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Panhandle PCR strategy to amplify the upstream unknown sequence of the Pr1 gene of *pythium guiyangense*☆

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Abstract

Objective: Based on a partialsubtilisin-like protease, Pr1 genomic sequence of *Pythium guiyangense* which has been cloned before, Panhandle PCR strategy was used to amplify the upstream flanking sequence adjacent to the known sequence of the Pr1 gene. **Methods:** The genomic DNA was firstly digested with *Bam*HⅠand then treated with calf intestinal alkaline phosphatase(CIAP). Next, a 5′ phosphorylated oligonucleotide was ligated to the 5′ ends of *Bam*HⅠ-digested DNA. After denaturation, intrastrand annealing and polymerase extension, a pan with a handle was formed,and lastly the nested PCR was performed. **Results:** A 864 bp product was amplified,which was adjacent to the known sequence of Pr1 gene.The gene has been accessed by GenBank (Accession:JQ975036). **Conclusion:** Panhandle PCR is a quick and convenient approach for amplifying and identifying unknown partner genes,which facilitates cloning full-length Pr1 gene.

Keywords： Panhandle PCR; Upstream regulation sequence; Subtilisin-like protease Pr1; Chromosome walking

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1. Introduction

The proteinaceous outer integument of insects forms an effective barrier against most microbes, the most entomophagous fungi can breach insects cuticle using extracellular proteases, among these extracellular proteases, subtilisin-like protease Pr1 provide the important factors of entomophagous fungi against insect cuticles [1-2]. *Pythium guiyangense* produces Pr1 during growth on cockroach cuticle, So isolation of the Pr1 gene is an important step to research *Pythium guiyangense*. Partial sequences of Pr1 had been cloned before, to get the full-length gene, we used a new approach called panhandle PCR to amplify the unknown partner sequence [3].

2. Materials and Methods

2.1 Materials

Pythium guiyangense strain, maintaimed on $KPYG_2$ and SFE plates, and kept at 24℃-26℃. *E.coli* JM109 strain kept in this lab, maintaimed on LB medium [4].

Taq polymerase, restriction enzyme *Bam*H Ⅰ, CIAP, ampicillin Na_2, T_4 DNA ligase, X-gal, IPTG, DL2000 marker, pMD18-T vector, high pure plasmid isolation kit and high pure PCR product purification kit bought from TaKaRa company.

All these primers were designed based on the known sequence (in Table 1 and Fig.1).

Fig.1.The position of the primers in the Pr1 gene

Table 1

The sequences of the primers

Gene name	Sequence
PD1:	5'-GCTTCCGATGTGCCGTTG -3'
PD2:	5'-CGCAGTGCTTCGTGGGTGT -3'
PD3:	5'-GTGTCCCGTGACCGTCTTCG -3'
PD4:	5'-GGGCACTTGTTGCGGGTTGT -3'
NDS:	5'(P)-GATCGGCTGGTATGACCCATTC CGAAAGTCCAAGAT-3'

2.2. Methods

2.2.1. Genomic DNA extraction, BamH Ⅰ Digestion and CIAP treatment

Total DNA was extracted from *Pythium guiyangense* strain using Benzyl chtoride method as described previously [5]. Digest 5mg genomic