Original article

Identification and Molecular Characterization of the cDNA Encoding *Cucumis melo* Allergen, Cuc m 3, a Plant Pathogenesis-Related Protein

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Abstract

Background: Melon (*Cucumis melo*) allergy is one of the most common food allergies, characterized by oral allergy syndrome. To date, two allergen molecules, Cuc m 1 and Cuc m 2, have been fully characterized in melon pulp, but there are few reports about the molecular characteristics of Cuc m 3.

Methods: The Cuc m 3 cDNA has been characterized by rapid amplification of cDNA ends (RACE), which revealed a 456 base-pair (bp) fragment encoding a 151-amino acid polypeptide with a predicted molecular mass of 16.97 kDa, and identified 79 and 178 bp untranslated sequences at the 5′ and 3′ ends, respectively.

Results: In silico analysis showed strong similarities between Cuc m 3 and other plant pathogen-related protein 1s from cucumber, grape, bell pepper, and tomato.

Conclusion: Here we report the identification and characterization of the Cuc m 3 cDNA, which will be utilized for further analyses of structural and allergenic features of this allergen.

Keywords: Allergen, Cuc m 3, Melon, Plant pathogenesis-related protein 1

Introduction

Melon (*Cucumis melo*) belongs to the gourd family, *Cucurbitaceae*, which also includes cucumber, pumpkin, squash, and watermelon. Honeydew, cantaloupe, and muskmelon represent some of the most common hybrids and cultivars of this family (1, 2). Recent studies have revealed that up to 7% of young children and about 4% of adults suffer from some type of food allergy (3). Melon (*C. melo*) allergy is one of the most common food allergies, characterized by oral itching, lip swelling, and labial edema (4). The initial report of the melon sensitivity was closely linked to the early description of the oral allergy syndrome (OAS). In 1970, Anderson et al. reported a case series of patients with ragweed allergy who experienced oral symptoms after eating various melons (eg, watermelon, cantaloupe, and honeydew)

and bananas (5). Ortolani et al. described an association between allergy to grass pollen and some vegetable hypersensitivity, such as tomato, melon, and watermelon (6). In a study of patients with pollen allergies, about one fifth of the patients showed IgE sensitivities to melon and pollen (7). Specific IgE assays suggest that some common antigenic epitopes exist between melon and grass pollen allergens (8, 9). Additionally, there are anecdotal reports of anaphylactic reactions to melon and ethanol-induced anaphylaxis after the ingestion of overripe melon (10, 11). More recently, Brehler et al. detected specific IgE antibodies to a wide variety of fruits, including melon, in 69% of serum samples from 136 patients with latex allergies (12).

To date, three melon allergens have been identified in melon pulp including Cuc m 1, a subtilisin-like protease (13), Cuc m 2, a profilin (4, 14), and Cuc m 3 (15), a plant pathogen-related protein 1 (PR-1). Cuc m 1 and Cuc m 2, which were recognized by more than 50% of the patients' sera, have been characterized by several research groups (15). Cuc m 3 belongs to the pathogen-related family and exhibits some amino acid similarities with the other members of this family in grape and cucumber. This allergen, as a PR-1 protein, is highly stable and insensitive to proteases that are found in the melon fruit juices (15). PR-1 is a dominant group of PRs induced by pathogens. Since their discovery in 1970, numerous researchers have attempted to assess the function of PR-1 proteins in plants, but with little success (15, 16). The aims of this study were to clone and characterize the cDNA encoding Cuc m 3, and use it to analyze structural and allergenic features of this allergen.

