Cloning and Expression of a Thiolase Gene from Phellinus igniarius

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Abstract

To explore the expression of the thiolase gene from Phellinus igniarius in the prokaryotic system. The thiolase gene of P.igniarius was amplified by PCR using specific primers. The products of PCR and prokaryotic expression vector pET22b were digested separately, and then linked for recombinant plasmid, transformed into E.coli DH5α. Recombinant plasmid was identified by digestion, and samples were sequenced. The successfully constructed recombinant plasmid was transferred into E.coli BL21. Finally, protein expression was detected with SDS-PAGE.Through the experiment, the thiolase was obtained and the prokaryotic expression vector was successfully transferred into Escherichia coli to achieve prokaryotic expression.

Keywords

Thiolase, Phellinus igniarius, Expression.

1. Introduction

Modern research has found that *Phellinus igniarius* has good effects in immune regulation and improving the survival rate of cancer patients. Animal experiments have found that polysaccharide from *Phellinus* igniarius can play an immunomodulatory role in tissues such as blood, digestive system, and spleen system, and exhibits anti-cancer and tumor growth inhibition.

Thiolase plays an important role in the biosynthesis and degradation of fatty acids, and it is a class of biocatalysts that are ubiquitous in both eukaryotes and prokaryotes.

2. Cloning and expression of thiolase 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 thiolase gene

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

PCR primer sequences:

P1 CCGCATATGAATGGCAGTTGCCAAGAGA

P2 CCCAAGCTTAGTTTCCTGAAACATCTTC

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 thiolase gene

The PCR product was analyzed by 1% agarose gel electrophoresis to obtain a target fragment of about 1400 bp.

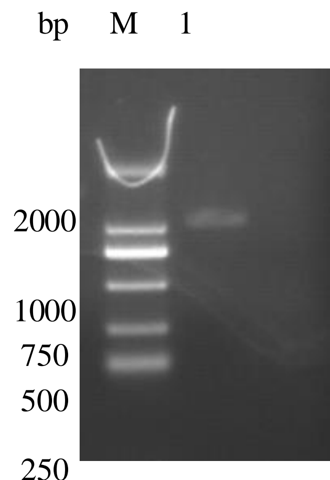

Figure 1. PCR product of thiolase gene Phellinus igniarius
M:Marker; 1: thiokinase gene

3.2 Construction of recombinant plasmid

The plasmid pET22b and the PCR product ligated after digested with *Bam*H I and *Hin*d III, 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 2 is the empty vector pET22b, and lanes 1, 3 and 4 are recombinant plasmids, which are slightly larger than the empty vector of pET22b, and the size is in accordance with expectations.

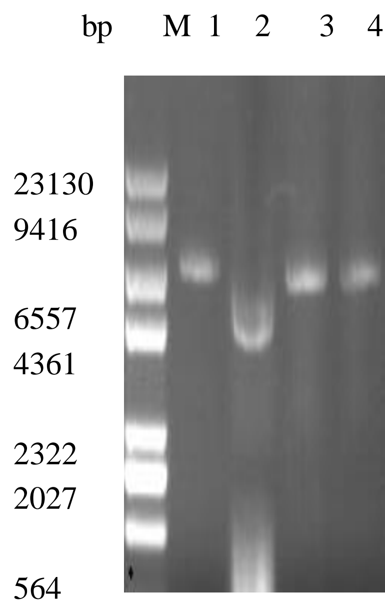

Figure 2 Construction of recombinant plasmid
M:Marke; 1,3,4: Recombinant plasmid; 2:pET22b

3.3 The expression of thiolase 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 40KD compared with the *Escherichia coli* BL21 strain. Consistent with the expected molecular weight, indicates that the target product thiolase protein was expressed successfully.

