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拟南芥 *AtPSK3* 基因的克隆及序列分析

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摘 要：根据拟南芥基因组数据库提供的信息，首次以特异引物经 PCR 技术克隆到拟南芥硫肽激素-α 的一个前体基因——*AtPSK3*，并对其进行了测序。序列分析表明，所获得的 *AtPSK3* 基因全长为 505 bp，含有一个内含子和两个没有 3′-或 5′-非转译区的外显子，与数据库提供的序列比较，同源性为 100%。

关键词：*AtPSK3* 基因；基因克隆；DNA 序列分析

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Study on the amplification and sequencing of *AtPSK3* from *Arabidopsis*

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Abstract: A Phytosulfokine-α gene(*AtPSK3*)was amplified from genomic DNA of *Arabidopsis* by PCR, based on the sequence information from *Arabidopdis* genome database, and its complete DNA sequence was analyzed. Results indicated that the *AtPSK3* gene contained 505 base pairs, consisting of one large intron and two exons without 3′-or 5′-UTR sequence, the sequence of which has a homology of 100 percent as compared with the reported sequence.

Key words: *AtPSK3* gene; gene clone; DNA sequence analysis

Phytosulfokine-α (PSK-α), a sulfated pentapeptide growth factor universally found in both monocotyledons and dicotyledons, was originally isolated from conditioned medium(CM)of asparagus (*Asparagus officinalis*) mesophyll cell cultures (Yang *et al.*, 2000). PSK-α has gained increasing attention recently because of its unique biological activities, such as strongly promoting the proliferation and differentiation of plant cells in low density culture at low concentration(Matsubayashi *et al.*, 1999; Hanai *et al.*, 2000), stimulating somatic embryogenesis and the adventitious bud and root formation from callus of plant (Kobayashi *et al.*, 1999; Yang *et al.*, 1999), and enhancing the growth and chlorophyl content of seedling (Yamakawa *et al.*, 1999).

The sequencing and analysis of *Arabidopsis* genome were completed at the end of 2000 (Rounsley *et al.*, 2000). It was reported that four genes encoding precursors of PSK-α had been identified from *Arabidopsis* with the BLAST program using the amino acid sequence of PSK-α (Yang *et al.*, 2001). Analysis of cDNAs for two of these, *AtPSK2* and *AtPSK3*, showed that both of them con-

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sist of two exons and one intron. *AtPSK2* and *AtPSK3* were expressed demonstrably not only in cultured cells but also in intact plants, suggesting that PSK-α may be essential for plant cell proliferation *in vivo* as well as *in vitro*. Overexpression of either precursor gene allowed the transgenic calli to grow twice as large as the controls.

In this paper, the *AtPSK3* gene encoding a precursor of Phytosulfokine-α was obtained by PCR from genomic DNA of *Arabidopsis* for the first time, using the sequence information from *Arabidopsis* genome database, and we hope the establishment of this system could provide an ideal model for further studies of its biological activities *in vitro*, especially its stimulative functions to plants.

1 Materials and methods

1.1 Plant materials

Arabidopsis seeds (ecotype Columbia) were supplied by Professor Jianru Zuo (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences).

After being immersed in hot water(50～52 ℃) for 30 min, *Arabidopsis* seeds were disinfected with 70%(v/v)ethanol for 3～5 min, washed three times with sterile distilled water, transferred to 10%(w/v)NaOCl for 15 min, followed by five rinses with sterile distilled water. The sterile seeds were cultured on 30 mL solid B_5 medium without hormone in 250 mL flask at 25 ℃ in the 16 h light/8 h dark cycles. About 20-day-old germinated seedlings of *Arabidopsis* were prepared for extraction of genomic DNA.

1.2 Extraction of genomic DNA

The genomic DNA was extracted from *Arabidopsis* seedlings according to the method of CTAB (Wang *et al.*, 2002). The DNA molecular size was analyzed by 0.8%(w/v)agarose gel electrophoresis.

1.3 PCR amplification

Primers were designed based on the nucleic acid sequence of *AtPSK3* gene in TAIR(The *Arabidopsis* Information Resource)Database(TAIR accession nos. AT3G49780.1)with the Primer Premier 5.0 program and synthesized by Shanghai Bioasia Biotechnology Co., Ltd. The primers were as follows: P1: 5'-TCGT TCTAGA TCAGTATGGGTAAGTTCACAAC -3' and P2: 5'-ATAC GAGCTC TTAGGGCTTGTGATTCTGAGT-3', which had *Xba*I site and *Sac*I site, respectively.

