Cloning and Expression of a 2-Oxoglutarate-Dependent-Dioxygenase from Phellinus Igniarius

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Abstract

The mycelia was obtained by culturing Phellinus igniarius as the material, and then cDNA was obtained by reverse transcription. The 2-oxoglutarate-dependent-dioxygenase (2-ODD) gene was obtained by PCR using specific primers. The PCR products and the prokaryotic expression vector were cleavage with same restriction enzyme and then linked to transform into E. coli DH5α. The plasmids were extracted and sequenced. The the successfully constructed plasmid was transferred into E. coli BL21 and induced to produce protein. Finally, the target protein was detected by SDS-PAGE. In the experiment, the 2-ODD gene of P. igniarius was successfully obtained, and reached the prokaryotic expression.

Keywords

2-oxoglutarate-dependent-dioxygenase, Phellinus igniarius, Expression.

1. INTRODUCTION

Phellinus igniarius is an ancient Chinese medicinal material. It is a parasitic fungus named after the parasitic mulberry tree. It is also a large fungus with special medicinal value and has anti-cancer effect. It also has a good effect on lowering blood fat, preventing arteriosclerosis, preventing and treating rheumatoid arthritis, and anti-allergy. These pharmacological effects are closely related to people's life and health. The research on Phellinus igniarius has a good medical prospect, and other pharmacological effects of Phellinus igniarius have yet to be discovered.

2-oxoglutarate-dependent-dioxygenase (2-ODD) is an important enzyme involved in various oxidation reactions in vivo, involving primary metabolites and secondary metabolites in living organisms. Synthesis, in plants involved in the synthesis of hormones ethylene, gibberellins and flavonoids, the most typical of which include Gibberellin synthase (GA200x), flavonol synthase (flavonol Synthase, FLS), etc.

2. CLONING AND EXPRESSION OF 2-ODD FROM PHELLINUS IGNIARIUS

2.1. Phellinus igniarius cultivation and mycelium collection

Phellinus igniarius strains stored in the laboratory were transferred to a PDA liquid medium, and cultured at 28 ° C for 8 days, and then the strain was activated. Transfer the activated strain to fresh PDA liquid medium and incubate for 8 days at 150 rpm. Filtered hyphae with filter, and the hyphae obtained by filtration were added to an 80 ml centrifuge tube, centrifuged at 9000 r/min for 15 min, and the unused liquid was discarded. The mycelium was washed 6 times used PBS buffer and then dried for using.

2.2. Extraction of total RNA and PCR amplification 2-ODD gene

Total RNA was extracted from Phellinus igniarius and obtained cDNA by reverse transcription. Specific primers were designed and the 2-ODD gene was amplified by PCR.

PCR primer sequences:

P1 CTACTCGAGATGCTTGCGTCGCCCATCCTT

P2 AACCTGCAGTCATAAATATGTTTCGCTCA

2.3. Construction of recombinant plasmid

The target gene and the expression vector were linked and then transformed into E. coli DH5α, and the plasmid was extracted and verified.

2.4. Expression of the engineering strain

The successful recombinant plasmid was transformed into E. coli BL21, and the expression of the target protein was detected by SDS-PAGE.

3. EXPERIMENTAL RESULT

3.1. PCR amplification of 2-oxoglutarate-dependent-dioxygenase gene

The PCR product was analyzed by 1% agarose gel electrophoresis to obtain a target fragment of about 1092 bp.

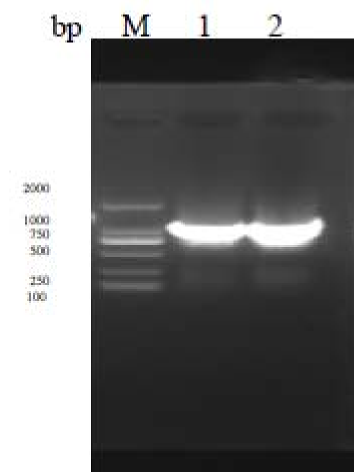

Figure 1. PCR product of 2-oxoglutarate-dependent-dioxygenase gene Phellinus igniarius

M: Marker; 1, 2: 2-oxoglutarate-dependent-dioxygenase

3.2. Construction of recombinant plasmid

The plasmid pSMART-V and the PCR product ligated after digested with XhoL I and Pst I, and then transformed into Escherichia coli DH5α. After overnight culture, a single colony was picked, the plasmid was extracted, and the result was analyzed by electrophoresis on the 1% agarose. In the figure, lane 3 is the empty vector pSMART-V, and lanes 1, 2 and 4 are recombinant plasmids, which 1 and 4 are slightly larger than the empty vector of pSMART-V, and the size is in accordance with expectations.

