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Identification of a *pr1*-like Gene of Entomopathogenic Fungus, *Beauveria bassiana* F-101 Isolated from *Thecodiplosis japonensis*

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***Beauveria bassiana* F-101, which has high toxicity toward *Acantholyda parki* as well as *Thecodiplosis japonensis*, was an isolate to develop an alternative control system against the major forest pests. Up to now, in *B. bassiana*, only one *pr1* gene has been isolated and characterized. Therefore, we here reported the identification of a *pr1*-like gene, which would be a factor of toxicity from *B. bassiana* F-101. The oligonucleotides for the amplification of the *pr1*-like gene, were chosen based on the conserved regions of the subtilisin family enzymes, *pr1* genes of *B. bassiana* and *Metarhizium anisopliae*, and proteinase K of *Tritirachium album*. The cloned PCR fragment had 1111 bp including 52 bp intron. The deduced Pr1-like peptide showed a low identity with Pr1s of entomopathogenic fungi such as *B. bassiana* Pr1 (BbPr1) and *M. anisopliae* Pr1 (MaPr1) as well as the proteinase K of *T. album* (TaPrK). Instead, the deduced peptide had a substantially high amino acid sequence identity (> 65%) with the serine proteases of *Magnaporthe grisea* (MgSPM1) and *Podospora anserina* (PaPspA). These results, therefore, appear to suggest that the putative Pr1-like peptide of *B. bassiana* F-101 belongs to the subtilisin-like serine protease family and may be a novel gene.**

Key words: *Beauveria bassiana* F-101, *Thecodiplosis japonensis*, Pr1, Subtilisin-like serine protease, Forest pest control

Introduction

The first physical barrier to insect infection of fungal entomopathogens is the insect cuticle. Most fungal pathogens use a combination of enzymatic attack and mechanical force to penetrate the host cuticles and access the haemolymph as nutrient source (Hajek and St. Leger, 1994). In enzymatic means, only several protease genes has been isolated and characterized as a putative pathogenic factor. The best understood model of fungal pathogenicity determinant is based upon *Metarhizium anisopliae* subtilisin-like protease (designated Pr1) (St. Leger *et al.*, 1992). This enzyme is adapted to extensively degrade insect cuticular protein (St. Leger *et al.*, 1987a). Ultrastructural studies with gold-labeled antibodies prepared against *M. anisopliae* Pr1 demonstrated that, while penetration of the epicuticle is primarily by enzymatic degradation, penetration of the procuticle involves both enzymatic degradation and the mechanical separation of the cuticle lamellae (Goettel *et al.*, 1989; Hassan and Charnley, 1989).

M. anisopliae strain ME-1 produces four isoforms of Pr1 during growth on cockroach cuticle. These were separated by isoelectric focusing and characterized according to their substrate specificity and inhibition patterns (St. Leger *et al.*, 1994). Characterization of a cDNA clone revealed that Pr1A is synthesized as a large precursor (40.3 kDa) containing an 18 amino acid signal peptide, an 89 amino acid propeptide, and the mature protein (28.6 kDa) containing 281 amino acids. In particular, the serine, histidine, and aspartate residues that comprise the active site of these proteases are conserved in Pr1 (St. Leger *et al.*, 1992). Besides the Pr1s of *M. anisopliae*, the *pr1* gene was also reported in the best studied entomopathogenic fungus, *Beauveria bassiana* (Joshi *et al.*, 1995). An extra-

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cellular alkaline serine protease, *B. bassiana* Pr1 has been characterized with high activity against the insect cuticle (Bidochka and Khachatourians, 1987; St. Leger *et al.*, 1987b). Its cDNA sequence revealed that *B. bassiana* Pr1 is synthesized as a large precursor (37.5 kDa) containing a signal peptide, a propeptide and the mature protein (26.8 kDa). The predicted amino acid sequence of *B. bassiana* Pr1 shows 53.6% and 59.1% identity, respectively, to *M. anisopliae* Pr1 and proteinase K (PrK) of *Tritirachium album*, a saprophytic fungus (Joshi *et al.*, 1995).

