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Isolation, cloning and molecular characterization of polygalacturonase I (*pga*I) gene from *Aspergillus niger* isolate from mango

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Isolation, cloning and molecular characterization of polygalacturonase (*pga*1) gene from the mango isolate of *Aspergillus niger* has been reported. The full length amplicon consisted of 1101 bp. The entire cDNA gene with the predicted protein of 367 amino acids had an estimated mol wt of 38.28 kDa with pI 4.40. When the nucleic acid sequence was compared with other *Aspergillus* spp., *pga*1 sequence showed the highest sequence similarity with *A. niger* and *A. fumigatus*. Comparison of the amino acid sequences revealed the presence of high degree of homology among the polygalacturonases (PGs) from different fungi. Bioinformatics analysis suggests that nucleic acid sequence of the isolated *pga*I gene shares 98% homology with the *pga*I gene of *A. niger*.

Keywords: *Aspergillus*, cloning, PCR, *pga*1 gene, polygalacturonase

Introduction

The metabolism of pectin substances containing the backbone of α-1,4-linked D-galacturonic acid residues is carried out in nature by an action of various pectinolytic enzymes, such as polygalacturonase, methylgalacturonase, pectin methyl esterase and pectin lyases. The pectinolytic enzymes have different role in nature depending on the organism producing them. They can act as enzymes important for fruit ripening[1]. They can also serve as enzymes involved in decay of plant tissue when produced by saprophytes. Polygalacturonases are the first cell wall degrading enzymes produced by fungal pathogens when cultured on isolated plant cell walls or during infections[2].

The main potential of the pectinolytic enzymes produced by the saprophytic fungus, *Aspergillus niger* is their wide applications in the food and beverage industries, especially for the preparation of juices[3]. Application of individual pectinases or defined combinations of pectinases, amongst which the polygalacturonases, esterases, pectate lyses can enhance the controlled breakdown of pectin and may lead to well defined pectic substances.

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Bussink *et al*[4,5] demonstrated the presence of 7 different *pga* genes from *A. niger*, of which three genes, viz., *pga*I, *pga*II and *pga*C, have already been characterized. In this paper, we report the isolation, cloning and molecular characterization of *pga*I gene isolated from the *A. niger* isolate from a mango fruit. Also the nucleic acid sequence of the *pga*I gene, its amino acid sequence and predicted mol wt of the protein from this isolate are mentioned.

Materials and Methods

Isolation of *A. niger*

The fungus, *A. niger* was isolated from the rind of an infected mango fruit. It was grown in Petridishes containing Potato Dextrose Agar (PDA) medium in an aseptic condition. Laboratory grown pure cultures of *A. niger* were used further for experimental purpose. Pure culture was inoculated on PDA in a Petridish and grown at room temperature (25°C±1°C) for 2-3 d for mycelia.

Preparation of cDNA

From the mycelia of *A. niger*, RNA was isolated as described by Sambrook *et al*[6]. cDNA was generated according to Gilliland *et al*[7]. In the first step, a reverse transcriptase reaction was performed on 5 µg of total RNA isolated from an *A. niger* using the reverse transcriptase enzyme, reaction buffers and a specific

3′ end primer as described by the supplier (Promega, USA). In the second step, the reverse transcriptase reaction was used for PCR employing the second, 5′end specific primer. The PCR program was used with the specific annealing temperature for consensus forward primer 5′ ATGCACTCTTACCAGCTTCTTGGC 3′ and reverse primer 5′ TCTCGCATTTATCGCTGGTCTTGC 3′ for *pga*I gene. The PCR amplification was carried out using the Piko Thermal Cycler of Finnzyme make. The master mix for PCR was prepared by adding 2 µL of Taq Buffer (10×), 2 µL dNTP's (10 m*M*), 0.4 µL of Taq Polymerase (3 U/µL), 7 µL of BSA (1 mg/mL), 0.2 µL of Tween 20, 2 µL of DNA-A and ß-DNA genome specific reverse and forward primers, 3 µL of template DNA from each biotypes and 3.4 µL of SMQ to make final volume to 20 µL. All the above components were mixed in a 0.2 mL PCR tube and PCR machine was set by giving the program for first denaturation at 94°C for 3 min, second denaturation at 94°C for 15 sec, annealing for 30 sec and extension at 72°C for 5 min. Each reaction proceeded with 38 cycles.

