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PCR cloning of a protein-coding part of the thioredoxin gene from *Streptomyces aureofaciens*

Short Communication

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Summary. The method of two-stage half-specific amplification was described and successfully used in the isolation of the protein-coding part of the thioredoxin gene from Streptomyces aureofaciens BMK. The efficiency of a new PCR modification for the specific amplification of the target DNA fragments (genes) with unknown sequences is compared with the used half-specific PCR. The determined target sequence demonstrates the highest homology with the thioredoxin genes from Corynebacterium nephridii C-1 and Anabaena 7119.

Keywords: Amino acids – Half-specific PCR – Thioredoxin – Gene isolation – *Streptomyces aureofaciens*

Introduction

The ability to accumulate exponentially specific fragments made PCR possible to clone in vitro target segments of DNA mainly when their cloning or screening is complicated in living cells. The screening of thioredoxin genes can serve as an example.

Thioredoxin is a disulfide-containing protein with a highly conservative structure. It is universal in the performance of numerous physiological functions, including participation in nucleic acid metabolism, probably as a result of which it prevents a lethal outcome of τ-irradiation of cells (Labudová and Kollárová, 1991).

The thioredoxin gene has never been isolated from *Streptomyces* strains, although the thioredoxin physiological role in their rich secondary metabolism, connected with the cell differentiation by means of certain regulation mechanisms is of great interest.

The genomic blot hybridization with the probes for thioredoxin gene highly conservative sequences demonstrates numerous bands due to their dissemination in different genes (Müller and Buchanan, 1989). The sequence of the first 17 amino acid residues from N terminus of the thioredoxin from *Streptomyces aureofaciens BMK* was determined: **GATVKVTNATFKSDVLE** (Dr. J. Kormanec, Max-Planck-Institute, Göttingen). So, two methods of half-specific PCR were used for cloning of the nucleotidic sequence coding the mature part of the thioredoxin: PCRI and PCRII, where PCRII is developed two-stage modification of simple half-specific PCRI.

As a result the determined thioredoxin gene and corresponding amino acid sequences have been compared with the known thioredoxin (genes) sequences.

Materials and methods

PCRI

The downstream specific 20 mer oligonucleotide was derived from the N terminus protein sequence (GATVKVT) and designed taking into account the codon preference of *Streptomyces* strains. The primer (N.P.1) sequence was homologous to the coding strand:

5′GGC GCG ACC GTC AAG GTC AC 3′

G G G

The universal sequencing primer (F.P.) with the sequence:

5′GT TTT CCC AGT CAC GAC 3′

was used as the upstream gene-unspecific primer in PCR.

An aliquot of the statistic library containing the SmaI-fragments of chromosomal DNA from *Streptomyces aureofaciens BMK* in pUC 18 cloning vector was used as a template for PCR. 100 ng of total recombinant plasmid DNA were denatured by heating for 8 min at 97°C. 30 cycles (1 min 95°C, 1 min 55°C, 5 min 71°C) of the half-specific PCR were provided in the presence of the template, 500 nM of each oligonucleotide (N.P.1 and F.P.), 200 μM dNTPs, 10 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100 and 2.5 units Taq polymerase (Promega) in total volume of 100 μl, followed by an elongation for 10 min at 72°C.

An aliquot of total PCR products was hybridized with the probed highly degenerated 20 mer with the sequence corresponding to the region of the known thioredoxins active site (A, G)(P, E, G)WCGPC constructed complementary to the coding strand. The positive product of PCR ($\approx$1, 7 kb fragments, Fig. 1) was eluated from separating gel and cloned in pUC 18 at SmaI-site. The nucleotide sequence was determined by using the Taq polymerase sequencing kit (Promega).

PCRII

The used specific primers I (N.P.1) and II (N.P.2) prepared as degenerated 20-mer oligonucleotides were derived from the determined sequence of the first 14 N terminus amino acid residues of a thioredoxin from *Streptomyces aureofaciens* BMK (GATVKVINATFKSD). The downstream oligonucleotides were designed taking into account the codon preference of *Streptomyces* homologous to the coding strand. N.P.2 had the sequence:

5′AAC GCG ACG TTG AAG TCG GA 3′ (N.P.2).

