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NOTE

Molecular Cloning and Sequence of *URA5* Gene Encoding Orotate Phosphoribosyl Transferase (OPRTase) from Entomopathogenic Fungus, *Metarhizium anisopliae*

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Abstract A *ura5* gene encoding Orotate Phosphoribosyl Transferase (OPRTase) of *Metarhizium anisopliae* was cloned by PCR methods and sequenced. The sequenced *ura 5* gene encodes a polypeptide of 234 amino acid residues. This deduced amino acid sequence showed high similarity to other fungi OPRTase and there was no intron sequence between ATG starting codon and TGA ending codon.

Key word: Polymerase chain reaction, entomopathogenic fungus, *Metharizium anisopliae*, orotate phosphoribosyl transferase

Entomopathogenic fungi are under consideration as potential biological control agents for crop pests. *M. anisopliae*, one of the entomopathogenic fungi, has been studied by many biologists [5, 6, 9] for use as a source of very promising mycoinsecticide. The pathogenicity and infectivity of this fungus is an active penetration of the insect integument resulting from the production of integument-degrading enzymes such as chitinase, protease, and lipase [13, 14]. Recently, in filamentous fungi, the recombinant DNA technique allowed for the development of new strains for the optimizing product of degrading enzymes and their stability [13]. The developing procedures for these strains require an efficient and reliable transfomation system.

Many transformation systems using dominant selectable markers for the transformation of wild-type strains result in low transformation frequencies. The best way to increase the transformation frequencies is to use auxotrophic mutants and their cloned genes for reliable transformation. Therefore, to use homologous transformation systems for moleculas breeding of *M. anisopliae* as a mycoinsecticide, we cloned the *ura5* gene encoding orotate phosphoribosyl transferase as a selective marker and sequenced the gene in this report. To our knowledge, this is the first report on *M. anisopliae*. First, using a fungal conserved sequence of *ura5* genes [1], we isolated a part of the *ura5* DNA from *M. anisopliae* (obtained from ATCC, U.S.A.) total DNAs by PCR process. PCR amplification was done by using a DNA thermal cycler (DNA thermal cycle 480, Perkin-Elmer cetus, New York, U.S.A.) and *Taq* DNA polymerase (Promega Co., U.S.A.).

Two primers, N1 (5'-CTT CTA GAC CNT AYT TYT TYA AYG C) and N2 (5'-T TYC TYC GNT TYC TRG CRT CCC TAG GAT) were made for PCR amplification according to the fungal conserved sequence of *ura5* genes [1]. Forty cycles were run using the conditions of 90°C for 1 min (melting step), 55°C for 1 min (annealing step), 72°C for 2 min (extention step), respectively. From the PCR DNA amplification, we obtained about a 250-bp DNA fragment (data not shown). The size of the DNA fragment corresponded to our expectation as predicted from the distance between prime sets. Next, in order to identify whether this DNA fragment originated from *M. anisopliae*, Southern analysis [10] was done with digested total DNA of *M. anisopliae*, probed with a 250-bp DNA fragment at low stringency. The result is shown in Fig. 1. From this result, we confirmed that this amplified 250 bp DNA fragment exists in *M. anisopliae* as a single copy and the signal region includes this fragment.

To isolate the full genomic gene of *ura5* from *M. anisopliae*, we carried out plaque hybridization of the *M. anisopliae* genomic library which is constructed into λDASH II vector/*Bam*H I (Stratagene Co., U.S.A.).

After screening this genomic library, we isolated a positive plaque (data not shown), purified this phage which contained a 4.4-kb insert, and recloned into the pUC119 *Sal*I site. Southern analysis of the restriction

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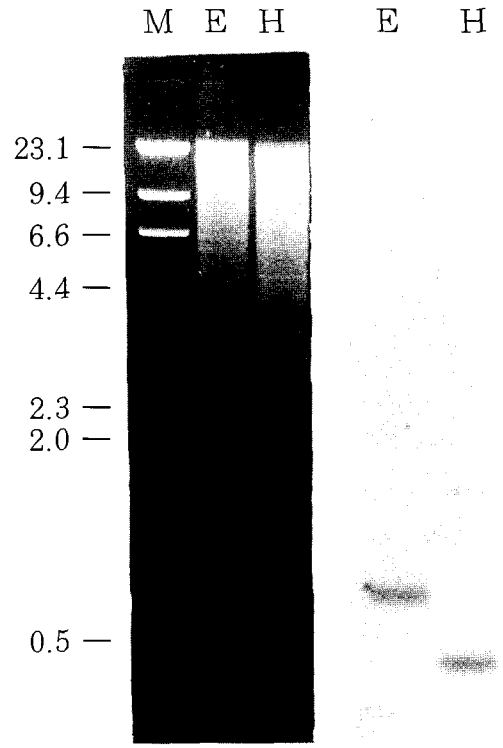

Fig. 1. Southern analysis of PCR fragment from *M. anisopliae*.
The genomic DNA of *M. anisopliae* was digested with *Eco*RI (E) and *Hind*III (H), and then hybridized with ^{32}P-labelled PCR fragment at 56°C (low stringency).