B. bassiana F-101 was isolated to develop an alternative control system against the major forest pests. Interestingly, this isolate had two valuable merits as forest insecticide; one is high pathogenicities against *Acantholyda parki* (the black-tipped sawfly) as well as *Thecodiplosis japonensis* (the pine needle gall midge) and the other is the germination ability at low temperature (Shin *et al.*, 1989, 2004). Isolation and analysis of the genes encoding proteases of the effective strain like *B. bassiana* F-101 is likely to be useful in studying their pathogenicity and synergistic relationship with other pathogen enzymes. Furthermore, according to our knowledge, only one *pr1* gene has been isolated and characterized (Joshi *et al.*, 1995). Therefore, we tried to identify a *pr1*-like gene which would be a factor of toxicity from *B. bassiana* F-101.

Materials and Methods

Fungal strain and media

B. bassiana F-101 was newly isolated from a dead larva of *T. japonensis* collected in Korean forest (Shin *et al.*, 2004). This isolate was cultured and maintained on a Sabouraud dextrose agar or broth (4% dextrose, 1% bactopeptone, w/wo 1.5% agar powder) plus 2% yeast extract (SDA+Y) medium at 25°C and a photoperiod of 15:9 (L:D) h (Vandenberg, 1996).

Genomic DNA isolation

The genomic DNA was isolated and purified by the modified method of Lee and Taylor (1990). Viable spores were collected from dextrose broth agar and used as inoculum. The fungus was cultured 50 ml dextrose broth in 250 ml flask for 7 days at 25°C in darkness with vigorous agitation. The cultured beer was harvested and the precipitate was ground well in a mortar in the presence of liquid nitrogen. The ground sample was then resuspended with DNA extraction solution [3% SDS, 50 mM EDTA, 50 mM Tris-HCl (pH 7.2), 1% 2-mercaptoethanol] at a ratio, 400 μl per 100 mg and incubated for 1 h at 65°C. Thereafter, phenol/chloroform extraction and isopropanol precipitation were performed. The dried DNA pellet was dissolved with TE buffer (pH 8.0) containing RNase A (10 mg/ml) and used as template DNA for PCR of the *pr1*-like gene.

Oligonucleotides and PCR

For the amplification of *pr1* gene from *Beauveria* spp. F-101, degenerated oligonucleotides, Pr1-F and Pr1-R were designed to amplify the *pr1* conserved region following the *pr1* gene of the previously reported *B. bassiana* (BbPr1), *M. anisopliae* (MaPr1) and the proteinase K of *T. album* (TaPrK). Their sequences and the GenBank accession numbers were shown in Fig. 1A and Table 1, respectively. Polymerase chain reaction (PCR) was performed with Ex-Taq™ DNA polymerase (Takara Co., Japan) using a DNA Thermal Cycler (Perkin Elmer Co., USA), based on a 30-cycle program, with each cycle consisting of denaturation at 94°C for 1 min, annealing at 65°C for 1.5 min, and extension at 72°C for 1 min. After amplification, the PCR products was purified using Qiaquick PCR purification kit (Qiagen Co., Germany) according to manufacturer's instruction and analyzed by 1.4% agarose gel electrophoresis.

Cloning and nucleotide sequence analysis

For the DNA sequence analysis of an amplified *pr1* par-

Table 1. The known *pr1* genes of entomopathogenic fungi and the serine proteases of other fungi similar to a *pr1*-like gene of *Beauveria bassiana* F-101

Species	Gene name	Gene description	GenBank Acc. No.	Reference
Beauveria bassiana F-101	Pr1-like	-	-	This study
Beauveria bassiana	BbPr1	Subtilisin-like serine protease	U16305	Joshi *et al.* (1995)
Metarhizium anisopliae	MaPr1	Cuticle-degrading serine protease	P29138	St Leger *et al.* (1992)
Tritirachium album	TaPrK	Proteinase K	X14689	Gunkle and Gassen (1989)
Magnaporthe grisea	MgSPM1	Putative vacuolar subtilisin-like serine protease	AB070268	Fukiya *et al.* (2002)
Podospora anserina	PaPspA	Subtilisin-like serine protease	AF047689	Paoletti *et al.* (2001)
Metarhizium anisopliae	MaPR1H	Subtilisin-like protease PR1H	AJ251921	Unpublished

tial gene, the PCR product was cloned in pGEM-T Easy vector (Promega Co, USA). The DNA sequence of a *pr1*-like gene was determined on an ABI sequencer Model 377 (ABI system, USA). The obtained sequence was compared with the known protease genes using BLAST search. The homology analysis by Clustal W was performed using MegAlign™ (Ver. 4.0) in Lasergene99 and multiple amino acid sequence alignment was performed by Clustal X (ver. 1.83).

