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HMG-CoA还原酶和FPP合酶基因拷贝数对紫穗槐-4.11-二烯酵母工程菌产量的影响

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摘要:

从青蒿中克隆了紫穗槐-4,11-二烯合酶基因(ADS),从酿酒酵母中克隆了HMG-CoA还原酶(HMGR)催化结构域和 FPP合酶(FPPS)基因。构建含ADS基因的酿酒酵母表达载体oYeDP60/G/AS,将其转入酿酒酵母,获得能产生紫穗 槐-4,11-二烯的酵母工程菌,以朱栾倍半萜为参照,产量为10 μ g·L⁻¹。另外构建了含HMGR和FPPS基因的酵母表 达载体pGBT9/A/HMG/G/FPP,将该载体和pYeDP60/G/AS共转入酿酒酵母,得到第二个能产生紫穗槐-4,11-二烯 的酵母工程菌,产量为 23.6 mg·L^{-1} ,结果表明增加HMG-CoA还原酶和FPP合酶基因的拷贝数能显著增加酵母工程 菌的紫穗槐-4,11-二烯产量。

关键词: 紫穗槐-4.11-二烯 HMG-CoA还原酶 FPP合酶 酵母工程菌

Increase of copy number of HMG-CoA reductase and FPP synthase genes improves the amorpha-4,11-diene production in engineered yeast

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Abstract:

The gene encoding amorpha-4,11-diene synthase was cloned from Artemisia annua L. Other two genes encoding the FPP synthase (FPPS) and HMG-CoA reductase (HMGR) were cloned from Saccharomyces cerevisiae. The cloned cDNAs were confirmed by DNA sequencing. Two expression vectors were constructed, one is named pGBT9/A/HMG/FPP harboring genes for HMG-CoA reductase and FPP synthase and the other is pYeDP60/G/AS, containing the gene encoding amorpha-4,11-diene synthase. Two kinds of engineered yeast were constructed: the first was named WHT [AS], which contained the plasmid pYeDP60/G/AS; the second was WHT [HMG+FPP+AS], in which the vectors pGBT9/A/HMG/FPP and pYeDP60/G/AS were introduced by cotransformation mediated with LiOAc and PEG4000. The positive clones were identified for further fermentations. The samples from fermentations were analyzed by GC-MS for amorpha-4,11-diene. The results show that engineered yeasts could produce amorpha-4,11diene. Moreover, the amorpha-4,11-diene production of WHT [HMG+FPP+AS] and WHT [AS] were 23.6 mg·L $^{-1}$ and 10 μ g·L $^{-1}$, respectively. Its concentrations were reported as equivalents of valencene. The results showed the copy number increase of HMGR and FPPS genes can improve the production of amorpha-4,11-diene in the fermentation of engineered yeasts.

Keywords: HMG-CoA reductase FPP synthase engineered yeast amorpha-4,11-diene

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