The PCR product was electrophoresed using 0.8% agarose gel. The gel was stained with ethidium bromide and visualized by using DNR make Gel Documentation System. An amplicon of approximately 1.1 kb was amplified.

Ligation and Identification of Insert DNA

The amplicon was eluted out from the agarose gel using the Gel Extraction Kit (Vivantis, Malaysia) by using the protocol given by the manufacturer.

Ligation of PCR product

The ligation of the amplicon was carried out as per the user's manual provided with the pGEM-T Easy vector kit (Promega, USA). *Escherichia coli* JM109 was used as the host cells. The ligation mixture was prepared by adding 5 µL of 2× Rapid ligation buffer, 2 µL of Vector (100-150 ng), 6 µL Insert (300-400 ng) and 1 µL T4 DNA Ligase (3 U/µL). Final volume made to 15 µL with SMQ water and a ligation reaction was set up. For getting the maximum number of transformants, the reaction was performed overnight at 4°C.

Preparation of Competent Cell Lines

A single colony of *E. coli* JM 109 was inoculated in 2 mL of LB medium and grown overnight at 37°C. About 500 µL of the overnight grown culture was added to 50 mL of fresh LB medium and grown for 2-3 h. Cells were harvested by centrifugation at 4,000 rpm for 10 min at 4°C. The cell pellet was suspended in 20 mL ice cold 100 m*M* $CaCl_2$ and recentrifuged. The pellet was resuspended in 1 mL of 100 m*M* $CaCl_2$. This was then dispensed in 200 µL aliquots to eppendorf tubes and kept at 4°C overnight.

A single cell culture was performed to get the pure culture carrying the insert DNA. A single colony of *E. coli* JM 109 was inoculated in 2 mL of LB medium and grown overnight at 37°C. 50 mL of LB medium was inoculated with a 5 mL overnight culture of the *E. coli* and grown at 37°C at 200 rpm. When an optimal density (OD_{600}) of 0.5-0.8 was reached, cells were chilled on ice for 15-20 min and centrifuged at 4000 rpm at 4°C for 15 min. The cells were then washed with one volume (50 mL) of ice cold sterile water and centrifuged again. The pellet was resuspended in 1 mL of 100 m*M* $CaCl_2$. This was then dispensed in 200 µL aliquots to eppendorf tubes and kept at 4°C overnight. The cells were finally suspended in 0.004 volumes (2 mL) of ice cold glycerol. Aliquots of 25-50 µL were placed into ice cold eppendorf tubes, snap frozen in liquid nitrogen and stored at –80°C.

Transformation of *E. coli*

The competent *E. coli* JM109 cells were transformed as described by Sambrook *et al*[6]. DNA (~50 ng) was added to the competent *E. coli* cells, mixed and kept on ice for 30 min. The cells were then incubated at 42°C for 2 min. To each tube 800 µL of LB broth was added and further incubated at 37°C for 1 h. The cells were pelleted by centrifugation and resuspended in 200 µL of LB broth and spread on LB medium plates containing appropriate antibiotic, IPTG (40 µg/mL) and 40 µg/mL X-gal. Total 13 transformed colonies were grown on the LB agar selective medium.

Isolation and Identification of Insert

Recombinant plasmid was isolated using alkaline lysis method and characterized by restriction digestion. The plasmid was digested with *Sac*II and *Not*I restriction endonucleases to isolate the insert from the vector plasmid. The desired fragment was also sequenced for further confirmation and characterization of the desired DNA (Fig. 1). The restricted fragment was eluted out from the agarose gel and sequenced.

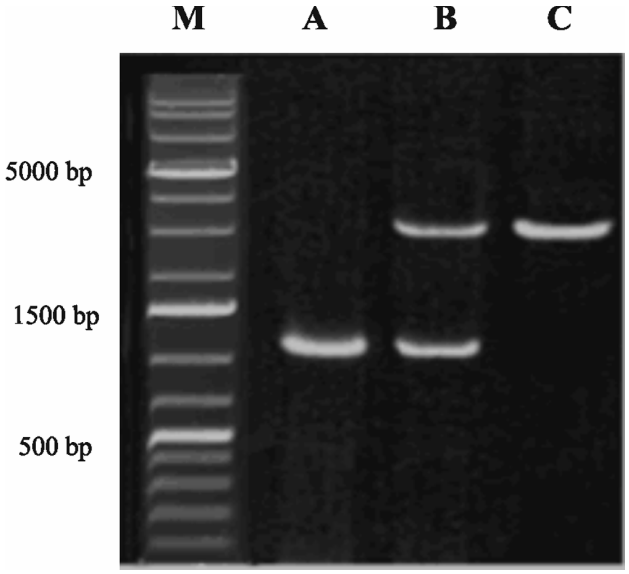

Fig. 1—Characterization of pGEMT carrying *pga*I: M = 1kb plus DNA ladder, A = PCR amplified product of *pga*I gene, B=T vector with insert plasmid (~3000 bp) and *pga*I gene insert (1101 bp after *Sac*II/*Not*I digestion), C=Plasmid (~3000 bp).

