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Engineering the yeast *Saccharomyces cerevisiae* for heterologous production of 2-hydroxypropiophenone

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Abstract

2-Hydroxypropiophenone (2-hpp) is a chiral 2-hydroxyketone with interest in pharmaceutical industry. Besides its decarboxylase activity, benzoylformate decaroboxylase (BFD) has a carboligase activity through which it can condense benzoylformate and acetaldehyde to yield 2-hpp. BFD was originally found in *Pseudomonas putida*. Here, we introduced the BFD coding gene from *P. putida* into the yeast *Saccharomyces cerevisiae* using CRISPR-Cas9 system. BFD was expressed under control of *TDH3* promoter. The engineered yeast is able to accumulate 2-hpp in the medium.

Keywords: 2-hpp, BFD, CRISPR/Cas9, *P. putida, S. cerevisiae*

Introduction

Wilcocks and Ward (1992) found out that *Pseudomonas putida* can use mandelate as the sole carbon source through mandelate pathway. They also discovered benzoylformate decarboxylase (BFD) is one of the enzymes in the mandelate pathway which catalyzes conversion of benzoylformate to benzaldehyde through a decarboxylation reaction. In the presence of acetaldehyde, benzoylformate decarboxylase enzyme can catalyze a carboligation reaction and condense benzoylformate and acetaldehyde to produce 2-hpp. The yeast *Saccharomyces cerevisiae* is an old friend of human. It is one of the GRAS (Generally Regard as Safe) organisms which is widely used in industry for production of biological products. In this study, we expressed BFD coding gene from *P. putida* into the yeast *S. cerevisiae* CEN.PK 113-7D. The BFD coding gene was integrated into the genome of yeast under control of *TDH3* promoter. For this purpose, a vector set for marker-free integration of genes into the genome of *S. cerevisiae* using CRISPR-Cas9 system with nearly 100% efficiency was utilized (Jessop-Fabre et al. 2016).

Materials and Methods

All vectors were obtained from Novo Nordisk Center of Biosustainability at Technical University of Denmark (DTU).

The gene and the promoter were amplified by VeraSeq Ultra DNA Polymerase (Qiagen). For the amplification of the gene and the promoter, 4 primers containing uracil were designed by Oligo7. The vectors were digested by restriction enzymes AsiSI/SfaAI (Thermo Fisher Scientific) and Nb.BsmI (NEB) and sticky ends were achieved. USER reaction (biobricks and ready to use vector) was placed into a 0.2 mL microtube and incubated in PCR machine in a gradient temperature (37 to 10 °C).

For confirmation of cloning, 10 colonies were randomly selected and used for colony PCR. For colony PCR, two designed primers form EasyClone-MarkerFree were used.

Integrative vectors were linearized by NotI restriction enzyme and transformed to *S. cerevisiae* CEN.PK 113-7D. Integration of pCfB2904 vector was confirmed by colony PCR. After fermentation, 2-hpp was extracted from 2 mL of samples with 2 mL dichloromethane by 5 min vortex. Samples were analyzed by GC from Agilent (Agilent Technologies 6890N, USA) equipped with HP-5 column and FID detector.