Materials and Methods

Isolation of RNA, first-strand cDNA synthesis, and amplification of melon Cuc m 3 cDNA

Total RNA was extracted from 1 g of fine powder ground from the inner layer of melon in liquid nitrogen by means of the guanidine thiocyanate extraction method [19]. First-strand cDNA was synthesized from 2 μg of total RNA using a first-strand cDNA synthesis Kit (Fermentas, Lithuania) with an oligo (dT) 18-containing primer (5′-CGCTACGTAACGGCATGACAGTGTTTTTTT TTTTTTTTTTT-3′). Amplification of the unknown sequence of the 3′ end from melon Cuc m 3 cDNA was carried out by 3′ rapid amplification of cDNA ends (RACE) primer (GeneRacerTM kit, Invitrogen, San Diego, CA, USA) and degenerate primers (Table 1) that were designed based on highly-conserved sequences in the coding regions of several members of PR-1 family found in Genebank. The amplification was performed with the following conditions: 3 min at 95 ℃, then the first 5 cycles of denaturation at 95 ℃ for 30 s, annealing at 53 ℃ for 120 s and elongation at 72 ℃ for 60 s; then 25 continuous cycles of denaturation at 95 ℃ for 30 s, annealing at 58 ℃ for 60 s and elongation at 72 ℃ for 45 s; and finally elongation for at 72 ℃ for 3 min on a Corbett Research thermocycler (Sydney, Australia). Ten microliters of the polymerase chain reaction (PCR) product were electrophoresed on a 2% ethidium-stained agarose gel and documented on a G-Box gel documentation system (Syngene, Cambridge, UK). After amplification, PCR products were cleaned using Gene Clean II kit (Q–Biogen, Illkirch, France), sub-cloned into the vector pTZ57R/T with the TA cloning kit (Fermentas, Lithuania), and sequenced (MWG Biotech AG, Ebersberg, Germany).

To determine the 5′ end of Cuc m 3 cDNA, a GeneRacerTM RNA oligo tail was ligated to the 5′ end of the purified cDNA.. These reactions created first-strand cDNA with known priming sites at the 5′ end. Then, the 5′ untranslated region (UTR) of the cDNA was amplified with a GeneRaceTM 5′ primer and a gene-specific primer (5′-GGCAAGATGTGTGGCCATTACAC-3′), which was designed based on the Cuc m 3 3' cDNA sequence. The cDNA 3' end was also amplified by PCR using a gene-specific primer (3′-CACAGAATTTCTCCACACCACTTGAG-3′) and a GeneRaceTM 3′ primer. All RACE-PCRs were carried out under the following program: 3 min at 94 ℃, then 38 cycles of denaturation at 95 ℃ for 60 s, annealing at 68 ℃ for 60 s, and elongation at 72 ℃ for 60 s; then a final elongation at 72 ℃ for 5 min on a Corbett Research thermocycler (Sydney, Australia). After amplification of the 5′ and 3′ cDNA ends, the PCR products were sub-cloned into the vector pTZ57R/T with the TA cloning kit (Fermentas, Lithuania). Clones carrying inserts were characterized by restriction analyses and sequencing. All of the amplifications for T/A cloning were performed with Pfu DNA polymerase, and poly-A tails were added using Taq DNA polymerase.

Table 1. The degenerate primers designed based on highly conserved sequence in the coding regions of several plant PR-1 sequences

Name	Primer sequence
F1	5′ GCNGTGVAVTTGTGGGTG 3′
F2	5′ CGKGCMCAAGTYGGVGTYGG 3′
F3	5′ GGTGGTTYRTYWCHTGCAACTA 3′
F4	5′ CAAAGTGAGGTGCACAAATAATCG 3′

Analysis of predicted protein sequence

The predicted molecular mass and isoelectric point were determined by the Gene Runner program v 3.05 (Hustington software). The deduced protein sequence of Cuc m 3 was next subjected to a protein-protein BLAST similarity search. Multiple sequence

alignment was performed by BioEdit software. The deduced amino acid sequences of PR-1s were obtained from the NCBI Protein Database with the following accession numbers: Q8S3W2 (Cucumis sativus), XP002273416 (Vitis vinifera), Q7XAJ6 (Vitis vinifera), ADB54823 (Vitis pseudoreticulata), EEE87889 (Populus trichocarpa), BAB78476 (Solanum torvum), AAK30143 (Capsicum annuum), ACB88202 (Solanum lycopersicum), and AAU15051.1 (Cynodon dactylon).

