

MR. KYOUNG YONG JEONG (Orcid ID : 0000-0001-9887-1426)

DR. KYUNG HEE PARK (Orcid ID : 0000-0003-3605-5364)

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Characterization of the major allergen, Que ac 1, from sawtooth oak pollen

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To the Editor

Oak is the most commonly found tree in Korea, occupying about 40% of the forest area, while the birch population is sparse. Recently, strong IgE reactivity to Bet v 1 was reported due to sensitization to oak in birch-free region.¹ Comparison of allergenicity of birch and oak pollen extracts revealed that sawtooth oak is the main cause of tree pollinosis in Korea.² Nevertheless, diagnosis and immunotherapy for pollinosis is performed with products prepared from species (*Q. alba*) that are not native to Korea.³ We are now reporting the full characterization of Que ac 1, a 17 kDa allergen, which was detected by IgE immunoblotting in a previous study.²

Native (n) Que ac 1 was purified in three chromatography steps; anion exchange chromatography, hydrophobic interaction chromatography, and gel filtration (Fig. 1A). Edman degradation of this protein resulted in multiple amino acid sequences, indicating the polymorphism of Que ac 1:

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Gly(Asp)Val(Glu/Pro/Ile)Phe(Tyr/Ile)Thr(Glu)His(Val)Glu(Lys)Ser(Asn)Thr(Ala)SerValIleProPr
oAlaArg(Ala)LeuPheLys.Purified protein was separated into 12 isoforms by 2D gel
electrophoresis, and most of these were confirmed to be PR-10 proteins by LC ESI MS/MS and
Edman degradation (Fig. 1D) (Table S1). Furthermore, Que ac 1 is highly polymorphic as
homologous allergens,⁴ and 22 isoforms were identified by RT-PCR (Fig. S1A). At least 3
isoallergens (1.0101, 1.0201, 1.0301) are present, and isoallergen Que ac 1.0101 was deposited in
GenBank under accession No. MN201198, Que ac 1.0201 under MN201199, and Que ac 1.0301
under MN201200. Que ac 1.01 accounts for 88.0% of Que ac 1, while 1.02 for 9.3% and 1.03 for
2.7%, respectively (Fig. S1A). Furthermore, many of the substitutions found in 1.02 and 1.03 are
present in individual 1.01 variants. A predominant isoallergen Que ac 1.0101, officially listed in
the WHO/international union of immunological societies allergen nomenclature subcommittee
according to the guideline,⁵ showed 58.1 to 83.0% sequence identity to homologous allergens
(Fig. S1B). Notably, it shares a homology of 58.1% to Bet v 1 and only 73.1% to Que a 1, which
are the most commonly used molecules for diagnosis of allergy to Fagales pollen.

Recombinant (r) Que ac 1.0101 showed a protein band at about 20 kDa, whereas nQue ac 1
manifests as a 17 kDa protein (Fig. 1B). The calculated molecular mass of nQue ac 1 was 17.309
kDa (pI 5.4) and that of its recombinant counterpart was 20.164 kDa (pI 5.53) including 26 amino
acids (MGHNHNHNHNHNHNAAGDDDDKASVD-) at the N-terminus. In CD analyses, similar
spectra of both native and recombinant Que ac 1 were recorded (Fig. 1C), although native protein
contained multiple isoforms (Fig. 1D). Both native and recombinant Que ac 1 were recognized by
IgE antibodies from 91.3% of the oak pollinosis patients' sera. IgE reactivity to rQue ac 1 and
nQue ac 1 showed a strong correlation with reactivity to sawtooth pollen extract (Pearson's
correlation coefficient = 0.96 and 0.93), while IgE reactivity to nQue ac 1 correlated strongly with
rQue ac 1 (Pearson's correlation coefficient = 0.91) (Fig. 2A-C). rQue ac 1 was able to inhibit
67.2% of IgE reaction to the whole pollen extract, while 57.6% was inhibited by rBet v 1, 88.7%
by nQue ac 1, and 94.7% by the pollen extract at 10 µg of inhibitor concentrations, respectively
(Fig. 2D). Basophil mediator release tests with Korean oak pollinosis patients were performed in
rat basophil leukemia (RBL)-2H3 cells.⁶ No statistical difference in mediator release assays was
observed between Que ac 1 and Bet v 1 (Fig. 2E).