The DNA sequence was confirmed by using NCBI Blast tools. The sequenced DNA showed high sequence similarity with *pga*I gene of *A. niger*.

Results and Discussion

In order to isolate the *pga*I gene, a cDNA clone of the endopolygalacturonase of *A. niger* from mango fruit was used. The cDNA was cloned into pGEM-T vector and verified using the restriction digestion (Fig. 1). The partial cDNA clone of *pga*I with 1101 bp fragment contains a 1093 bp ORF, having a potential to encode a protein of 367 amino acid residues. The initiation codon ATG is at the positions of 1 bp and the stop codon TGA are at 1044 bp (Fig. 2).

Sequence Analysis of *pga*I

Multiple sequence alignment between *pga*I gene and *pga* genes from *A. niger* (NCBI Acc.no. XM 001389525) and *A. fumigatus* (Acc.no. XM 746347) was carried out by using ClustalW analysis tool (Fig. 2). The results of the Blast search using the amino acid sequence in the NCBI database showed that the gene isolated from the *A. niger* isolate has 1101 nucleotides. The length of cloned *pga*I is very similar to those of other reported from *Aspergillus* spp., which ranges from 1107 to 2495 nucleotides. ClustalW tool for multiple and pair-wise sequence alignments and phylogenetic tree construction aligning of sequences with the nucleic acid sequences of polygalacturonases from other two *Aspergillus* spp. revealed that the sequence of *pga*I from *A. niger* isolate shares 98 and 78% similarity with the polygalacturonase genes of *A. niger* (XM 001389525; Pel *et al*[9]) and *A. fumigatus* (XM 746347; Nierman *et al*[10]), respectively (Fig. 2).

Deduced Amino Acid sequence of *pga*I and Comparison with Other Polygalacturonase Genes

Amino acid sequence of the isolated *pga*I gene was predicted by using Genescan bioinformatics tool. The protein mass and isoelectric point was calculated by using Protein Calculator online tool (http://scansite.mit.edu/cgi-bin/calcpi). Deduced amino acid sequence was obtained by using ExPasy bioinformatics tool.

Based on a comparison of the nucleic acid sequence from *pga*I with sequences of the other fungal polygalacturonases, it is clear that polygalacturonase *pga*I, synthesized as cDNA clone, codes for a portion of 367 amino acids (Fig. 3). The deduced amino acid sequence of the mature enzyme codes for a protein of 38.28 kDa mol with an isoelectric point of 4.40.

A comparison of three polygalacturonases (endopolygalacturonase I, endopolygalacturonase II, endopolygalacturonase C) produced by *A. niger* was reported by Visser *et al*[8]. The multiple sequence alignment of deduced amino acid sequence of polygalacturonase enzyme (*pga*I) with other