Results

cDNA cloning and sequencing of the Cuc m 3 gene

PCR of the melon cDNA containing the defined sequence at the 3′ end by the degenerate 5′ forward primer (F2) and the 3′ primer (5'-CGCTACGTAACGGCATGACAGTG-3') resulted in a 680-bp product (Fig. 1) that was cloned into pTZ57R/T. Four of these clones were sequenced (Table 1). After sequencing, two primers were designed based on the known sequence to obtain the full sequence of Cuc m 3, including the 5′ and 3′ ends. The amplified 3′-end sequence confirmed the 3′-end sequence generated by degenerate forward and defined reverse primers. By the 5′-RACE technique, a 480-bp fragment was amplified with an overlapping sequence with the obtained 3-end sequence (Fig. 1).
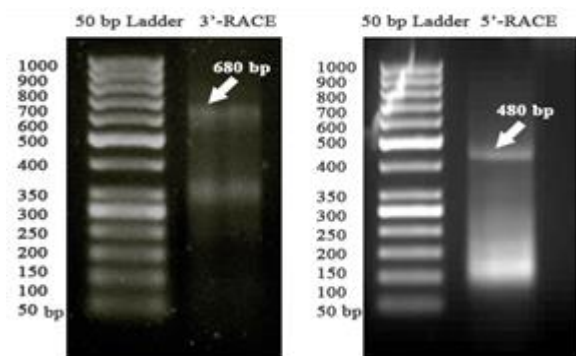
Fig. 1. Amplification of 5´-end (right) and 3´-end (left) of the Cuc m 3 cDNA by RACE-PCR.

Finally, a 456 bp nucleotide sequence representing the complete cDNA sequence of Cuc m 3, including 79 and 178 bps of the 5′ and 3′ UTRs, respectively, was obtained by the cluster analysis of all the above fragments (Fig. 2).

The nucleotide sequences for the coding region and 5′ UTR of Cuc m 3 were deposited in the GenBank database under the accession numbers of EU556704.1 and EU679402.1, respectively. The amino acid sequence of this protein can be accessed through the NCBI Protein Database, Accession Number ACB45874.1.
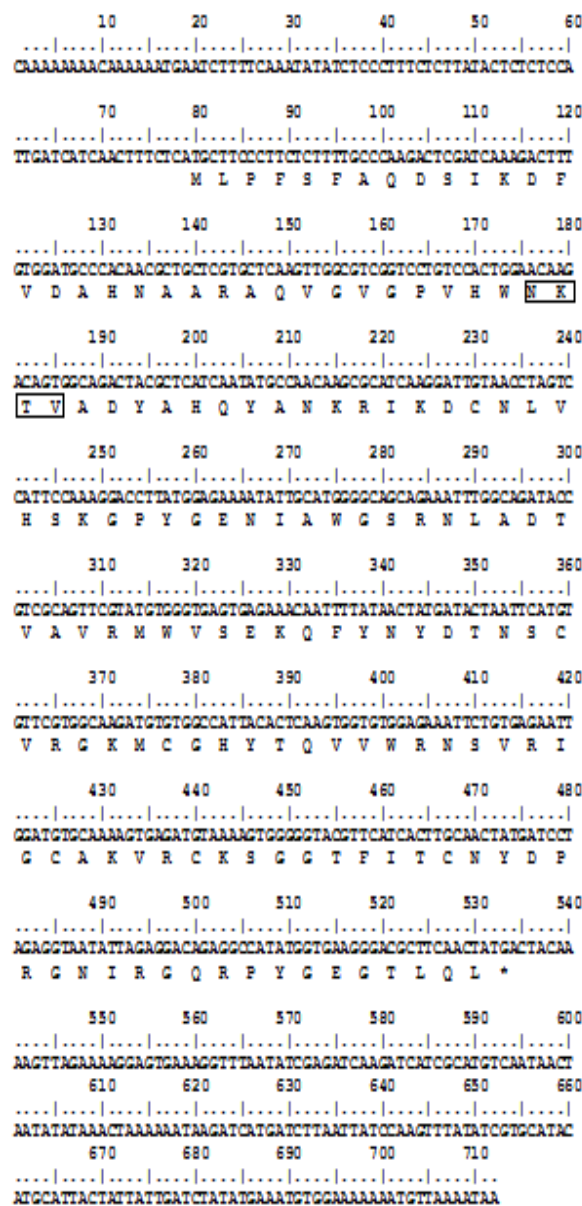
Fig. 2. Nucleotide sequence of the complete Cuc m 3 cDNA and its deduced amino acid sequence. The potential N-linked glycosylation site (NKTV) was shown within an open box. Asterisk indicates the stop codon. The position of F1 primer has been underlined.