In conclusion, we purified, cloned, and produced rQue ac 1. Further study is needed to determine

whether Korean pollinosis patients would benefit from immunotherapy with the primary sensitizer rather than the cross-reactive allergen, as there was no significant difference in the reactivities with respect to basophil mediator release induced by Que ac 1 and Bet v 1.

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CONFLICTS OF INTEREST

KY Jeong, KH Park and JW Park have stocks in Prolagen. JW Park reports serving as an unpaid chief technology officer for Prolagen. KY Jeong receives a consulting fee from Prolagen. Other authors have no potential conflicts of interest to disclose.

AUTHORS

Kyoung Yong Jeong,¹Jongsun Lee,¹Ji Eun Yuk,¹ Jong Han Park,² Fatima Ferreira,³Min Kyu Sang,⁴Yong Seok Lee,⁴ Lothar Vogel,⁵ Kyung Hee Park,¹ Jung-Won Park¹

¹Dept. of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, Seoul, Korea

²Department of Medicine, Yonsei University College of Medicine, Seoul, Korea

³Department of Bioscience, University of Salzburg, Salzburg, Austria

⁴Department of Life Science and Biotechnology, College of Natural Sciences, Soonchunhyang University, Asan, Korea

⁵Paul Ehrlich Institut, Federal Agency of Sera and Vaccines, Langen, Germany

AUTHOR CONTRIBUTIONS

KY Jeong performed purification, polymorphism analysis, production of recombinant proteins, and CD analysis. J Lee did ELISA. JE Yuk, JH Park, performed RBL assay. MK Sang and YS Lee performed cloning. L Vogel provided RBL cells and taught how to perform RBL assay. KH Park and JW Park collected serum samples. KY Jeong, KH Park, and JW Park analyzed the data. KY Jeong and JW Park designed the study and wrote the manuscript. F Ferreira and JW Park critically revised the manuscript.

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ETHICAL APPROVAL

Collection of serum samples was approved by the Institutional Review Board of our institution (4-2017-1197), and informed consent was obtained.

ORCID

Kyoung Yong Jeong <http://orcid.org/0000-0001-9887-1426>

Jongsun Lee <https://orcid.org/0000-003-1526-2241>

Ji Eun Yuk <http://orcid.org/0000-0003-2517-2653>

Jong Han Park

Fatima Ferreira <http://orcid.org/0000-0003-0989-2335>

Min Kyu Sang

Yong Seok Lee

Lothar Vogel

Kyung Hee Park <http://orcid.org/0000-0003-3605-5364>

Jung-Won Park <http://orcid.org/0000-0003-0249-8749>

Correspondence

Kyoung Yong Jeong, PhD.

Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.

Tel: 82-2-2228-0808

jeongky@yuhs.ac

Jung-Won Park, MD. PhD.

Department of Internal Medicine, Institute of Allergy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.