Results and Discussion

We first performed PCR toward the genomic DNA of *B. bassiana* to identify a *pr1*-like gene. Oligonucleotides were chosen based on the conserved regions of the subtilisin family enzymes, *pr1* genes of *B. bassiana* and *M. anisopliae,* and proteinase K of *T. album* (Fig. 1A and Table 1). Moreover, forward primer sequence includes the conserved sequence in bacterial subtilisin (Tyr-Ile-Val-Lys-Phe-Lys) (Gunkle and Gassen, 1989). In *B. bassiana* F-101, PCR using oligonucleotides, Pr1-F and Pr1-R had several fragments but the largest band was about 1 kb. And so we cloned full PCR products into pGEM-T easy, selected a construct containing *pr1*-like sequence in size of about 1 kb by sequencing. Finally, the cloned PCR fragment had 1111 bp including 52 bp intron (Fig. 1B and 2). A partial *pr1*-like gene of *B. bassiana* F-101 was compared to the known *pr1* gene of entomopathogenic fungus and the serine protease gene of other fungus. Surprisingly, the deduced Pr1-like peptide showed a low identity with Pr1s of entomopathogenic fungi such as *B. bassiana* Pr1 (BbPr1) and *M. anisopliae* (MaPr1) as well as the proteinase K of *T. album* (TaPrK) after an alignment by Clustal W (Table 2). In detail, the amino acid sequence of Pr1-like peptide has only 38.2, 44.2 and 37.8% sequence identity with BbPr1, MaPr1 and TaPrK, in other hand, BbPr1 has 59.5 and 63.6% identity with MaPr1 and TaPrK. However, in subsequent GenBank search performing by BLASTX, this deduced peptide had a significant

A

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(A) Forward direction
BbPr1        94 ATTGCCGGCAAGTACATTGTCAAGCTCAAGGAC  127
MaPr1       112 ATTGCCGACAAGTATATTGTCAAGTTCAAGGAT  144
TaPrK       748 GTTGCCAACAAGTACATTGTCAAGTTCAAGGAG  780
            Pr1-F  ⇒ 5´-CAAGTACATTGTCAAGYTCAAGG-3´ (Y:C,T)

(B) Reverse direction
BbPr1        952 ACTAATACCATCTCGGGCACTTCGATGGCCACTCCC  948
MaPr1        979 ACAAACTCCATCTCTGGTACCTCCATGGCTACTCCC  994
TaPrK       1669 ACCCGCTCCATCTCTGGAACTTCCATGGCTACTCCC 1704
            Pr1-R  ⇒ 3´-GGTAGAGMCCGTGAAGCTACCG-5´  (M:A,C)
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B

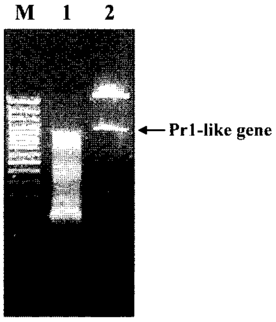

Fig. 1. Oligonucleotide design (A) for the detection of a *pr*1-like gene by the conserved sequences of the previously known *pr*1 genes. Bb, Ma and Ta indicate *B. bassiana*, *M. anisopliae* and *T. album.* Their GenBank accession numbers were listed in Table 1, and 1% agarose gel electrophoresis (B) of PCR-amplified product of the *pr1*-like gene. Lane M, 100 bp ladder; lane 1, PCR product of genomic DNA of *B. bassiana* F-101; lane 2, *Eco*RI digested *pr1*-like gene in pGEM-T easy.

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   1 CAAGTACATTGTCAAGTTCAAGGAGCATGTCGATGAGCCGAAGGCCAAGACTCACCACTC
      K  Y  I  V  K  F  K  E  H  V  D  E  P  K  A  K  T  H  H  S

  61 CTGGGTCTCGGATATCCATACCGATGGCAAGCAGCAGCGCCTCGAGCTTCGCAAGCGAGG
      W  V  S  D  I  H  T  D  G  K  Q  Q  R  L  E  L  R  K  R  G

 121 CCTCTTTGACTCAGTCGAAGATGCATTTGATGGCGTGAAGCACACCTTTGATCTCGGCTC
      L  F  D  S  V  E  D  A  F  D  G  V  K  H  T  F  D  L  G  S