Nucleotide and amino acid sequence analysis of Cuc m 3 cDNA

Sequencing analysis revealed a 453-bp open-reading frame (EU556704), which encodes Cuc m 3, a 151-amino-acid polypeptide (ACB45874.1) with a predicted molecular mass of 16.97 kDa and a theoretical isoelectric point value of 9.47 (Fig. 2). One

potential N-glycosylation site was identified at amino acids 33-36 (NKTV) using the Gene Runner program.

The deduced protein sequence was subjected to a protein-protein BLAST similarity.

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                                 10        20        30        40        50        60        70
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACB45874(Cucumis melo)           ----------------------------MLPFSFAQDSIKDFVDAHNAARAQVG--------------
Cuc m 3 fragments Ref. 12        -----------------------------------------....................---------
Q8S3W2(Cucumis sativus)          -----------------------------------....PQ...GV..V...........----------
XP002273416(Vitis vinifera)      ------------MGLFKISLVFIC-LVGLALVHT.C..N.QQ.YLN..T..........------------
BAF95881(Vitis hybrid)           -----------MGLCRSPLALLC-FMGLALAHICC..N.PQ.YLN..T..........-------------
Q7XAJ6(Vitis vinifera)           -----------MGLCRSPLALLC-FMWLALAHICC..N.PQ.Y.N..............------------
ADB54823(Vitis pseudoreticulat   -----------MGLCKISSSLVVGFMGLVLAHI.Y..N.AQ..L...VP..E..................
EEE87889.1(Populus trichocarpa   -----------MMSSNISLAFFT-LITLSLILP.R...NPQ.YL.....A...-----------------
BAB78476(Solanum torvum)         --------------------VACFITFAIFHS.Q..TPQEN.LN.....RR------------------
AAK30143(Capsicum annuum)        --------------MGHSNIALIVCFITFAIFHSTQ..N.PQ.YLN.....R.----------------
ACB88202(Solanum lycopersicum)   --------------MGYSNIALIICFLTFAIFHS.Q..N.PQ.YLN.....R.----------------
AAU15051.1(Cynodon dactylon)     ---------------------STQLPSDEPLNGLNDKA.Q.ILNE.MF..KEH---------------
P35782 (Vespa cabro)             NNYCKIKCRSGIHTLCKYGTSTKPNCGKNVVKASGLTKQENLEILKQ..EF.QK.ARGLETRGNPGPQPP
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                                        80        90       100       110       120       130       140
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACB45874(Cucumis melo)           --VGPVHWNKTVADYAHQYANKRIKDQNLVHSKGPY--GENIAWGSRNLA----GTVAVRMWVSEKQFY
Cuc m 3 fragments Ref. 12        --....--.....-..R......X.....AT---------------------------
Q8S3W2(Cucumis sativus)          ---.IE.D....SF.Q....R.LN..R..N.G........PD.S---AKD.QI..D..P.