Tel: 82-2-2228-1961, Fax: 82-2-362-8647

parkjw@yuhs.ac

Figure legends

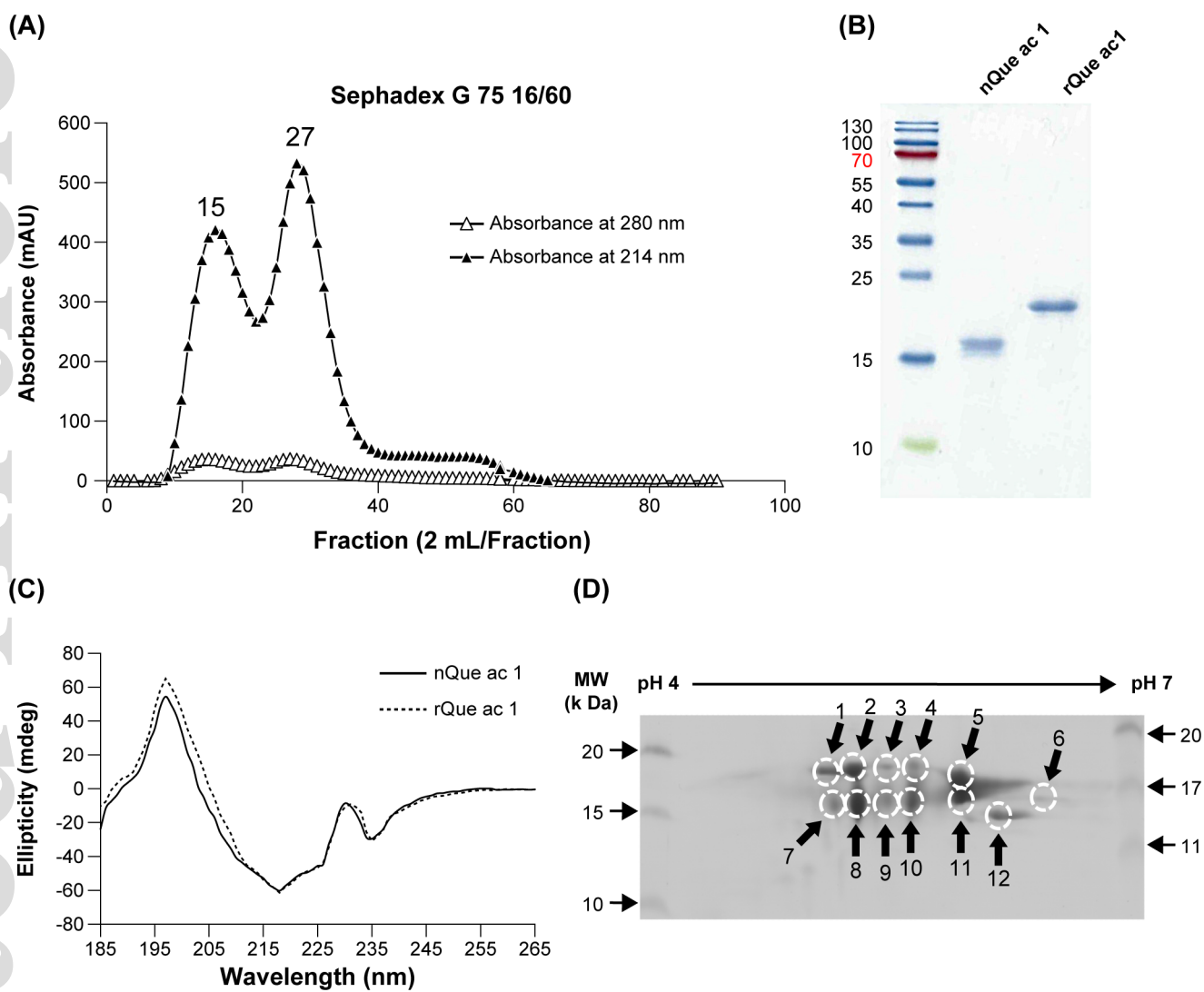
Figure 1. Purification and secondary structure of native and recombinant Que ac 1. Purification of native Que ac 1 by gel filtration chromatography. Two major peaks were visible after gel filtration (A). Analysis of the purity of the purified native Que ac 1 and recombinant Que ac 1 by SDS-PAGE (B). Circular dichroism spectra of native and recombinant Que ac 1 (C). Multiple isoforms of Que ac 1 (D). Purified native Que ac 1 was separated by 2D gel electrophoresis, and each spot was analyzed by tandem mass spectrometry and Edman degradation.

Figure 2. IgE reactivity of Que ac 1. IgE reactivity between native Que ac 1 and pollen extract (A), between recombinant Que ac 1 and pollen extract (B), and between native Que ac 1 and recombinant Que ac 1 were compared. IgE inhibition was also performed (D). Mediator release capacity of Que ac 1 and Bet v 1 in RBL cells (E).