 181 TGGCTTCCGCGGTTATGCTGGCCGCTTTGACGACGACGTCATTGAGCAGATCCGCAACCA
      G  F  R  G  Y  A  G  R  F  D  D  D  V  I  E  Q  I  R  N  H

 241 CCCTGACGTACGTATTACCTCAGTTTTTGCCACGCTTCATCATTTAACATGTCGACTAGG
      P  D                                                      V

 301 TCGAGGCCATCGAGCGTGACTCCATTGTACACACCATGGTCCCCATTCACCAAGGAGACT
       E  A  I  E  R  D  S  I  V  H  T  M  V  P  I  H  Q  G  D  C

 361 GTCACCGAGGACACTTGCAAACCCGAGACGGAGAAGCAGGCTCCTTTGGGGTCTTGCCCG
       H  R  G  H  L  Q  T  R  D  G  E  A  G  S  F  G  V  L  P  V

 421 TATCTCCCATCGCGAGACTCTCGGATTTGGTAACTTTAACAAGTATCTATACGCTAAAGG
       S  P  I  A  R  L  S  D  L  V  T  L  T  S  I  Y  T  L  K  G

 481 GTGGTCAGGGTGTTGATGCCTACGTTATTGACACTGGCACCAGCATTGCCCACGTCGATT
       G  Q  G  V  D  A  Y  V  I  D  T  G  T  S  I  A  H  V  D  F

 541 TCGAGGGCCGCGCCAAGTGGGGTTACACTGCCCCCGACGGCGATGTGGATGAGGATGGCA
       E  G  R  A  K  W  G  Y  T  A  P  D  G  D  V  D  E  D  G  N

 601 ACGGTCACGGCACTCACTGCTCCGGCACCATCGCCGGCAAGAAGTACGGTGTTGCCAAAA
       G  H  G  T  H  C  S  G  T  I  A  G  K  K  Y  G  V  A  K  K

 661 AGGCCAACGTTTACGCCGTCAAGGTGCTCCGCTCTAACGGCTCGGGCTCCATGTCGGATG
       A  N  V  Y  A  V  K  V  L  R  S  N  G  S  G  S  M  S  D  V

 721 TGATCAAGGGTGTCGACTGGGCTGCCAAGTCCCACGAGGAACAGGTCAAGAAGGCCAAGG
       I  K  G  V  D  W  A  A  K  S  H  E  E  Q  V  K  K  A  K  D

 781 ACGGTAAGCGCAAGGGCTTCAAGGGCTCCGTCGCCAACATGTCTCTTGGTGGTGGCAAGA
       G  K  R  K  G  F  K  G  S  V  A  N  M  S  L  G  G  G  K  T

 841 CTACTCTGCTCGACATGGCTGTCGACGCCGCCGTTGCCAAGGGCCTCCATTTTGCTGTTG
       T  L  L  D  M  A  V  D  A  A  V  A  K  G  L  H  F  A  V  A

 901 CTGCTGGTAACGATAACGCCGATGCCTGCAACTACTCTCCCGCTGCCGCCAAAAAGGCCG
       A  G  N  D  N  A  D  A  C  N  Y  S  P  A  A  A  K  K  A  V

 961 TCACCGTCGGTGCCTCCACCATTGATGACAGCCGTGCCTACTTCTCCAACTTCGGCAAGT
       T  V  G  A  S  T  I  D  D  S  R  A  Y  F  S  N  F  G  K  C

1021 GCACTGACATCTTTGCCCCCGGCCTGAACATCCAGTCCACCTGGATCGGCTCCAAGTATG
       T  D  I  F  A  P  G  L  N  I  Q  S  T  W  I  G  S  K  Y  A