XP002273416(Vitis vinifera)      ---..SMT..N...S..QN...Q..Q....N...........GS.T----.D..NI..G..PN
BAF95881(Vitis hybrid)           ---..MS.DN...A..QN...Q..G......L....PS.T----.D..N..G..SN
Q7XAJ6(Vitis vinifera)           ---.SIT..D...A..QN....S..........LK..GS.T----.D..NI..G..PN
ADB54823(Vitis pseudoreticulat   ---..MS.DN...A..QN.I.Q..G......L....PS.T----.D..NI..G..SN
EEE87889.1(Populus trichocarpa   ---..LI.DT..QA..QN...Q.AG....I.G......L..S.AD.S----..D..K...D..AY
BAB78476(Solanum torvum)         ---..MI.DN.I.A..QN...Q..G..MMQ....D......AAFPQ.N----AAG..K..ED..W
AAK30143(Capsicum annuum)        ---..MI.DNRL.AF.QN...Q..G..RMQ....D......AAFPQ.N----AAG..K..N...W
ACB88202(Solanum lycopersicum)   ---..MI.DNRL.AF.QN...Q.AG..RMQ....D......AAFPQ.N----AAG..K..D..W
AAU15051.1(Cynodon dactylon)     ---.P.LI..T.L.KFSQD..ESKL..KM...DS....LMF..GAIS----WKTT.DT.SD..KS
P35782 (Vespa cabro)             AKSMNTLV..DEL.QI.QVW..QCNYGHDNCRNSAK.SV.Q..E..TSADNFVNVSNM.K..ED.VKD
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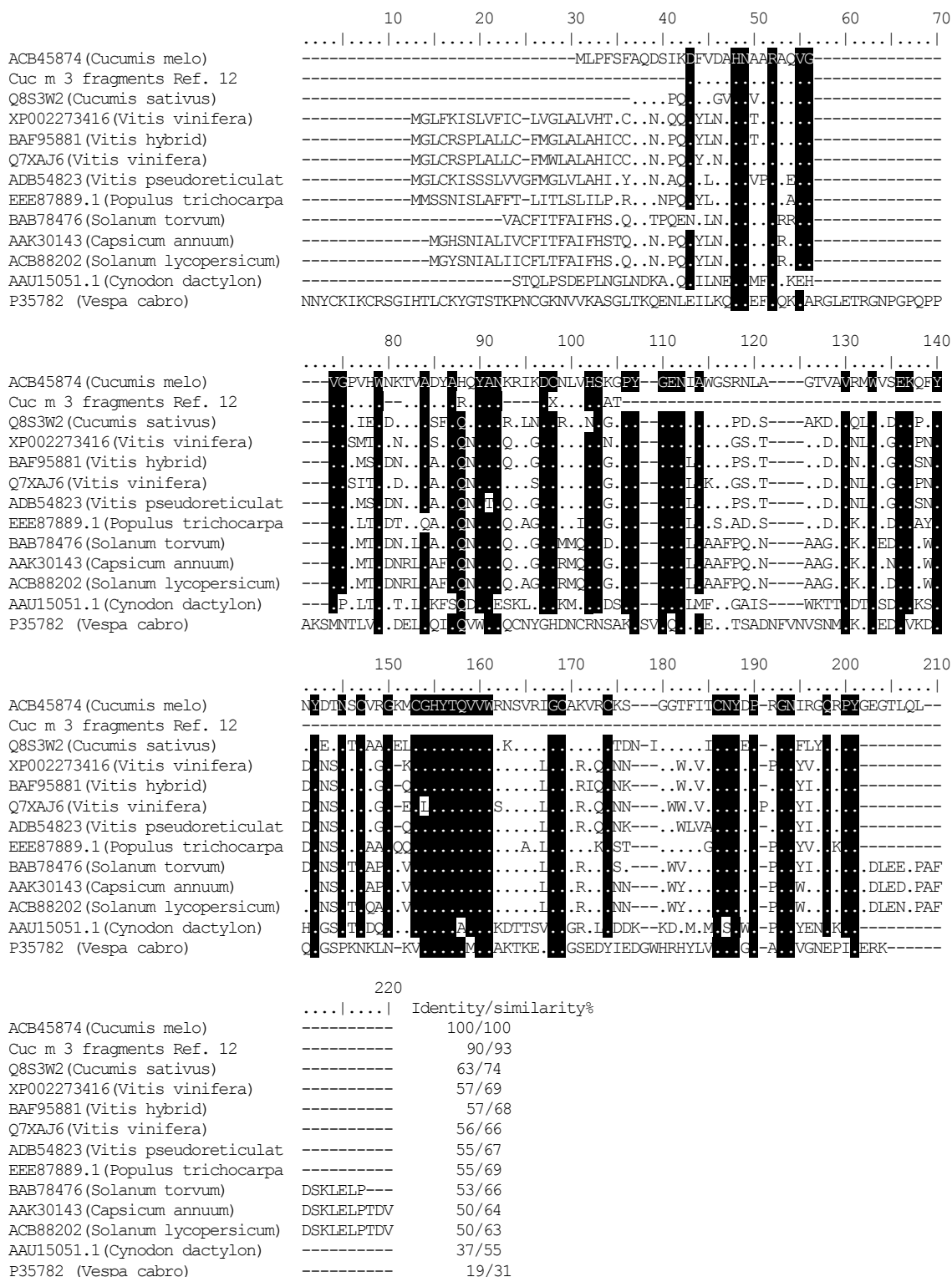
```
                                       150       160       170       180       190       200       210
                                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACB45874(Cucumis melo)           NYDTNSQVRGKMCGHYTQVVWRNSVRIGGAKVRCKS---GGTFITCNYLE-RGNIRGQRPYGEGTLQL--
Cuc m 3 fragments Ref. 12        --------------------------------------------------------------------
Q8S3W2(Cucumis sativus)          ..E..IT.AA.EL.............K.......TDN-I......L..E..-.FLY..---------
XP002273416(Vitis vinifera)      D.NS...G.-K............L...R.QNN---..W.V......-.P..YV..---------
BAF95881(Vitis hybrid)           D.NS...G.-Q............L...RIQ.NK---..W.V........-...YI..---------
Q7XAJ6(Vitis vinifera)           D.NS...G.-E.I........S....L...R.Q.NN---.WW.V......-.P...YI..---------
ADB54823(Vitis pseudoreticulat   D.NS...G.-Q............L...R.Q.NK---..WLVA........-...YI..---------
EEE87889.1(Populus trichocarpa   D.NS...AA.QQ.............A.L....K.ST---.....G.....-.P..YV..K.---------
BAB78476(Solanum torvum)         D.NS.T.AP..V............L...R..S.---.WV....-.YI...-......DLEE.PAF
AAK30143(Capsicum annuum)        ..NS...AP..V............L...R....NN---..WY.......-.P...W...-......DLED.PAF
ACB88202(Solanum lycopersicum)   ..NS.T.QA..V............L...R...NN---..WY.......-.P...W...-......DLEN.PAF
AAU15051.1(Cynodon dactylon)     H.GS.TKDQ......A....KDTTSV..GR.L.DDK--KD.M.M.S.W.-.P..YEN.K..---------