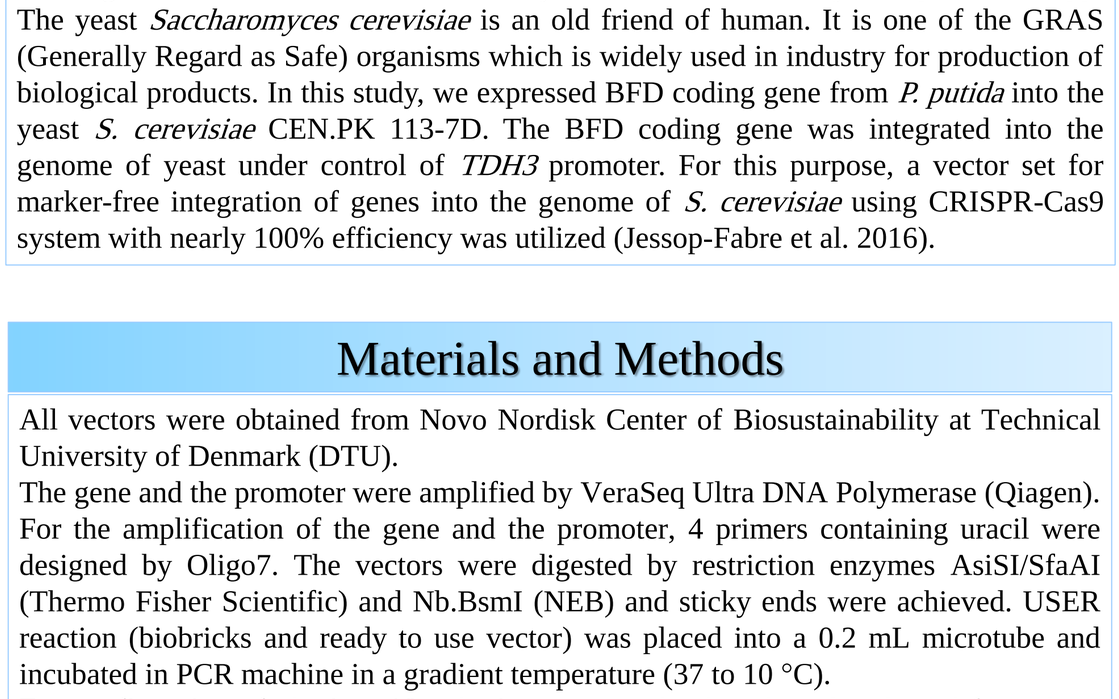

Fig. 1. Production, extraction and analysis of 2-hpp produced by engineered yeast strain.

Results

It was necessary to purify the integrative vector from agarose gel after digestion to prevent rejoining digested fragments. Concentration of PCR products and digested vector was measured with Nano Drop OneC (Thermo Scientific). USER cloning was successful and positive colonies were observed. The vectors were then transformed into *Escherichia coli* and the transformation was confirmed by colony PCR. The integrative vector was linearized by NotI and the integrative part was purified from agarose gel and transformed to *S. cerevisiae* CEN.PK 113-7D with 2 other vectors (pCfB3045 and pCfB2312).

GC analysis confirmed production of about 0.2 g/L 2-hpp after 12 hours.

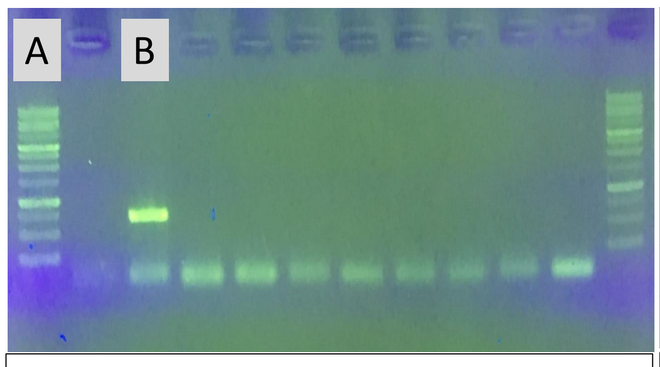

Fig. 2. B is 911 bp band on agarose gel which conformed integration in the genome. A is 10 kbp Ladder.

Biobricks	Concentration
MdlC Gene	118.4 ng/µL
TDH3 promoter	139.9 ng/µL
Ready to use pCfB2904 vector	893.9 ng/µL
Closed pCfB2904 vector	73.5 ng/µL
pCfB3045 vector	537.3 ng/µL

Table 1. Concentration of biobricks and vectors.

Discussion, Conclusion and Suggestions

The yeast *S. cerevisiae* as one of the best and most widely used cell factories was used for heterologous production of 2-hpp. The *MdlC* gene from *P. putida* which encodes BFD enzyme was integrated into the chromosome XI of *S. cerevisiae* CEN.PK 113-7D under the control of *THD3* promoter as a strong promoter in yeast. CRISPR-Cas9 system is a powerful tool for metabolic engineering of the yeast and enables easy genome engineering of industrial diploid and polyploid stains. After fermentation and analysis with GC about 0.2 g/L 2-hpp was produced in 12 h. It is important to characterize all parameters in fermentation process to get maximum production. Also, it is suggested to find the rare codons of the gene and use the codon optimized gene.