PCR amplification was performed using GeneAmp PCR system 2 400(Perkin-Elmer). Total volume of PCR reaction system was 50 μL, including 1 × *Taq* DNA polymerase buffer, 10 μL genomic DNA of *Arabidopsis* as template, 100 nM primer P1 and P2, respectively, 200 μM dNTP, 1.5 mM $MgCl_2$ and 2.5 U *Taq* DNA polymerase(TaKaRa). Hot start procedure was adopted. The parameters of the PCR reaction were: 96 ℃ for 5 min, 94 ℃ for 1min, 55 ℃ for 1 min, 72 ℃ for 1min with 1 cycle, 94 ℃ for 1min, 58 ℃ for 1min, 72 ℃ for 1min with 29 cycles, and a final extention for 72 ℃ for 10 min.

After detected by 1.0%(w/v)agarose gel electrophoresis, the PCR products were purified by DNA Purification Kit (Dingguo Bioengineering Co., Ltd.), double-digested with *Xba*I and *Sac*I (TaKaRa), followed by gel-purification. Then the ligation of the DNA fragments with the plasmid pUC19 which was treated by the same methods was preformed with T4 DNA ligase at 16 ℃ over night. The ligation products were used to transform *E. coli* JM109. White colonies were selected on solid LB medium(pre-spread with 40 μL of 20 mg/mL X-Gal and 4 μL of 200 mg/mL IPTG)containing 100 mg/L ampicillin.

1.4 Sequence analysis

After identified by PCR amplification, the recombinants were sequenced by Shanghai Bioasia Biotechnology Co., Ltd.

2 Results and discussion

2.1 Extraction of *Arabidopsis* genomic DNA

An over 21 kb genomic DNA of *Arabidopsis* was obtained by the method of CTAB(Plate Ⅰ-A), which was large enough to be used for the *AtPSK3*

gene amplification.

2.2 Cloning *AtPSK3* gene

The genomic DNA from *Arabidopsis* was used as template for PCR reaction and a fragment about 530 bp was obtained (Plate Ⅰ-B, lane 2 and lane 3). After optimization, we got more *AtPSK3* gene and less nonspecific amplification products (Plate Ⅰ-B, lane 2) than before (Plate Ⅰ-B, lane 3). We may draw a conclusion that the specific amplification products are consistent with the length of the reported *AtPSK3* gene.

2.3 Identification of the recombinant clones

The products double-digested with *Xba*Ⅰ and *Sac*Ⅰ were recovered from an agarose gel and ligated into plasmid pUC19. Many colonies were obtained after transformation. Plasmids were isolated from these bacteria and analyzed by PCR. An about 530 bp DNA fragment was obtained when the recombinant plasmid pUC-*AtPSK3* was used as template for PCR reaction (PlateⅠ-C, lane 2); nothing but some nonspecific amplification products was got when the pUC19 plasmid was used as template (PlateⅠ-C, lane 1). PlateⅠ-C shows that the recombinant plasmid maybe contains the target gene.

2.4 Analysis of nucleotide sequence of the *AtPSK3* gene

To further confirm the inserted fragment, a DNA sequence analysis was carried out. The result (Fig. 1) proved that the obtained fragment indeed was the *AtPSK3* gene. It contained 505 base pairs, consisting of one intron (265 bp) and two exons (117 bp and 123 bp) without 3′-or 5′-UTR sequence. The sequence of the *AtPSK3* gene we obtained from the *Arabidopsis* had a homology of 100% as compared with that of the *AtPSK3* gene reported in *Arabidopsis* genome database.

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1: TCTAGATCAGTATGGGTAAGTTCACAACCATTTTCATCATGGCTCTCCTTCTTTGCTCTA    60
2:          ATGGGTAAGTTCACAACCATTTTCATCATGGCTCTCCTTCTTTGCTCTA

1: CGCTAACCTACGCAGCAAGGCTGACTCCGACGACAACCACCGCTTTGTCCAGAGAAAACT   120
2: CGCTAACCTACGCAGCAAGGCTGACTCCGACGACAACCACCGCTTTGTCCAGAGAAAACT

1: CCGTCAAGGTTCGTTAACTTCTTTGTCTTTTTCAGTATAGTACTAGTCGAAACATATCTG   180
2: CCGTCAAGgttcgttaacttctttgtctttttcagtatagtactagtcgaaacatatctg

1: CAATTGCAAAACAAAGAATTAATCTATCGCAGTATATGTCAAAGTTTCTATATATAGTAC   240
2: caattgcaaaacaaagaattaatctatcgcagtatatgtcaaagtttctatatatagtac

1: AAAACAAAAAACCAAAAAGAGTTTGCATGCATGCTCCTTAAGATTTGTTTCGTGTAATAG   300
2: aaaacaaaaaaaccaaaaagagtttgcatgcatgctccttaagatttgtttcgtgtaatag

1: ATTATATAATATCACACGATTTGTTTATTTGTTACCGCGGTAGTTTAGAAATTAACACCG   360
2: attatataatatcacacgatttgtttatttgttaccgcggtagtttagaaattaacaccg