P35782 (Vespa cabro)             Q.GSPKNKLN-KV.....M..AKTKE...GSEDYIEDGWHRHYLV...G.-A..VGNEPI.ERK------
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                                 220
                                 ....|....|   Identity/similarity%
ACB45874(Cucumis melo)           ----------     100/100
Cuc m 3 fragments Ref. 12        ----------      90/93
Q8S3W2(Cucumis sativus)          ----------      63/74
XP002273416(Vitis vinifera)      ----------      57/69
BAF95881(Vitis hybrid)           ----------      57/68
Q7XAJ6(Vitis vinifera)           ----------      56/66
ADB54823(Vitis pseudoreticulat   ----------      55/67
EEE87889.1(Populus trichocarpa   ----------      55/69
BAB78476(Solanum torvum)         DSKLELP---      53/66
AAK30143(Capsicum annuum)        DSKLELPTDV      50/64
ACB88202(Solanum lycopersicum)   DSKLELPTDV      50/63
AAU15051.1(Cynodon dactylon)     ----------      37/55
P35782 (Vespa cabro)             ----------      19/31
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Fig. 3. Comparison of amino acid sequence deduced from Cuc m 3 cDNA (ACB45874) with other plant PR-1 from *Cucumis sativus* (Q8S3W2), *Vitis vinifera* (XP002273416), *Vitis hybrid* (Vitis hybrid), *Vitis vinifera* (Q7XAJ6), *Vitis pseudoreticulata* (ADB54823), *Populus trichocarpa* (EEE87889), *Solanum torvum* (BAB78476), *Capsicum annuum* (AAK30143), *Solanum lycopersicum*(ACB88202) and *Cynodon dactylon* (AAU15051.1) and *Vespa cabro* (P35782) . The amino acid sequence identity and similarity with Cuc m 3 were indicated at the end of the alignment. Black shading indicates identical amino acids**.**

search, which identified a sperm-coating protein (SCP)-like extracellular protein domain in the deduced Cuc m 3 amino acid sequence. A protein homology search using BLAST software demonstrated that Cuc m 3 shared 63, 57, 55, 53, 50, 50, and 37% identical residues with pathogenesis-related protein PR-1 of cucumber (Cucumis sativus), grape (Vitis vinifera), black cottonwood (Popoulus trichaocarpa), Turkey berry (Solanum torvum), bell pepper (Capsicum annuum), tomato (Solanum lycopersicum), and Bermuda grass (Cynodon dactylon), respectively. The highest degrees of 74% to 55% amino acid sequence similarities were with proteins in the PR-1 family (Fig. 3). All proteins with significant matches had sizes similar to the Cuc m 3 protein, ranging from 141 to 176 amino acids.

Discussion