DNA Multiple Sequence Alignment:

```
XM001389525     ATGCACTCTTACCAGCTTCTTGGCCTGGCCGCTGTCGGCTCCCTCGTCTCTGCCGCCCCC 60
pgaI            ATGCACTCTTACAGGCTTCATGGCCTGGCCGCTGTCGGCTCCCTCGTCTCTGCCGCCTTC 60
XM746347        ATGCGTTCTGTTAAGCTTTTTGGCCTGGCTGCGCTGGGCTCCCTCGGCGCTGCTGCCCCT 60
                ****    ***  ****  ********* **  * ********** * **** ***

XM001389525     GCTCCTTCTCGCGTCTCCGAGTTCGCTAAGAAGGCCTCTACCTGCACCTTCACCTCTGCC 120
pgaI            GCTCCTTCTCGCGTCTCCGAGTTCGCTATGAAGGCCTCTACCTGCACATTCACCTCTGCC 120
XM746347        GCTCCGTCTCGCGTGTCGGATCTGACCAAGAGGTCTTCAACTTGTACCTTCACCGCAGCC 120
                ***** ******** ** **  *  * * ** * * ** ** ** ** ****** * ***

XM001389525     TCTGAGGCCAGCGAGAGCATCTCCAGCTGCTCCGATGTTGTCCTGAGCAGCATCGAGGTC 180
pgaI            TCTGAGGCCAGCGAGAGCATCTCCAGCTGCTCCGATGTTGTCCTGAGCAGCATCGAGGTC 180
XM746347        TCCCAGGCTACCGAGAGCGCTTCTGGTTGCTCCGAGATTGTCCTGGACAACATCGAGGTT 180
                **  **** * *******   **  * ********  ********  ** *********

XM001389525     CCCGCTGGCGAGACCCTGGACCTGTCCGATGCTGCTGATGGCTCCACCATCACCTTCGAG 240
pgaI            CCCGCTGGCGAGACCCTGGACCTGTCCGATGCTGCTGATGGCTCCACCATCACCTTCGAG 240
XM746347        CCTGCCGGTGAGACACTGGATCTCTCGGATGTTGATGATGGAACCACCATCGTCTTCGAA 240
                ** ** ** ***** ***** ** ** **** ** ******  ********  ******

XM001389525     GGCACCACTTCCTTCGGATACAAGGAATGGAAGGGTCCCCTGATCCGCTTCGGTGGTAAG 300
pgaI            GGCACCACTTCCTGCGGATACAAGGAATGGAAGGGTCCCCTGATCCGCTTCGGTGGTAAG 300
XM746347        GGCACCACCACCTTTGGCTATAAAGAGTGGAGCGGCCCCTTGATCCGCTTCGGCGGCAAG 300
                ********  ***  ** ** ** ** ****  ** *** ************* ** ***

XM001389525     GATCTGACCGTCACCATGGCCGACGGCGCTGTCATCGACGGTGACGGTTCCCGCTGGTGG 360
pgaI            AATCTGACCGTCACCATGGCCGACGGCGCTGTCATCGACGGTGACGGTTCCCGCTGGTGG 360
XM746347        GACATCACCATCAAGCAGAACTCCGGCGCTGTGATTGACGGAGAAGGCTCCCGCTGGTGG 360
                 *  * *** ***    *  *  ********* ** ***** ** ** ************


XM001389525     GACAGCAAGGGTACCAACGGTGGCAAGACCAAGCCCAAGTTCATGTACATCCACGACGTT 420
pgaI            GACAGCAAGGGTACCAACGGTGGCAAGACCAAGCCCAAGTTCATGTACATCCACGACGTT 420
XM746347        GACGGCGAGGGCACCAATGGCGGCAAGACCAAGCCCAAGTTCATGTACGCGCACAGCCTC 420
                 *** ** **** ***** ** ***************************   ***  * *


XM001389525     GAGGACTCGACCTTCAAGGGCATCAACATCAAGAATACTCCCGTCCAGGCCATCAGTGTC 480
pgaI            GAGGACTCGACCTTCAAGGGCATCAACATCAAGAATACTCCCGTCCAGGCCATCAGTGTC 480
XM746347        GAGGACTCGACCATCACCGGGCTATCCATCAAGAACACACCTGTGCAGGCCATTAGTGTT 480
                ************ ***  **  *   ********* ** ** ** ******** *****

XM001389525     CAGGCTACCAACGTCCACCTGAACGACTTCACCATCGACAACTCCGACGGTGATGACAAC 540
pgaI            CAGGCTACCAAAGTCCACCTGAACGACTTCACCATCGACAACTCCGACGGTGATGACAAC 540
XM746347        CAGGCGACCAACCTCTACCTGATCGACATCACCATCGATAACTCCGACGGGGATGACAAC 540
                ***** *****  ** ****** **** ********** *********** *********

XM001389525     GGTGGCCACAACACCGACGGTTTCGACATCAGCGAGTCCACCGGTGTCTACATCAGCGGT 600
pgaI            GGTGGCCACAGGTCCGACGGTGTCGACATCAGCGAGTCCACCGGTGTCTACATCAGCGGT 600
XM746347        GGCGGGCACAACACTGACGGGTTTGACATCAGCGAGAGTACCGGGGTGTATATCCGCGGC 600
                ** ** ****   * *****  * ************   ***** ** ** *** ****
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(*Contd*)

DNA Multiple Sequence Alignment: —*Contd*

