Full-Length cDNA Sequence of a Gene Related to the Thermotolerance of *Agaricus bisporus*

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Abstract: A gene special primer was designed and synthesized based on the cDNA sequence data of the DNA fragment 028-1 related to the thermotolerance of *Agaricus bisporus*. The 5'-end cDNA fragment of the gene was obtained using the technique of rapid amplification of cDNA ends (RACE). After sequencing and splicing, the full-length cDNA sequence of the gene was obtained. The sequence is 1.37 kb long, and no obvious homologous sequence was found in GenBank.

Key words: *Agaricus bisporus*, thermotolerance, RACE, cDNA sequence, DNA cloning

1 Introduction

Agaricus bisporus (Lange) Imbach is a cultivated mushroom with rich nutrition, high economic value and ecological significance. The mushroom fruits under a stable temperature, which is a major factor affecting its growth. To improve the thermotolerance of *A.bisporus*, develop its year-round cultivation under natural climate conditions, and decrease the cultivation cost for controlling the temperature, we planed to breed thermotolerance commercial strains of *A.bisporus* by genetic engineering. In the past work, DD-RTPCR technology was adopted to analyze the gene expression difference of A.bisporus under normal and higher temperature cultivation, and several thermotolerance related gene fragments were obtained.[1, 2]
The technique of rapid amplification of cDNA ends (RACE) is a simple and effective method for rapid amplified the 5' and 3' ends of cDNA from low abundance transtript based on the PCR technology and partial known cDNA sequence, and is also named anchored PCR or one-side PCR.[3] Because the special fragments obtained in the DD-RTPCR were already the 3'-end sequence of cDNA, in this study, we planed to design primers based on the known sequence, amplify the 5'-end of the gene by RACE technique, and obtain the full-length cDNA sequence of the thermotolerance related gene.

2 Materials and Methods

2.1 Strains

The thermotolerant *A. bisporus* strain 02 was provided by the Fujian Research Institute of Light Industry. Under normal conditions, the fungus was grown in potato dextrose (PD) liquid medium at 24°C for two weeks. For growth at increased temperature, the mycelium was first grown at 24°C and then transferred to a 32°C incubator for 12 hrs. *Escherichia coli* JM109 was obtained from Promega.

2.2 Molecular techniques

Total RNA was extracted by the One-step method using Trizol reagent according to the user's manual. Reverse

transcription and rapid amplification of cDNA ends (RACE) were carried out using the SMART™ RACE cDNA Amplification Kit (Clontech) using the manufacturer's protocols. The gene special primer (GSP1) used in RACE was designed based on the 028-1 sequence and the RACE requirements, and synthesized by Sangon Shanghai. Its sequence was: 5'- AGGCC CGCGA GTTCC AGACC ACCCG A -3', with 70% of GC content and a predicted Tm value of 70°C. Cloning of the RACE product was done with the pGEM-T Easy Vector Systems (Promega) according to the user's instructions. The PCR product of the recombinant was sequenced by Sangon Shanghai.

2.3 Reagents

The SMART™ RACE cDNA Amplification Kit was from Clontech, pGEM-T Easy Vector Systems from Promega, and Trizol, endonucleases, PCR Kits and other reagents from Sangon Shanghai.

3 Results and Analysis

3.1 Extraction and reverse transcription of total RNA

Mycelia of strain 02 growing at normal temperature for two weeks were induced by culture at 32°C for 12hrs and total RNA was isolated. After formaldehyde denaturation gel electrophoresis, three RNA bands of 28S, 18S and 5S were observed indicating that the total RNA samples were of good quality. Approximately 200ng of total RNA was subjected to reverse transcription as described in Methods, and 5'-RACE Ready cDNA was obtained.

3.2 Rapid amplification of cDNA ends (RACE)

Using 5'-RACE Ready cDNA as template, PCR amplification was carried out with 5' Unique Primer Mix (UPM) supplied by the kit and the synthesized 028-1 Gene Special Primer (GSP1). A DNA fragment approximately 1.2kb in size was obtained, while no bands were amplified in reactions of the negative controls containing only UPM or GSP1 (Figure 1). According to the theory of RACE, the 1.2kb DNA fragment should be the complete 5'-end sequence of the gene at which the 028-1 fragment is located.

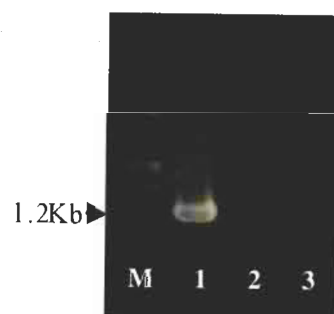

Figure 1. 5'-Race patterns of thermotolerance-related gene
Lane 1, UPM + GSP1; Lane 2, UPM only; Lane 3, GSP1 only; Lane M, Lambda DNA/EcoRI+HindIII Markers

3.3 Cloning and identification of RACE product

The PCR product of the 5'-RACE fragment obtained above was gel purified, ligated to the cloning vector pGEM-T Easy, and transferred into *E. coli* JM109. The recombinant plasmid DNA was PCR amplified with

UPM and GSP1, and was digested with endonuclease *Not*I. The target band of ~1.2kb was seen in both cases. No bands appeared at the corresponding position in both negative controls suggesting the successful cloning of the DNA fragment (Figure 2).