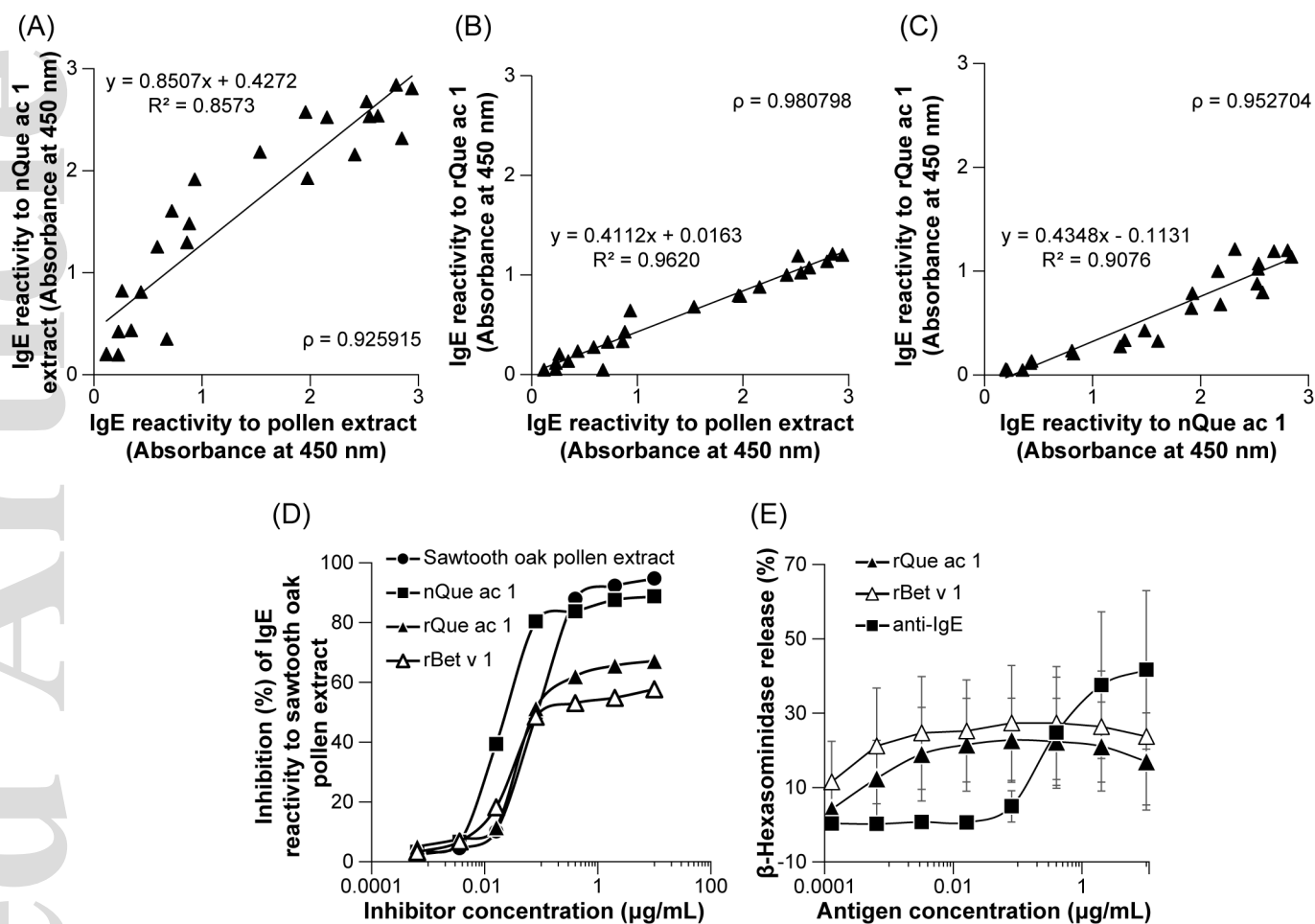
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all_14875_f2.tif

Table S1. Identification of Que ac 1 isoforms by tandem mass analysis and Edman degradation

Spot	Diagnostic peptides	Protein name	Species*	Sequence coverage	Mascot score	Edman degradation
1	K.IVASPDGGSIVK.T	Que a 1	<i>Quercus alba</i>	7%	58	GlyValIleThrTyrGluSerGluAspAlaSerValIleXxxProAlaXxxLeuPheXxx
		Cas s 1	<i>Castanea sativa</i>			
2	K.AFVLDSNLIPIK	Que a 1	<i>Quercus alba</i>	25%	93	GlyValIleThrTyrGluSerGluAspAlaSerValIleProProAlaArgLeuPhePhe
	K.SIEIVEGNNGPGTIK.K	Cas s 1	<i>Castanea sativa</i>			
	K.IVASPDGGSIVK.T					
3	K.IVASPDGGSIVK.S	Que a 1	<i>Quercus alba</i>	7%	37	
		Cas s 1	<i>Castanea sativa</i>			
4	K.STEIIEGNNGPGTIK.K	Que a 1	<i>Quercus alba</i>	9%	15	
5	K.AFVLDSNLIPIK.V	Que a 1	<i>Quercus alba</i>	17%	108	GlyValTyrThrHisGluSerGlnGluThrSerValIleProProAlaXxxLeuPheXxx
	K.STEIIEGNNGPGTIK.K	Pru ar 1-like	<i>Quercus lobata</i>			
	K.STEIIEGNNGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
6	K.STEIIEGNNGPGTIK.K	Que a 1	<i>Quercus alba</i>	10%	135	
	K.STEIIEGNNGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
7	K.STEIIEGNNGPGTIKK.I	Que a 1	<i>Quercus alba</i>	17%	64	
	K.IVASPDGGSIVK.S	Cas s 1	<i>Castanea sativa</i>			