```
XM001389525    GCTACCGTCAAGAACCAGGACGACTGCATTGCCATCAACTCTGGCGAGAGCATCTCTTTC 660
pgaI           GCTACCGTCAAGAACCAGGACGACTGCATTGCCATCAACTCTGGCGAGAGCATCTCTTTC 660
XM746347       GCTACCGTCAAGAACCAGGATGACTGCATTGCCATCAACTCCGGCGAGAACATCGAATTC 660
               ******************** ******************** ******* ****   ***

XM001389525    ACCGGCGGTACCTGCTCCGGTGGCCACGGTCTCTCCATCGGCTCTGTCGGTGGCCGTGAT 720
pgaI           ACCGGCGGTACCTGCTCCGGTGGCCACGGTCTCTCCATCGGCTCTGTCGGTGGCCGTGAT 720
XM746347       TCCGGTGGTACCTGCTCTGGCGGCCACGGTCTCTCCATTGGCTCGGTTGGCGGTCGCGAC 720
                **** *********** ** ***************** ***** ** ** ** ** **

XM001389525    GACAACACCGTCAAGAACGTGACCATCTCCGACTCCACTGTCAGCAACTCCGCCAATGGT 780
pgaI           GACAACACCGTCAAGAACGTGACCATCTCCGACTCCACTGTCAGCAACTCCGCCAATGGT 780
XM746347       GACAACACCGTCAAGAACGTCACCATCACCGACTCCACCGTGACCGATTCGGCCAACGGC 780
               ******************** ****** ********** ** * * * * ** ***** **

XM001389525    GTCCGCATCAAGACCATCTACAAGGAGACCGGCGATGTCAGCGAGATCACCTACTCCAAC 840
pgaI           GTCCGCATCAAGACCATCTACAAGGAGACCGACGATGTCAGCGAGATCACCTACTCCAAC 840
XM746347       GTGCGTATCAAGACGGTCTACGACGCCACCGGCTCTGTTAGCCAAGTCACCTACTCCAAC 840
               ** ** ********  ***** * *  **** *  *** *** *  **************

XM001389525    ATCCAGCTCTCCGGCATCACCGACTACGGTATCGTCATCGAGCAGGACTACGAGAACGGC 900
pgaI           ATCCAGCTCTCCGGCATCACCGACTACGGTATCGTCATCGAGCAGGACTACGAGAACGGT 900
XM746347       ATCAAGCTGTCAGGTATCACGGACTATGGTATCGTCATCGAGCAGGACTACGAGAACGGC 900
               *** **** ** ** ***** ***** ********************************

XM001389525    TCTCCCACCGGCACCCCCTCCACCGGTATCCCCATCACTGATGTCACTGTTGACGGTGTC 960
pgaI           TCTCGCACCGGCACCCCCTCCACCGGTATCCCCATCACTGATGTCACTGTTGACGGTGTC 960
XM746347       AGCCCGACCGGTACCCCTACCACTGGCGTTCCGATCACCGACCTGACTATCGACGGTGTG 960
                *  ***** *****  **** **  * ** ***** **  * *** * ********

XM001389525   ACCGGCACTCTTGAGGATGACGCCACCCAGGTCTACATTCTCTGCGGTGACGGCTCTTGC 1020
pgaI          ACCGGCACTCTTGAGGATGACGCCACCCAGGTCTACATTCTCTGGGTAGACGGCTCTTGC 1020
XM746347      ACTGGTACCGTCGAGTCCGACGCCGTCGAGGTGTACATTCTTTGCGGAGACGGTAGCTGC 1020
              ** ** **  * ***   ******  * **** ******** ** *  *****    ***

XM001389525   TCTGACTGGACCTGGTCCGGTGTTGACCTCTCTGGTGGCAAGACCAGCGATAAATGCGAG 1080
pgaI          TCTGACTGGACCTGGTCCGGTGTTGACCTCTCTGGTGGCAAGACCAGCGATAAATGCGAG 1080
XM746347      AGTGACTGGACCTGGGAGGGCGTGGATATCACTGGCGGGGAGAAGAGCTCCAAGTGCGAG 1080
                *************   ** ** **  ** **** **  ***  ***   ** ******