1081 CCATCAACGCCATCTCGGGCACTTCGATGGC 1111
       I  N  A  I  S  G  T  S  M
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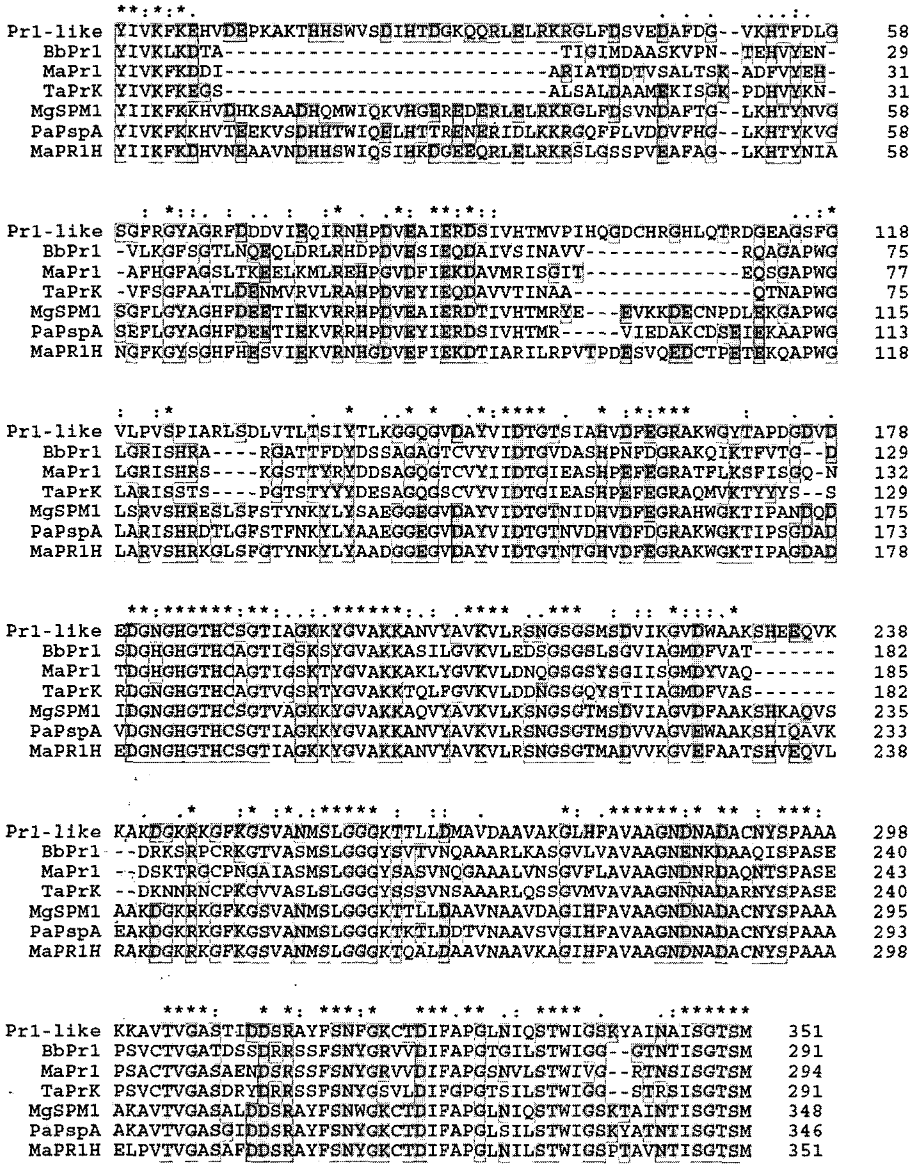
Fig. 2. Partial nucleotide sequence and the deduced amino acid sequence of a *pr*1-like gene from *B. bassiana* F-101. Introns are underlined.

Table 2. The deduced amino acid identity of a *pr1*-like gene of *Beauveria bassiana* F-101

% amino acid identity								
Gene	GenBank Acc. No.	Pr1-like	BbPr1	MaPr1	TaPrK	MgSPM1	PaPspA	MaPR1H
Pr1-like	-							
BbPr1	U16305	38.2						
MaPr1	P29138	44.2	59.5					
TaPrK	X14689	37.8	63.6	62.9				
MgSPM1	AB070268	70.1	40.3	45.9	42.3			
PaPspA	AF047689	69.1	41.8	46.3	44.3	78.0		
MaPR1H	AT251921	66.4	41.8	46.3	43.3	75.6	73.4	



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         **:*:*.                                     . .  .   ...:.
Pr1-like YIVKFKEHVDEPKAKTHHSWVSDIHTDGKQQRLELRKRGLFDSVEDAFDG--VKHTFDLG    58
   BbPr1 YIVKLKDTA----------------------------TIGIMDAASKVPN--TEHVYEN-    29
   MaPr1 YIVKFKDDI---------------------------ARIATDDTVSALTSK-ADFVYEH-    31
   TaPrK YIVKFKEGS---------------------------ALSALDAAMEKISGK-PDHVYKN-    31
  MgSPM1 YIIKFKKHVDHKSAADHQMWIQKVHGEREDERLELRKRGLFDSVNDAFTG--LKHTYNVG    58
  PaPspA YIVKFKKHVTEEKVSDHHTWIQELHTTRENERIDLKKRGQFPLVDDVFHG--LKHTYKVG    58
  MaPR1H YIIKFKDHVNEAAVNDHHSWIQSIHKDGEEQRLELRKRSLGSSPVEAFAG--LKHTYNIA    58