1: ACGTTCATATGTTGTTGTATATATTATGTATAGGAAATTGAAGGAGACAAGGTTGAAGAA   420
2: acgttcatatgttgttgtatatattatgtatagGAAATTGAAGGAGACAAGGTTGAAGAA

1: GAAAGCTGCAACGGAATTGGAGAAGAAGAATGTTTGATAAGACGAAGCCTTGTTCTTCAC   480
2: GAAAGCTGCAACGGAATTGGAGAAGAAGAATGTTTGATAAGACGAAGCCTTGTTCTTCAC

1: ACCGATTACATTTATACTCAGAATCACAAGCCCTAA GAGCTC                     522
2: ACCGATTACATTTATACTCAGAATCACAAGCCCTAA
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Fig. 1　Comparison of nucleotide sequence

1: Sequence of insert fragment (underlined: *Xba*Ⅰ and *Sac*Ⅰ sites); 2: Sequence of reported *AtPSK3* gene (capital: exon, lowercase: intron).

Anthocyanin is an important kind of dye for food, makeup and medicine, and it is also a remedy for many diseases. In recent years, anthocyanin is obtained by the culture of the *Roselle* calli to shorten the yield period, unfortunately however, the *Roselle* calli's growth period is still too long. The

transfer of *AtPSK3* gene into *Roselle* cell is a potential method to stimulate the proliferation of *Roselle* cell in low density. In this paper, we have successfully isolated the *AtPSK3* gene from *Arabidopsis* genomic DNA by PCR, and the sequence analysis revealed that the obtained *AtPSK3* gene is identical to that in *Arabidopsis* genome database. We hope it could be the first step towards constructing a transgenic *Roselle* cell line with rapid proliferation.

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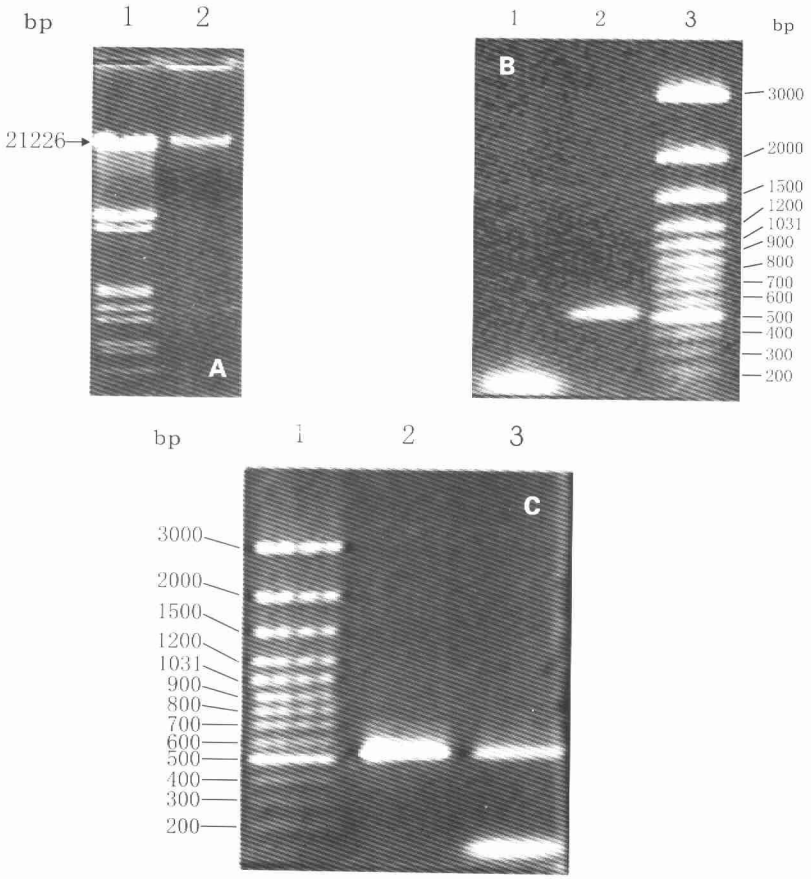

A. 0.8% agarose electrophoretic analysis of *Arabidopsis* genomic DNA: 1. Marker: Lambda DNA/*EcoR* Ⅰ + *Hind* Ⅲ; 2. *Arabidopsis* genomic DNA.
B. Electrophoretic analysis of PCR amplification product: 1. PCR Marker: GeneRuler ™100bp DNA Ladder Plus; 2. Amplification product after optimization; 3. Amplification product before optimization.
C. Identification of recombinant pUC-*AtPSK3* by PCR: 1. Nonspecific products of PCR using pUC19 plasmid as template; 2. Specific products of PCR using recombinant plasmid as template; 3. PCR Marker: GeneRuler ™100 bp DNA Ladder Plus.