XM001389525   AACGTTCCTTCCGGTGCTTCTTGCTAA 1107
pgaI          AACGTTCCTTCCGGTGCTTCT------ 1101
XM746347      AATGTTCCCTCAGGTGCTTCTTGCTAG 1107
              ** ***** ** *********
```

Fig. 2—Multiple sequence alignment of *pga*I gene along with polygalacturonases NCBI XM 001389525 and XM 746347 accessions.

Deduced Amino Acid Sequence:

```
ATGCACTCTTACAGGCTTCATGGCCTGGCCGCTGTCGGCTCCCTCGTCTCTGCCGCCTTC
 M  H  S  Y  R  L  H  G  L  A  A  V  G  S  L  V  S  A  A  F
GCTCCTTCTCGCGTCTCCGAGTTCGCTATGAAGGCCTCTACCTGCACATTCACCTCTGCC
 A  P  S  R  V  S  E  F  A  M  K  A  S  T  C  T  F  T  S  A
TCTGAGGCCAGCGAGAGCATCTCCAGCTGCTCCGATGTTGTCCTGAGCAGCATCGAGGTC
 S  E  A  S  E  S  I  S  S  C  S  D  V  V  L  S  S  I  E  V
CCCGCTGGCGAGACCCTGGACCTGTCCGATGCTGCTGATGGCTCCACCATCACCTTCGAG
 P  A  G  E  T  L  D  L  S  D  A  A  D  G  S  T  I  T  F  E
GGCACCACTTCCTGCGGATACAAGGAATGGAAGGGTCCCCTGATCCGCTTCGGTGGTAAG
 G  T  T  S  C  G  Y  K  E  W  K  G  P  L  I  R  F  G  G  K
AATCTGACCGTCACCATGGCCGACGGCGCTGTCATCGACGGTGACGGTTCCCGCTGGTGG
 N  L  T  V  T  M  A  D  G  A  V  I  D  G  D  G  S  R  W  W
GACAGCAAGGGTACCAACGGTGGCAAGACCAAGCCCAAGTTCATGTACATCCACGACGTT
 D  S  K  G  T  N  G  G  K  T  K  P  K  F  M  Y  I  H  D  V
GAGGACTCGACCTTCAAGGGCATCAACATCAAGAATACTCCCGTCCAGGCCATCAGTGTC
 E  D  S  T  F  K  G  I  N  I  K  N  T  P  V  Q  A  I  S  V
CAGGCTACCAAAGTCCACCTGAACGACTTCACCATCGACAACTCCGACGGTGATGACAAC
 Q  A  T  K  V  H  L  N  D  F  T  I  D  N  S  D  G  D  D  N
GGTGGCCACAGGTCCGACGGTGTCGACATCAGCGAGTCCACCGGTGTCTACATCAGCGGT
 G  G  H  R  S  D  G  V  D  I  S  E  S  T  G  V  Y  I  S  G
GCTACCGTCAAGAACCAGGACGACTGCATTGCCATCAACTCTGGCGAGAGCATCTCTTTC
 A  T  V  K  N  Q  D  D  C  I  A  I  N  S  G  E  S  I  S  F
ACCGGCGGTACCTGCTCCGGTGGCCACGGTCTCTCCATCGGCTCTGTCGGTGGCCGTGAT
 T  G  G  T  C  S  G  G  H  G  L  S  I  G  S  V  G  G  R  D
GACAACACCGTCAAGAACGTGACCATCTCCGACTCCACTGTCAGCAACTCCGCCAATGGT
 D  N  T  V  K  N  V  T  I  S  D  S  T  V  S  N  S  A  N  G
GTCCGCATCAAGACCATCTACAAGGAGACCGACGATGTCAGCGAGATCACCTACTCCAAC
 V  R  I  K  T  I  Y  K  E  T  D  D  V  S  E  I  T  Y  S  N
ATCCAGCTCTCCGGCATCACCGACTACGGTATCGTCATCGAGCAGGACTACGAGAACGGT
 I  Q  L  S  G  I  T  D  Y  G  I  V  I  E  Q  D  Y  E  N  G
TCTCGCACCGGCACCCCCTCCACCGGTATCCCCATCACTGATGTCACTGTTGACGGTGTC
 S  R  T  G  T  P  S  T  G  I  P  I  T  D  V  T  V  D  G  V
ACCGGCACTCTTGAGGATGACGCCACCCAGGTCTACATTCTCTGGGTAGACGGCTCTTGC
 T  G  T  L  E  D  D  A  T  Q  V  Y  I  L  W  V  D  G  S  C
TCTGACTGGACCTGGTCCGGTGTTGACCTCTCTGGTGGCAAGACCAGCGATAAATGCGAG
 S  D  W  T  W  S  G  V  D  L  S  G  G  K  T  S  D  K  C  E
AACGTTCCTTCCGGTGCTTCT
 N  V  P  S  G  A  S
```

Fig. 3—Nucleotide sequence of *pga*I gene isolated from *A. niger* and deduced amino acid sequence of the protein.

Protein Multiple Sequence Alignment:

```
XM001389525   MHSYQLLGLAAVGSLVSAAPAPSRVSEFAKKASTCTFTSASEASESISSCSDVVLSSIEV 60
pgaI          MHSYRLHGLAAVGSLVSAAFAPSRVSEFAMKASTCTFTSASEASESISSCSDVVLSSIEV 60
XM746347      MRSVKLFGLAALGSLGAAAPAPSRVSDLTKRSSTCTFTAASQATESASGCSEIVLDNIEV 60
              *:* :* ****:*** :** ******::: ::*******:**:*:** *.**::**..***

XM001389525   PAGETLDLSDAADGSTITFEGTTSFGYKEWKGPLIRFGGKDLTVTMADGAVIDGDGSRWW 120
pgaI          PAGETLDLSDAADGSTITFEGTTSCGYKEWKGPLIRFGGKNLTVTMADGAVIDGDGSRWW 120
XM746347      PAGETLDLSDVDDGTTIVFEGTTTFGYKEWSGPLIRFGGKDITIKQNSGAVIDGEGSRWW 120
              **********. **:**.*****: *****.*********::*:.  .******:*****

XM001389525   DSKGTNGGKTKPKFMYIHDVEDSTFKGINIKNTPVQAISVQATNVHLNDFTIDNSDGDDN 180
pgaI          DSKGTNGGKTKPKFMYIHDVEDSTFKGINIKNTPVQAISVQATKVHLNDFTIDNSDGDDN 180
XM746347      DGEGTNGGKTKPKFMYAHSLEDSTITGLSIKNTPVQAISVQATNLYLIDITIDNSDGDDN 180
              *.:************* *.:****:.*:.************:::* *:**********

XM001389525   GGHNTDGFDISESTGVYISGATVKNQDDCIAINSGESISFTGGTCSGGHGLSIGSVGGRD 240
pgaI          GGHRSDGVDISESTGVYISGATVKNQDDCIAINSGESISFTGGTCSGGHGLSIGSVGGRD 240
XM746347      GGHNTDGFDISESTGVYIRGATVKNQDDCIAINSGENIEFSGGTCSGGHGLSIGSVGGRD 240
              ***.:**.********** *****************.*.*:****************** 

XM001389525   DNTVKNVTISDSTVSNSANGVRIKTIYKETGDVSEITYSNIQLSGITDYGIVIEQDYENG 300
pgaI          DNTVKNVTISDSTVSNSANGVRIKTIYKETDDVSEITYSNIQLSGITDYGIVIEQDYENG 300
XM746347      DNTVKNVTITDSTVTDSANGVRIKTVYDATGSVSQVTYSNIKLSGITDYGIVIEQDYENG 300
              *********:****::*********:*. *..**::*****:****************** 

XM001389525   SPTGTPSTGIPITDVTVDGVTGTLEDDATQVYILCGDGSCSDWTWSGVDLSGGKTSDKCE 360
pgaI          SRTGTPSTGIPITDVTVDGVTGTLEDDATQVYILWVDGSCSDWTWSGVDLSGGKTSDKCE 360
XM746347      SPTGTPTTGVPITDLTIDGVTGTVESDAVEVYILCGDGSCSDWTWEGVDITGGEKSSKCE 360
              * ****:**:****:*:******:*.**.:****  *********.***::**:.*.***

XM001389525   NVPSGASC 368
pgaI          NVPSGAS- 367
XM746347      NVPSGASC 368
              *******
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Fig. 4—Multiple sequence alignment of polygalacturonase (*pga*I) amino acid sequence along with NCBI XM 001389525 and XM 746347 accessions.

polygalacturonases (*pga*) shares 96% amino acid sequence similarity with the protein from *A. niger* (XM 001389525) and 76% similarity from the *A. fumigatus* (XM 746347) (Fig. 4).

Further work on expression, catalytic activity and mutagenesis of *pga*I gene is under consideration.

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