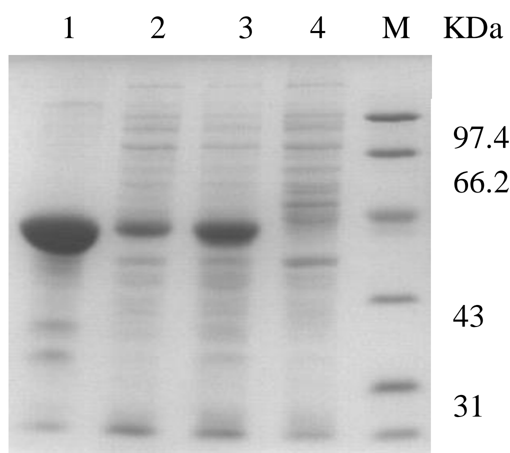

Figure 5 SDS-PAGE analysis of the expression of thiolase
1: precipitation; 2: supernatant; 3: *Escherichia coli* BL21 transferred into the recombinant plasmid; 4: *Escherichia coli* BL21; M: standard protein molecular weight

3.4 Gene sequence of thiolase from Phellinus igniarius

The sequence is as follows:

ATGAATGGCAGTTGCCAAGAGAAAGCTGCTGATTCAAGTGAAAATGGGATGAACAAG
ATTGGCGGAGAAACCATGATCCGCGTTTGCATGCACGTGACTGATCCTCCACCATCCA
GTCAGTCGGTTCCATGTTTAAACGCAGCCTGGGAGAAAAAGCATGTTGCGAACCTCTG
TTTGCCTTTGCCTTTGCCTTCAACGTCTCTTCTTTCACCAACAAGCATCGTTCAAATAAT
GGAAAGAGTTAGATCTCTGGCTTCCCATTTCACTGGTCAATCAGGCCGCGCTGCGCTTG
AGCGAAAGAGTCCAGATGATGTGGTCATCACACTCGCTGTGCGCTCACCTCTGTGCAA
GGCTCACAAAGGAGGGTTTAAAGATACTCGTTCCGACGAGCTCCTCGCAGCGATGTAC
AAGGCTGTCATAGAGAGGTCGGGTATTGATCCAAAGCTCATTGAGGATATCTGCGTTG
GCAATGTCCTTACTTCCGGTCCCACGTATGAGGCTCGTGCTGCAGCTCTCGCCGCAGGG
ATACCCGAGACGACACCCGTGCAAGTCATAAACAGATTTTGCTCTAGCGGACTGATGG
CCGTAACGACAATTGCCAATCAAATCCGTTCCGGTCAGATTGAAATTGGCCTTGCTGTC
GGTCTGGAGAGCATGTCCTTCAACCCAGATCAAGCAGCTCCGCCTTTGAGTCAACAAA
TAATGGCACATCCGCTTGCCAAGGACTCTGTAGAGCCAATGGGGTGGACCTCTGAAAA
TGTCGCAAAAGATTTCACTATCCCTCGAGAGGACATGGATGCATTTGCAGCACTGTCTT
TCCAGAGGGCCGAAGCGGCGCAGAAAGCTGGCATCTTCGAGAGCGAGATTGTCCCTCT
TACCGTCCCAAAGCATGATCCAGCAACGGGTAGGACGGAGACAATCACAGTTACTCGC
GATGACGGGATTCGCTACGGTACCACAAAAGAAGTTCTTGGAAAACTCAAGAGCGCTT
TCCCACAATGGGGTGACGGCAAGACCACAGGCGGAAATGCCAGCCAGATTACGGACG
GCGCAGCCGCAGTACTGATGATGACTCGTCGTAAAGCAGAAGAACTAGGTTTGAAAAT
TCTTGGCAAGCACGTCACAACGGCTGTCGCGGGTCTAGCACCTCGTATCATGGGTATTG
GTCCGGTGTATGCTATACCGAAAGCATTAAAGAATGCTGGTATCACAATCGAGGATGT
TGATCTATTTGAGATCAACGAGGCCTTCGCGTCGATGTACGTGTATTGTGTCCGCGAAC
TGGGGCTTGACATCAATAAAGTCAATGTGAATGGAGGCGCGATTGCTCTTGGGCATCC
ATTAGATATGTTTTTTCTTCATCAGAAGATGTTTCAGGAAACTTAG

4. Conclusions

In this study, the thiolase gene was cloned from *Phellinus igniarius*, and the soluble expression of thiolase in *E. coli* was realized, which laid a foundation for further study of the function of mulberry thiolase.

Acknowledgments

This research achievement is financed by Weifang Medical University Domestic Visting Scholar Program, No.20187-16; No. 20187-32.

This research achievement is financed by WeiFang Medical University students' science and technology innovation project, No. KX2017043; KX2017033.

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