3.4 Sequencing, splicing and analysis of cloned DNA fragment

After sequencing of the recombinant with primer T7 and SP6, the DNA data of the ~1.2kb 5'-RACE fragment about was btained. Based on its overlap sequence with the known 0.4kb 028-1 DNA fragment, the 1.37Kb full-length cDNA sequence of the gene at which fragment 028-1 located was obtained after splicing (Figure 3). The letters "TATA" shown in bold font may possibly be the "TATA box" of the gene, and the "ATG" may possibly be the translation origin of the gene. No obvious homological sequence of the gene was found in GenBank, suggesting it to be an unreported gene.

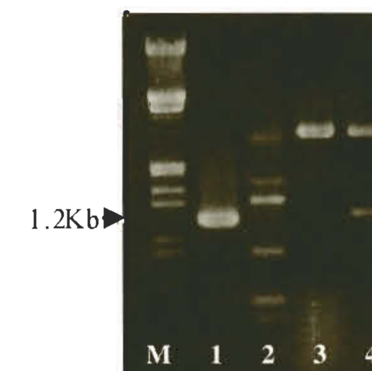

Figure 2. Identification of the recombinant DNA by PCR (with primers UPM+GSP1) and restriction analysis (with *Not*I)
Lane 1, PCR patterns of recombinant plasmid; Lane 2, PCR patterns of pGEM-T Easy vector; Lane 3, Restriction patterns of pGEM-T Easy Vector; Lane 4, Restriction patterns of recombinant plasmid; Lane M, Lambda DNA/EcoRI+HindIII Markers.

4 Discussion

In recent years, the successful application of RACE suggested it to be an effective and rapid method for cloning full-length cDNA especially from trace amounts of mRNA. It was also used in other work such as constructing cDNA libraries[4] and finding homologous genes. However, it is not easy to use this technique successfully. Several factors such as reverse transcription, PCR, gene length, poorly designed primers and non-specific products may result in failure. In our attempts to obtain full-length cDNA by RACE, the gene special primers for three thermotolerance-related fragments were designed and synthesized, but only the primer used in this study produced the 5'-end fragment and resulted in the full-length cDNA sequence of the gene.

It was reported that the amino acid sequence encoded by the 028-1 DNA fragment showed 24% homology with the vitamin-K-dependent protein S of several organisms.[5] However, the full-length cDNA sequence had no obvious homology to sequences deposited in GenBank, suggesting that a relationship based on the 24% homology mentioned above could not be confirmed. At the same time, our results suggested that the gene might be a previously unreported gene.

In the next stage of our research, the expression plasmid construction and transformation of the gene, and protein analysis, will be carried out to find a possible relationship between the gene and the thermotolerance of *A.bisporus*.

5'TTCTTCACGTGCTGCCACCAAACCAACAAATCTACTGGATTGGACTTCGCTCACTAGCTTTGAT
CCCAA**TATA**CTGTCCCTTCTGGAAGAAACTCCACAACCACAGCCTACGGCTACTTCTAGCGCT**AT**
GGACCTTGATCTATTTAGTACTCTTGACGAAGGACCCGATTCATCTTCCCCGTTCACGACGATCGC
GAGTAATCCAGTGTTCATGTCCTTTGCCTCGAGTTTTGACACTATGACACTACCCGAAAACTCATC
GGCTCCCATGAACAAGTCCGGGAATAGGTCAAATTCGCAATCCTTTAACTTTGATATGAATTCGTT
GGGCTCCTGGCCCACACCCACGCCTCCGGCTCAAGATACGTCGCTTGACGACCTGTTCGCAGGC
TATGTTCCGAACCAGACTGTTGACTTTTCCACATTCGCCACTACCCCTTCAATAAGCCCTGTCACC
CACCAAGCAACTGTCAACAACCCTGCCTTCACCAATGTCAACGGTAAAAACTATTCATACCTAAC
AAGGCAATCTTCCGATGCTGCACGATTATCGTCTATTCCGTCACCAACGTCACAGAGCACCGTCG
GCTCAGACCCATCACCAAGGAGGGTGATCACTCTCCTCACGTTTCCAACTCCGCTACTGATACGG
GCTATCACAAGAAGTCCGAGTGTCCAAAGACAAAGTCTGAGCTGTTAAAACGGATAAATGAAGC
GGGATCGTCACCGTTCGCTCGCGCGGGTTTGTCGAAAGCCCTTGATCGACATGGATCCATGGTAT
CCTGTGCGGGAACTAATCTTCCGAAGACGGCGAAAAATGACATGAACGTAGAAGTGCTTAGCGC
TTGGAGAAGTATCACATCCAATCCCGCATTCAAGGATGCCAATATTGACATCAATGACCTATGCGC
ACAGTTCACTGACAAAGCCAAGTGCGATGGTACCAAGGTTGTACTTGAGCCGCAAGGGATGCAT
GATATCATTGAATCGCTCGCCTCCAAGAACCACTGATAATGCCTGGGTTGGTGAAGAAGGGAGA
GATCATCCGTGTATCCATTATCATCGATTGTATTGTGGGGCGCTGTGGAGTTCCATATCCGGATCCG
CCCATACTTCCGCCATTTTTCACCCATACCTCCGACTCGGGTGGTCTGGAACTCGCGGGCCTGCAT
AACGATCAGGCTCTTGTCATCGACAAGGGCCAGCGGCTGCGTGCGATTATAGCGCAATCCAGGA
GTCAAAAAGTGTGAGGATGTCGTCTGCTATTGTCTGACTTTCTCGTGTATTCAATGTTGTCTTCAC
TACTGTGAGGGATACTTATGTACTATTATATGCTGTCGTCTTTCTTTGGGCCTTTTAAAAAAAAAAA
3'

Figure 3. Full-length cDNA sequence of 028-1 gene related to thermotolerance in *A. bisporus*

Acknowledgement

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