In this work, we report the complete cDNA sequence of Cuc m 3 from *Cucumis melo* using 5´ and 3´RACE and degenerate primers designed based on the partial amino acid sequences of members of the PR-1 family (15). Previously reported partial amino acid sequences (40 amino acids) indicate 10% differences with the deduced amino acid derived from the complete Cuc m 3 cDNA. The complete amino acid sequence of Cuc m 3 showed a strong similarity (more than 60% sequence identity) with the other members of PR-1 family from grape and cucumber. This fact, combined with the presence of an SCP-like extracellular protein domain in the deduced Cuc m 3 amino acid sequence, implies that this allergen belongs to the plant pathogenesis-related protein 1 (PR-1) family. This PR protein accumulates after infections with pathogens, and may act as an anti-fungal agent or be involved in cell wall loosening. Many plant allergens from food and pollen have been found to be PR proteins (15-18). Because PR proteins can be induced by stress conditions, such as pathogen infection (19), they could be potential cross-reacting allergens of plant foods and their quantitative presence can vary with growing, harvesting, and storage conditions. Their weak homology to group 5 allergens from insect venoms 3 could link food allergy and hypersensitivity to insect stings in some patients (20). In conclusion, in this report we identified and characterized the cDNA encoding Cuc m 3, which can be utilized in in vitro expression and potentially aid in more detailed analyses of structural and allergenic features of this allergen than were previously possible.

Acknowledgements

References

1. Rodriguez J, Crespo JF, Burks W, Rivas-Plata C, Fernandez-Anaya S, Vives R, et al. Randomized, double-blind, crossover challenge study in 53 subjects reporting adverse reactions to melon (Cucumis melo). J Allergy Clin Immunol. 2000 Nov;106(5):968-72.

2. Obando-Ulloa JM, Eduardo I, Monforte AJ, Fernández-Trujillo JP. Identification of QTLs related to sugar and organic acid composition in melon using near-isogenic lines. Scientia Horticulturae. 2009 Aug;121(4):425-33.

3. Palomares O, Vereda A, Cuesta-Herranz J, Villalba M, Rodriguez R. Cloning, sequencing, and recombinant production of Sin a 2, an allergenic 11S globulin from yellow mustard seeds. J Allergy Clin Immunol. 2007 May;119(5):1189-96.

4. Sankian M, Varasteh A, Pazouki N, Mahmoudi M. Sequence homology: a poor predictive value for profilins cross-reactivity. Clin Mol Allergy. 2005 Sep;3:13.

5. Anderson LB, Jr., Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. J Allergy. 1970 May;45(5):310-9.

6. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. Ann Allergy. 1988 Dec;61(6 Pt 2):47-52.

7. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. Clin Exp Allergy. 1994 Apr;24(4):367-74.

8. Garcia Ortiz JC, Cosmes Martin P, Lopez-Asunolo A. Melon sensitivity shares allergens with Plantago and grass pollens. Allergy. 1995 Mar;50(3):269-73.

9. Garcia Ortiz JC, Ventas P, Cosmes P, Lopez-Asunsolo A. An immunoblotting analysis of cross-reactivity between melon, and plantago and grass pollens. J Investig Allergol Clin Immunol. 1996 Nov-Dec;6(6):378-82.

10. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med. 1987 Jun;316(26):1622-6.

11. Mallon DF, Katelaris CH. Ethanol-induced anaphylaxis following ingestion of overripe rock melon, Cucumis melo. Ann Allergy Asthma Immunol. 1997 Mar;78(3):285-6.

12. Brehler R, Theissen U, Mohr C, Luger T. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. Allergy. 1997 Apr;52(4):404-10.

13. Cuesta-Herranz J, Pastor C, Figueredo E, Vidarte L, De las Heras M, Duran C, et al. Identification of Cucumisin (Cuc m 1), a subtilisin-like endopeptidase, as the major allergen of melon fruit. Clin Exp Allergy. 2003 Jun;33(6):827-33.

14. Rodriguez-Perez R, Crespo JF, Rodriguez J, Salcedo G. Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome. J Allergy Clin Immunol. 2003 Mar;111(3):634-9.

15. Asensio T, Crespo JF, Sanchez-Monge R, Lopez-Torrejon G, Somoza ML, Rodriguez J, et al. Novel plant pathogenesis-related protein family involved in food allergy. J Allergy Clin Immunol. 2004 Oct;114(4):896-9.

16. Hoffmann-Sommergruber K. Plant allergens and pathogenesis-related proteins. What do they have in common? Int Arch Allergy Immunol. 2000 Jul;122(3):155-66.

17. Chow LP, Chiu LL, Khoo KH, Peng HJ, Yang SY, Huang SW, et al. Purification and structural analysis of the novel glycoprotein allergen Cyn d 24, a pathogenesis-related protein PR-1, from Bermuda grass pollen. Febs J. 2005 Dec;272(24):6218-27.

18. Arilla MC, Ibarrola I, Puente Y, Daza JC, Martinez A, Asturias JA. Cloning, expression and characterization of mugwort pollen allergen Art v 2, a pathogenesis-related protein from family group 1. Mol Immunol. 2007 Jul;44(15):3653-60.

19. Niderman T, Genetet I, Bruyere T, Gees R, Stintzi A, Legrand M, et al. Pathogenesis-related PR-1 proteins are antifungal. Isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against Phytophthora infestans. Plant Physiol. 1995 May;108(1):17-27.

20. Van Loon LC, Van Strien EA. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol. 1999 Apr;55(2):85-97.