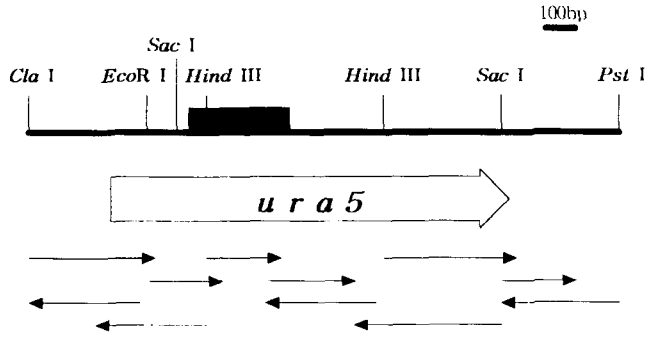

Fig. 2. Restriction edonuclease map and sequencing strategy for *ura5* gene of *M. anisopliae*.
Arrows indicate the length and direction of sequencing. Probe binding region is indicated by a thick line.

enzyme digested plasmid showed that the PCR amplified DNA, labeled with ^{32}P, hybridized with a 1.2-kb *Pst*I-*Cla*I fragment. This 1.2-kb *Pst*I-*Cla*I fragment was sequenced on both strands by a dideoxy nucleotide chain termination method of Sanger *et al.* (Fig. 2) [11].

The result is shown in Fig. 3. This 1.2-kb *Pst*I-*Cla*I fragment contains an open reading frame, which putatively codes for 234 amino acid residues. The translational start region is similar to the motif in other fungal promoter sequences [12]. There is a CT rich region upstream of the translational start site composed of 16 uninterrupted

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                                                        ggggca  205
ttcgacagactcgtaagcgtaaatccttgaaatctctttatccttgacatatgcccaaacataataca -137
tttctaattttgcaaacatccctctttctttcccagacggcgttataccgtattgagctgcctgtca  -69
atcgactcaagtagtctgttcctgaacccactaactacttgtgtttctttctcaaccataaaaccaac   -1
ATG TCT GGC CAA CTT GCG TCC TAC AAG CAA GAG TTC CTC AAG GCA GCC ATC    51
 M  S  G  Q  L  A  S  Y  K  Q  E  F  L  K  A  A  I
GAA GGC GGC GTC CTG AAG TTC GGA AGC TTC GAG CTC AAG TCC AAG AGA ATA   102
 E  G  G  V  L  K  F  G  S  F  E  L  K  S  K  R  I
TCC CCT TAC TTC TTT AAT GCT GGA GAA TTC CAC ACT GCT CAT CTC GCC GGT   153
 S  P  Y  F  F  N  A  G  E  F  H  T  A  H  L  A  G
GCT ATT TCG TCC GCC TTT GCC AAG ACT ATT ATT GAC GCT CAG CAA AAT GCC   204
 A  I  S  S  A  F  A  K  T  I  I  D  A  Q  Q  N  A
GGC CTA GAC TTT GAC ATT ATC TTT GGC CCC GCA TAC AAG GGA ATC CCT CTC   255
 G  L  D  F  D  I  I  F  G  P  A  Y  K  G  I  P  L
TGC TCT GCC ATC ACC ATT AAG CTG GGT GAG ATT TCC CCC CAG AAT TTG GAC   306
 C  S  A  I  T  I  K  L  G  E  I  S  P  Q  N  L  D
ACG GTC TCC TAC TCC TTC GAC AGA AAA GAA GCC AAG GAC CAT GGT GAG GGC   357
 T  V  S  Y  S  F  D  R  K  E  A  K  D  H  G  E  G
GGC AAC ATT GTC GGC GCC TCT CTC AAG GGC AAG AAA ATA CTC ATT GTT GAT   408
 G  N  I  V  G  A  S  L  K  G  K  K  I  L  I  V  D
GAT GTT ATC ACT GCT GGT ACT GCA AAG AGA GAA GCC ATT GAC AAG ATC AGA   459
 D  V  I  T  A  G  T  A  K  R  E  A  I  D  K  I  R
AAG GAG GGT GGT ATT GTC GCG GGC ATC GTT GTT GCC CTC GAC CGC AAG GAG   510
 K  E  G  G  I  V  A  G  I  V  V  A  L  D  R  K  E
AAG CTG CCT GCC GCA GAT GGT GAC GAC TCC AAG CCT GGA CCT AGC GCT ATT   561
 K  L  P  A  A  D  G  D  D  S  K  P  G  P  S  A  I
GGC GAG CTG AGA AAG GAG TAT GGT ATT CCG ATC TTT GCT ATC CTC ACG TTG   612
 G  E  L  R  K  E  Y  G  I  P  I  F  A  I  L  T  L
GAT GAT ATT ATC GCG GGT ATG AAA AGC TTC GCT TCT GAC GAC GAC ATC AAG   663
 D  D  I  I  A  G  M  K  S  F  A  S  D  D  D  I  K
CGG ACC GAG GAG TAC CGC CAG AAG TAC AAG GCT ACC GAT TAG ataaagaaatcg  737
 R  T  E  E  Y  R  Q  K  Y  K  A  T  D  *
ttgctatgtggatgtccgggagctcaaaccaaaaattaatataaaaaaaaaaaacggggagattgga  804
tatgcgatggcggtttatgcgggtgtctaggtacaataaataatggaccgcacttcaccaacctacc  871
gtaggatctttgtaggagagcttgttatctagatttattctcttcaggttgagtatggatacctagg  938
taattacgtttcgttagttctgaaaacgtcacctagctttgtttgacacgtagatcggtcccctggc 1005
tgcag                                                               1010
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Fig. 3. Nucleotide sequences of the *M. anisolpiae ura5* gene.
Within the 5' untranslated region, the CT-rich region is boxed. The putative TATA box and CAAT box are underlined. The primer sequences used in this study have bold boxes. The nucleotide sequence is in the GenBank data bases under accession number BankIt 156170 AF038545.