C C C C

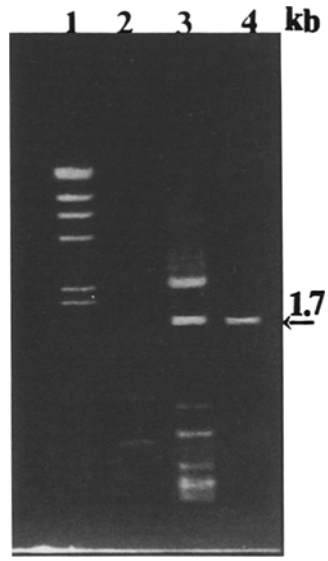

Fig. 1. 1% agarose gel. Lane *1*, standard DNA marker: Hind III digested λ DNA; lane *2*, PCR specificity control: template is pUC 18 DNA, the primers are N.P.1. and F.P.; lane *3*, total products of desired half-specific PCR I; lane *4*, PCR II homogenic product

As it was in PCR I the universal sequencig primer (F.P.) was used as the upstream unspecific primer in PCR II.

The 1st stage of PCR II was performed as PCR I at the same amplification conditions. An aliquot of total PCR I products (100 ng) was used as DNA template for the 2nd step of PCR II, which was performed under the same conditions as PCR I (or 1st step of PCRII) but with the second pair of oligonucleotides: N.P.2 and F.P..

The purified homogenic product of the 2nd step of PCR II (Fig. 1) was cloned at the Sma I-site of pUC 18 in *E. coli* JM 101 and than sequenced by dideoxy chain-termination method using (α-^{35}S)dATP (Amersham) and "Taq-Track" – system ("Promega"). Sequence comparisons and data bank searches were performed with the Beckman Microgenic computer program.

Results and discussion

With respect to the presence of the partial information about the protein sequence the efficiency of the simple half-specific PCR I and introduced modified 2-stage half-specific PCR II has been investigated in the amplification of the protein-coding part of the thioredoxin gene from *Streptomyces aureofaciens BMK*. While only ~5% of PCR I products have corresponded to the thioredoxin gene (positive ~1, 7 kb DNA fragment), Fig. 1 demonstrates the presence of the homogenic product in the case of PCR II.

The successful technique of PCR II described in this paper involves the specific amplification of target undetermined sequences using the partial information about the corresponding protein sequence. Thus, represented 2-stage cascade PCR, which does not need the selection by specific hybridization of the target PCR product, has increased the specificity of a half-specific amplification up to 100%.

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  Gly Ala Thr Val Lys Val Thr Asn Ala Thr Phe Lys Ser Asp
1 GGC GCG ACG GTC AAG GTG ACG AAC GCG ACG TTC AAG TCC GAC

  Val Leu Glu Ser Asp Lys Pro Val Leu Val His Phe Glu Gly
  GTC CTG GAG AGC GAC AAG CCC GTA CTG GTG CAC TTC GAG GGA

  Pro Trp Cys Gly Pro Cys Lys Met Val Ala Pro Val Leu Asp
  CCA TGG TGC GGC CCA TGC AAG ATG GTC GCG CCC GTC CTG GAC

  Glu Ile Ala Asn Glu Tyr Glu Gly Lys Val Lys Val Ala Lys
  GAG ATC GCG AAC GAG TAC GAG GGC AAG GTC AAG GTC GCG AAG

  Val Asn Thr Asp Glu Asn Pro Gln Leu Ala Ser Gln Tyr Gly
  GTC AAC ACG GAC GAG AAC CCC CAA CTG GCG AGC CAG TAC GGC

  Val Arg Ser Ile Pro Thr Leu Leu Met Phe Lys Gly Gly Glu
  GTG CGC AGC ATC CCG ACG CTG CTC ATG TTC AAG GGC GGC GAG

  Val Ala Ala Asn Met Val Gly Ala Ala Pro Lys Thr Arg Leu
  GTC GCG GCG AAC ATG GTG GGC GCC GCG CCC AAG ACG CGC CTC

  Ala Ala Phe Leu Asp Ala Ser Leu ***
  GCG GCC TTC CTG GAC GCG TCG CTC TGA 321
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Fig. 2. The determined nucleotide (and corresponding amino acid) sequence of thioredoxin gene from *Streptomyces aureofaciens BMK*

The comparison of the determined nucleotide and corresponding protein sequences (Fig. 2) with the known sequences exhibits the highest homology with thioredoxin from *Corynebacterium nephridii* C-1 and *Anabaena* 7119: 80% ($\approx$69% at the DNA level) and 76% (64%) respectively, at evolutionarily stable positions (Meng and Hogenkamp, 1981; Lim et al., 1986).

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