!#$敲除组件与目标基因!#$发生了正确重组。

4.2

2.4

G418 DNA L1-L2 A-D A-KanB KanC-D A-BleB BleC-D sfa1-1 sfa1-2

A YS1 B YS2 1D 3B 5H 5S

2.5

pSH47 Cre Kan' Blé' sfa1 loxP L1-L2 A-D PCR 196bp 1666bp

pSH47 PCR sfa1 YS1-sfa1

2.6

YS1-sfa1

突变株乙醇产量较出发菌株提高。

图B

和

乙醇发酵产乙醇曲线

讨论

突变株乙醇产率提高，说明...

表

图

YS1-sfa1

YS1

YS1-sfa1

YS1

YS1-sfa1

YS1

乙醇含量的变化

图

YS1-sfa1

YS1

YS1-sfa1

YS1

8.0%
基因缺失在促进乙醇合成方面起到了一定作用。由于酿酒酵母乙醇代谢受到ADH1、ADH2和ADH3等其它相关基因的调节,同时酵母乙醇耐受能力还受到诸如超氧化物歧化酶、超氧化物歧化酶和质膜脂肪酸等因素的影响,所以基因若能结合其它相关基因的敲除会对酵母乙醇合成产生更显著的效果。本研究通过系统两种筛选标记共转化方法成功地获得基因缺失突菌株。此方法能在不影响其它基因的基础上准确敲除目的基因,一次性就可得到双倍体缺陷型菌株,方便易行。需要指出的是,如果连续敲除基因,还需考虑酶发挥作用的范围及单个或多个位点本身重组对菌株的影响。该方法获得的突变菌株其突变发生在染色体水平,能够稳定遗传,并具备无需添加其它生长因子就能生长的优势。

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