           : *::. : .. :  :* . .*: **:*::                        ..:*
Pr1-like SGFRGYAGRFDDDVIEQIRNHPDVEAIERDSIVHTMVPIHQGDCHRGHLQTRDGEAGSFG   118
   BbPr1 -VLKGFSGTLNQEQLDRLRHDPDVESIEQDAIVSINAVV-------------RQAGAPWG    75
   MaPr1 -AFHGFAGSLTKEELKMLREHPGVDFIEKDAVMRISGIT-------------EQSGAPWG    77
   TaPrK -VFSGFAATLDENMVRVLRAHPDVEYIEQDAVVTINAA---------------QTNAPWG    75
  MgSPM1 SGFLGYAGHFDEETIEKVRRHPDVEAIERDTIVHTMRYE---EVKKDECNPDLEKGAPWG   115
  PaPspA SEFLGYAGHFDEETIEKVRRHPDVEYIERDSIVHTMR-----VIEDAKCDSEIEKAAPWG   113
  MaPR1H NGFKGYSGHFHESVIEKVRNHGDVEFIEKDTIARILRPVTPDESVQEDCTPETEKQAPWG   118

         :  :*           .  *  ..* *  .*:**** .  * :*:***    :   .  .
Pr1-like VLPVSPIARLSDLVTLTSIYTLKGGQGVDAYVIDTGTSIAHVDFEGRAKWGYTAPDGDVD   178
   BbPr1 LGRISHRA----RGATTFDYDSSAGAGTCVYVIDTGVDASHPNFDGRAKQIKTFVTG--D   129
   MaPr1 LGRISHRS----KGSTTYRYDDSAGQGTCVYIIDTGIEASHPEFEGRATFLKSFISGQ-N   132
   TaPrK LARISSTS----PGTSTYYYDESAGQGSCVYVIDTGIEASHPEFEGRAQMVKTYYYS--S   129
  MgSPM1 LSRVSHRESLSFSTYNKYLYSAEGGEGVDAYVIDTGTNIDHVDFEGRAHWGKTIPANDQD   175
  PaPspA LARISHRDTLGFSTFNKYLYAAEGGEGVDAYVIDTGTNVDHVDFDGRAKWGKTIPSGDAD   173
  MaPR1H LARVSHRKGLSFGTYNKYLYAADGGEGVDAYVIDTGTNTGHVDFEGRAKWGKTIPAGDAD   178

          **:******:**:..:.******:.: .**** ..***  : :: *:::.*
Pr1-like EDGNGHGTHCSGTIAGKKYGVAKKANVYAVKVLRSNGSGSMSDVIKGVDWAAKSHEEQVK   238
   BbPr1 SDGHGHGTHCAGTIGSKSYGVAKKASILGVKVLEDSGSGSLSGVIAGMDFVAT-------   182
   MaPr1 TDGHGHGTHCAGTIGSKTYGVAKKAKLYGVKVLDNQGSGSYSGIISGMDYVAQ-------   185
   TaPrK RDGNGHGTHCAGTVGSRTYGVAKKTQLFGVKVLDDNGSGQYSTIIAGMDFVAS-------   182
  MgSPM1 IDGNGHGTHCSGTVAGKKYGVAKKAQVYAVKVLKSNGSGTMSDVIAGVDFAAKSHKAQVS   235
  PaPspA VDGNGHGTHCSGTIAGKKYGVAKKANVYAVKVLRSNGSGTMSDVVAGVEWAAKSHIQAVK   233
  MaPR1H EDGNGHGTHCSGTIAGKKYGVAKKANVYAVKVLRSNGSGTMADVVKGVEFAATSHVEQVL   238