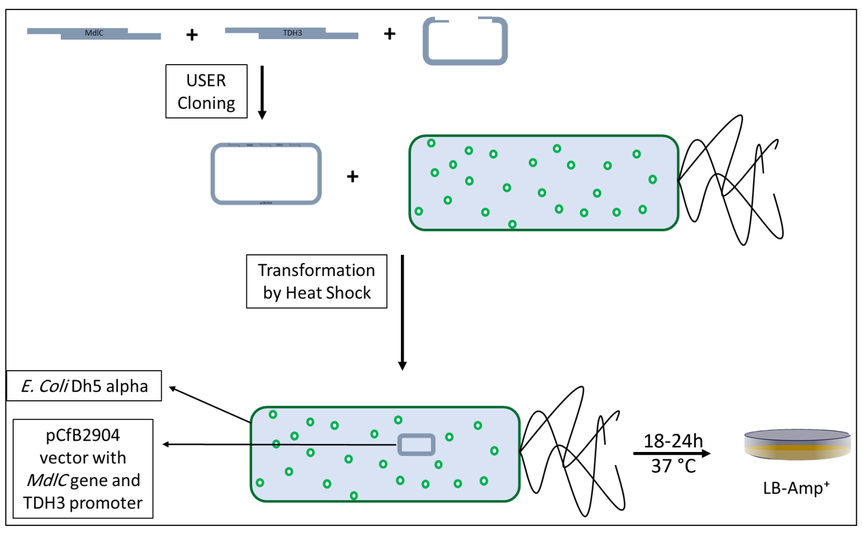

Fig. 3. USER cloning and transformation to *E. coli* DH5alpha.

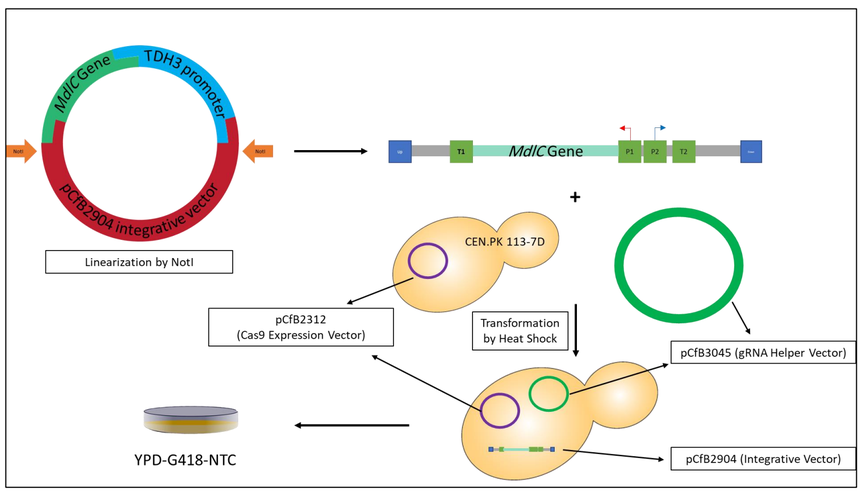

Fig. 4. Linearization of integrative vector and transformation to *S. cerevisiae* CEN.PK 113-7D with other vectors.

References

- Jessop-Fabre MM, Jakočiūnas T, Stovicek V, Dai Z, Jensen M, Keasling J, Borodina I. 2016. "EasyClone-MarkerFree: A vector toolkit for marker-less integration of genes into *Saccharomyces cerevisiae* via CRISPR-Cas9." Biotechnology Journal, 11: 1110-1117.
- Wilcocks R, Ward OP. 1992. "Factors affecting 2-hydroxypropiophenone formation by benzoylformate decarboxylase from *Pseudomonas putida*." Biotechnology and Bioengineering, 39: 1058-1063.
- Ward OP, Wilcocks R, Prosen E, Collins S, Dewdney NJ, Hong Y. 1992. "Acyloin formation by benzoylformate decarboxylase from *Pseudomonas putida*." Applied and Environmental Microbiology 58: 1699-1704.