Effects of adhE Gene Knock-out on Hydrogen Evolution of Klebsiella oxytoca HP1

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Abstract: Klebsiella oxytoca HP1 is a potential H_2-producing bacterium we screened from a hot spring. In the present study, metabolic engineering strategy was carried out to construct ethanol pathway mutants by homologous recombination, in order to improve hydrogen production yield. Metabolic fluxes of the wild and the mutants were analysed and compared.

Key words: metabolic engineering; hydrogen production; Klebsiella oxytoca HP1; adhE
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Klebsiella oxytoca HP1[1] is a potential H_2-producing bacterium we screened from a hot spring. However, the yield of H_2 from glucose of this bacterium is less than 2 mol/mol glucose, which is only half of the maximum yield of dark hydrogen fermentation. For industrial-scale H_2 production by K. oxytoca, enhancement of its H_2 production ability is needed[2-3].

In the present study, metabolic engineering strategy was carried out to construct ethanol pathway mutants by homologous recombination.

1 Experiments

The Construction Strategy of the homologous recombination plasmid pTA-Str is show in Fig. 1. An internal fragment of K. oxytoca HP1 adhE gene was amplified by PCR from genomic DNA with degenerate oligonucleotide primers designed from conserved regions of adhE gene sequences. By inserting an aadA cassette into the coding region of operon gene adhE +694 ~ +695 district in pTA, the homogenetic integration plasmid vector pTA-Str which was used to transform K. oxytoca HP1 wild-type strain was constructed and a mutant with resistance to Streptomycin was obtained.

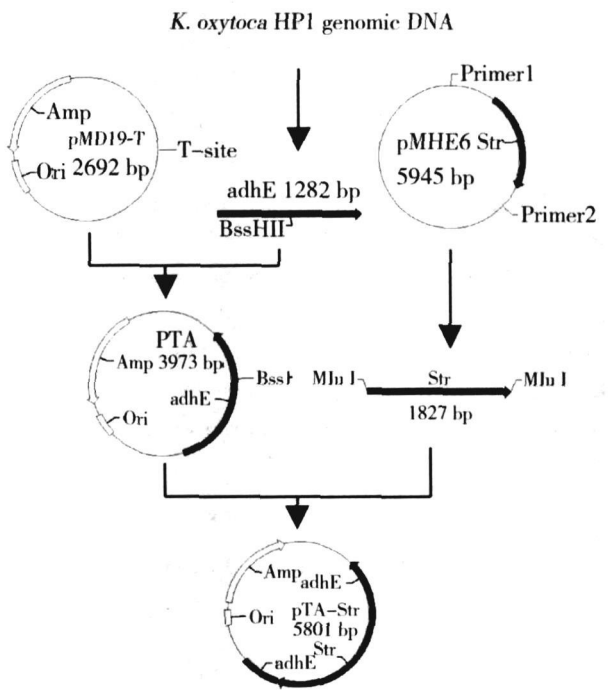

Fig. 1 Strategy of vector construction for adhE gene knockout

Metabolites were analyzed by HPLC and gas chromatograph[4].

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2 Results

PCR analysis of the genomic DNA from constructed mutants indicated that the appropriate deletion and insertion indeed occurred. Batch cultures were carried out to examine metabolic engineering operation. H_2-producing metabolic network of *K. oxytoca* HP1 is showed in Fig. 2.

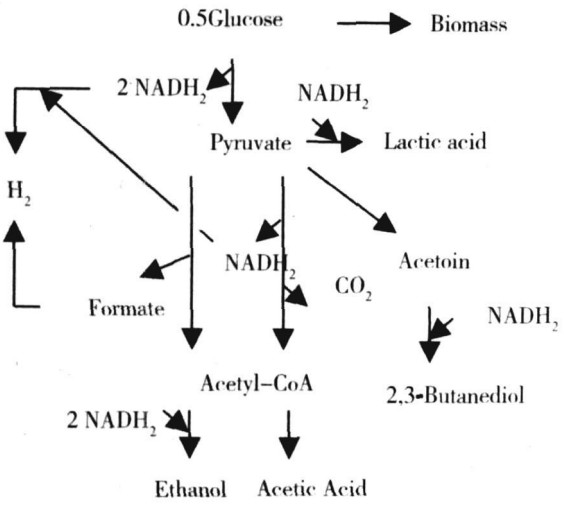

Fig. 2 Metabolic network of *K. oxytoca* HP1

The deletion mutant displayed similar growth compared to the wild-type. Biomass and metabolites formed were list in Table 1. Comparison to the wild-type, ethanol, acetate, lactate and H_2 of the mutant were decrease by 69.9%, and increased by 56.5%, 105.9%, 9.4%, respectively. It seemed that the reductant saved in ethanol pathway was not captured by hydrogenase of *K. oxytoca* HP1 for hydrogen production, but redirected to lactate-producing pathway. Ethanol pathway node may be a rigid node for H_2 production of *K. oxytoca* HP1 on glucose fermentation.

Tab. 1 Metabolic fluxes of the wild and the mutant of glucose fermentation

Metabolites	Acetate	Ethanol	Lactate	H_2	Biomass
Wild	64.68	107.48	25.64	13.2	178.4
mutant	101.2	32.4	52.8	11.2	195.2
%	56.5	-69.9	105.9	-15.2	9.4

The results reported here represent a step toward using metabolic engineering to developing strains of H_2-producing bacteria with high yield.

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Klebsiella oxytoca HP1乙醇脱氢酶基因敲除对产氢的影响

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摘 要: *Klebsiella oxytoca* HP1是从温泉筛选得到的很有潜力的产氢菌株。为了进一步提高产氢能力, 应用同源重组技术构建了乙醇途径缺少菌株, 并分析和比较了野生菌和工程菌的代谢流量分布。

关键词: 代谢工程; 发酵产氢; *Klebsiella oxytoca* HP1; adhE基因
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