           .  .*   :* :*.:***** :  ::  .      *: .******:* ** : ***:
Pr1-like KAKDGKRKGFKGSVANMSLGGGKTTLLDMAVDAAVAKGLHFAVAAGNDNADACNYSPAAA   298
   BbPr1 --DRKSRPCRKGTVASMSLGGGYSVTVNQAAARLKASGVLVAVAAGNENKDAAQISPASE   240
   MaPr1 --DSKTRGCPNGAIASMSLGGGYSASVNQGAAALVNSGVFLAVAAGNDNRDAQNTSPASE   243
   TaPrK --DKNNRNCPKGVVASLSLGGGYSSSVNSAAARLQSSGVMVAVAAGNNNADARNYSPASE   240
  MgSPM1 AAKDGKRKGFKGSVANMSLGGGKTTLLDAAVNAAVDAGIHFAVAAGNDNADACNYSPAAA   295
  PaPspA EAKDGKRKGFKGSVANMSLGGGKTKTLDDTVNAAVSVGIHFAVAAGNDNADACNYSPAAA   293
  MaPR1H RAKDGKRKGFKGSVANMSLGGGKTQALDAAVNAAVKAGIHFAVAAGNDNADACNYSPAAA   298

             ****:   * *: ***:*   ***.** .: **** .    .:******
Pr1-like KKAVTVGASTIDDSRAYFSNFGKCTDIFAPGLNIQSTWIGSKYAINAISGTSM   351
   BbPr1 PSVCTVGATDSSDRRSSFSNYGRVVDIFAPGTGILSTWIGG--GTNTISGTSM   291
   MaPr1 PSACTVGASAENDSRSSFSNYGRVVDIFAPGSNVLSTWIVG--RTNSISGTSM   294
   TaPrK PSVCTVGASDRYDRRSSFSNYGSVLDIFGPGTSILSTWIGG--STRSISGTSM   291
  MgSPM1 AKAVTVGASALDDSRAYFSNWGKCTDIFAPGLNIQSTWIGSKTAINTISGTSM   348
  PaPspA AKAVTVGASGIDDSRAYFSNYGKCTDIFAPGLSILSTWIGSKYATNTISGTSM   346
  MaPR1H ELPVTVGASAFDDSRAYFSNYGKCTDIFAPGLNILSTWIGSPTAVNTISGTSM   351
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Fig. 3. Alignment of the deduced *B. bassiana* F-101 Pr1 amino acid sequence with the previously reported protease gene of entomopathogenic fungi and other fungi. The GenBank accession number of each compared sequence is listed in Table 1. The star (*), colon (:) and period (.) denote identical, conserved and semi-conserved residues according to Clustal X alignment (ver. 1.83).

amino acid sequence identity in the range of over 65% to other subtilisin-like serine protease. Specially, this peptide has the highest identity (70.1%) with the protease of *Magnaporthe grisea* (MgSPM1) (Fukiya *et al.*, 2002) and 69.1% with the protease of *Podospora anserina* (PaPspA) (Paoletti *et al.*, 2001). In addition, it has 66.4% identity with one isoform of *M. anisopliae* Pr1 (MaPR1H, putative subtilisin-like protease). *M. grisea* is the causal agent of rice blast disease and is also one of the best characterized plant pathogenic fungi. The *spm1* gene of *M. grisea* is a vacuolar serine protease and may have an important role in pathogenicity. *Podospora anserina* is a saprophytic fungus, and its PspA peptide is a subtilisin-like serine protease and specially, is induced during this cell-death reaction (vegetative incompatibility). These two high identity-scored proteases seem to be quite different from *B. bassiana*. Further, the function of PR1H in *M. anisopliae* pathogenicity is not revealed. Clustal X alignment showed that the putative Pr1-like peptide was longer than other Pr1 genes in size but similar to other serine protease group (Fig. 3). Also, in the conserved sequence of subtilisin-like protease (Tyr-Ile-Val-Lys-Phe-Lys), a Pr1-like peptide was more conserved than BbPr1. These results, therefore, suggest that the putative Pr1-like peptide of *B. bassiana* F-101 indeed belongs to the subtilisin-like serine protease family and may be a novel gene.

In conclusion, this identification of the *pr1*-like protease as the major cuticle-degrading proteases produced by *B. bassiana* which is relatively unknown about proteases, may be a crucial clue to understanding penetration of host cuticles, a ubiquitous process among entomopathogenic fungi. Also, the *pr1*-like gene may be a useful source for improving pathogenicity by inserting it into *B. bassiana* chromosome as like the Pr1 of *M. anisopliae* (St. Leger *et al.*, 1996). Further studies will be conducted in order to clone the full *pr1*-like gene from cDNA of *B. bassiana* F-101 and express to investigate its protease activity.

Acknowledgements

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