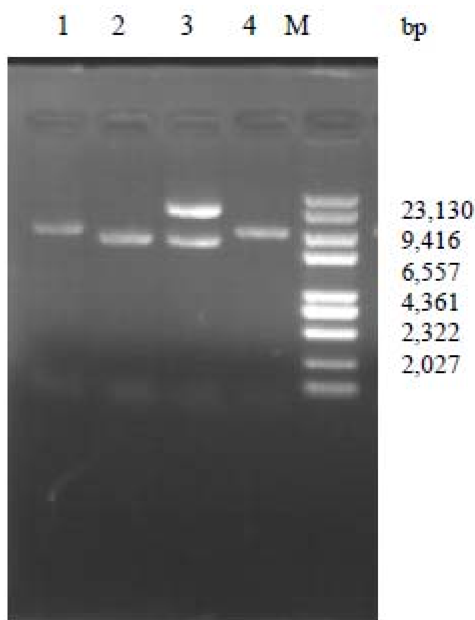

Figure 2. Construction of recombinant plasmid

M: Marke; 1, 3, 4: Recombinant plasmid; 2:pET22b

3.3. The expression of 2-ODD gene

The Escherichia coli BL21 strain containing the recombinant plasmid was cultured and induced, and the cells were collected, and then sonicated to collect supernatant and precipitation respectively, and analyzed by SDS-PAGE. It can be seen from the figure that the engineered strain obtained specific expression at 50KD compared with the Escherichia coli BL21 strain. Consistent with the expected molecular weight, indicates that the target product 2-ODD protein was expressed successfully.

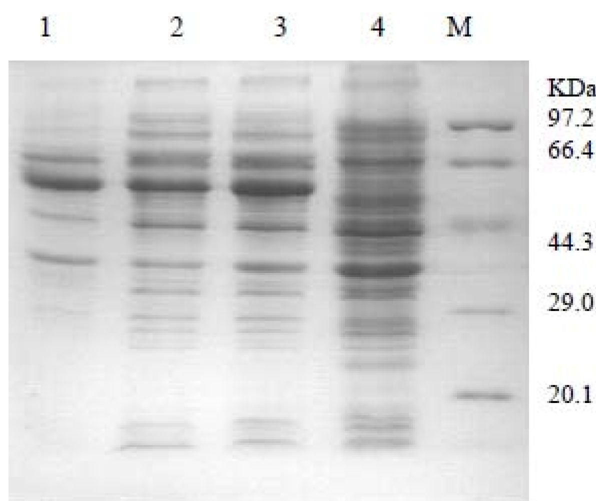

Figure 3. SDS-PAGE analysis of the expression of 2-ODD

1: precipitation; 2: supernatant; 3: Escherichia coli BL21 transferred into the recombinant plasmid; 4: Escherichia coli BL21; M: standard protein molecular weight

3.4. Gene sequence of 2-ODD from Phellinus igniarius

The sequence is as follows:

ATGCTTGCGTCGCCCATCCTTCGCTCACACGTCCCAGGTCGTTTTTCACTGAGTAAAGCACACCGTT
GTGCGTTCCGCGGGCTTGCGACACACGCGTCTGGATTTCGCATCCCGGTTATAGACTTCAATCGTTATC
AGATCGCAGCGTCGACGGACCAGAGGCAAGAAACTGCCAATGAAGTAGTAGATGGCTTCAAAGAGGC
TGGCTTTATCTACATCAAGAACCATGGAATCCCTGACTCAGTGATCACCAATGTCTTTCAGAAGAGCG
CATCTTTCTTCAAACTACCGGAAGATGTCAAGTCAAAGCTTGAGTGGGAGGACCCGCGTGCGAATCGT
GGTTACGTTCAGATAGGCAGAGAACGTGTAACTCAGTCGAGCGATCCCGATGAAATTGCAGCCATGCG
TTCCAAGTCTCCGGACTACAAGGAGACTATGGAAATTGGTCGTGACTGGGATCCTGTTTGGAGAAATC
GTTGGCCTCAGGAATCAGATTGCCCTGGCTTTAAGGATACAATGCTCAACTTCTTCCAGACATGTCAT
GAACTTCATGTCTCGGTTATGAGCTCGATAGCTCTTGGACTCAGTTTGAACGAAAGATTCTTCGACGA
TAAAATCAACGAGCAATACCATAACCTCCGACTCCTGTCTTATCCTCCTGTCAAAACCGAGATTCTTCG
TCAAGAGGGGCAGGCGAGAGCTGGAGCTCACTCAGACTATGGCACCCTGACTCTCCTATTTCAGGATT
CGGTCGGAGGTTTGGAAGTGCAGAATCCACATACTGGAGAATTTCACCCAGCGGTCCCAATCCCCGGT
ACAATCGTCATTAACGTCGGTGATCTTCTGAGCCGCTGGTCAAACGACATTTTACGCTCGACGCTCCA
TCGCGTCGTCGCACCACCTGCGAAACAGATCAGCGAGACCGAACAGGTCACCCCCGCTCGCCAGTCAA
TCGCATTTTTTTGCAATCCTGATTTCGGTGCACTCATCGACTGCTTGCCGAATTGTTACGGTCCGGAC
AAGGCGAAGAAATATGAACCAGTGACGACAGAGCAGTACATTGTACAACGGTTGAGCGAAACATATTT
ATGA

4. CONCLUSIONS

In this study, the 2-oxoglutarate-dependent-dioxygenase gene was cloned from Phellinus igniarius, and the soluble expression of 2-oxoglutarate-dependent-dioxygenase in E. coli was realized, which laid a foundation for further study of the function of 2-oxoglutarate-dependent-dioxygenase of Phellinus igniarius .

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