pyrimidine residus. In many fungal genes, the transcriptional start site is located in or just downstream of the CT rich region [3, 12]. In this gene, there is a putative TATA box upstream of the CT rich region and a putative CAAT box approximately 32 bases downstream of the CT box.

In the ORF region, there is no intron sequence as judged by the research of the other published *ura5* genes. The amino acids sequence of the *M. anisopliae ura5* gene was deduced and compared to this protein from other fungal OPRTases (Fig. 3) [2, 7, 8, 15].

Five proteins show high homology. In particular, OPRTase of *M. anisopliae* most closely resembled that of *T. reesei* (83% identity). Hershey and Taylor [4] proposed a putative site to bind phosphoribosyl pyrophosphate in OPRTase. OPRTase of *M. anisopliae* is also found in this binding site composed of polypeptide sequence, Val, Asp, Asp, Val, Ile, Thr, Ala, Gly, Thr.

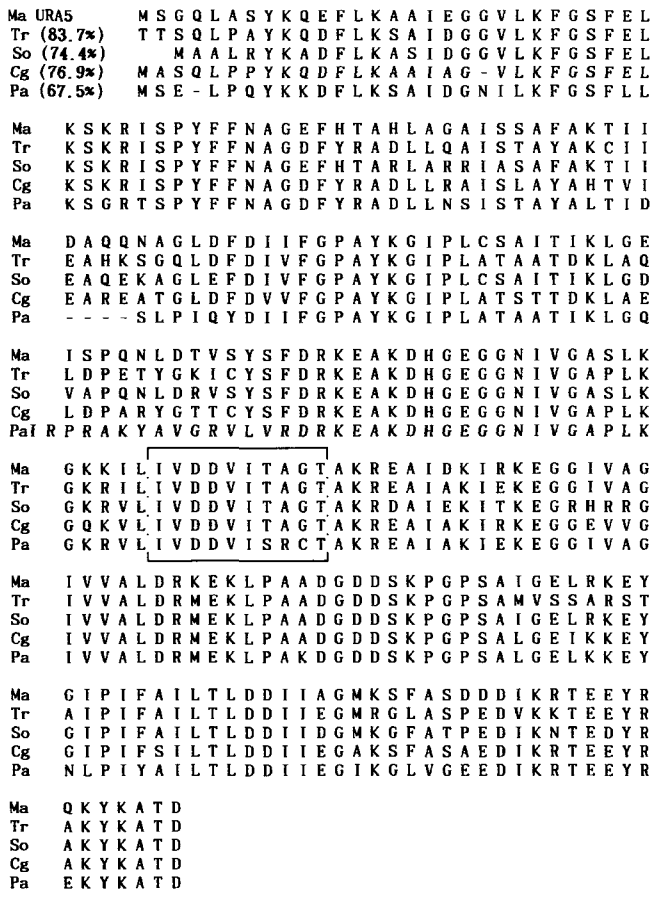

Fig. 4. Comparision of the *M. anisopliae* URA5 (OPRTase) with other fungal OPRTases.

Strains abbreviation: Ma, *M. anisopliae* in this study; Tr, *T. ressei* [2]; So, *S. onacrospora* [7]; Cg, *C. graminicola1* [8]; Pa, *P. anserina* [15]. The box indicates the polypeptide sequence of the putative phosphoribosyl pyrophosphate binding site proposed by Hershey and Taylor [4].

At present, we are using this *ura5* gene for molecular breeding of *M. anisopliae* as a mycoinsecticide.

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