8	K.AFVLDSNLIPIK.V	Que a 1	<i>Quercus alba</i>	25%	184	GlyValIleThrThrGluSerGluAspAlaSer
	K.STEIIEGNGGPGTIK.K	Pru ar 1-like	<i>Quercus lobata</i>			ValIleProProAlaXxxLeuPheXxx
	K.STEIIEGNGGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
	K.IVASPDGGSIVK.S					
9	K.AFVLDSNLIPIK.V	Que a 1	<i>Quercus alba</i>	25%	179	
	K.STEIIEGNGGPGTIK.K	Pru ar 1-like	<i>Quercus lobata</i>			
	K.STEIIEGNGGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
	K.IVASPDGGSIVK.S					
10	K.AFVLDAADNLIPIK.V	Que a 1	<i>Quercus alba</i>	25%	102	GlyValTyrThrXxxGluSerGlnGluThrXx
	K.STEIIEGNGGPGTIK.K	Pru ar 1-like	<i>Quercus lobata</i>			xXxxIleProXxxAla
	K.STEIIEGNGGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
	K.IVASPDGGSIVK.S					
11	K.AFVLDSNLIPIK.V	Que a 1	<i>Quercus alba</i>	25%	135	GlyValTyrThrHisGluSerGlnGluThrSer
	K.STEIIEGNGGPGTIK.K	Pru ar 1-like	<i>Quercus lobata</i>			ValIleProProAlaArgLeuPhePhe
	K.STEIIEGNGGPGTIKK.I	Cas s 1	<i>Castanea sativa</i>			
	K.IVASPDGGSIVK.S					
12	K.STEIIEGNGGPGTIKK.I	Que a 1	<i>Quercus alba</i>	17%	21	
	K.IVASPDGGSIVK.S	Cas s 1	<i>Castanea sativa</i>			

**Quercus alba*, White oak; *Quercus lobata*, Valley oak; *Castanea sativa*, Sweet chestnut.

[Figure S1]

(A)

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Que ac 1-134 MGVYNYESQETSVIPPARLFKAFVLDSNLIISKVLPHAVKSTEIIIEGNGG 50
Que ac 1-051 ...N..... 50
Que ac 1-129 ...F...EDA.....P...E.Q.I..... 50
Que ac 1-128 ...I.H.....P.....I..... 50
Que ac 1-124 ...FTH..... 50
Que ac 1-146 ...IT..... 50
Que ac 1-043 ...F..... 50
Que ac 1-143 .....H.....P.....I..... 50
Que ac 1-132 .....H..... 50
Que ac 1-006 ...F..... 50
Que ac 1-016 ...I...P..... 50
Que ac 1-025 ...I.....P.....I..... 50
Que ac 1-029 ...I.....A..... 50
Que ac 1-030 ...F.....I..... 50
Que ac 1-038 ...N..... 50
Que ac 1-069 ...F.....P.....I..... 50
Que ac 1-071 ...TH..... 50
Que ac 1-088 ...NTH.....P.....I..... 50
Que ac 1-180 ...F.H.....P.....I..... 50
Que ac 1-183 ...F.H.....P.....I..... 50
Que ac 1-205 ...N.H.....P.....I..... 50
Que ac 1-234 ...ITH.....P.....I..... 50
*** .:.*:::*****.*** *: * ** *****

Que ac 1-134 PGTIKKFTFGEASKVKYAKHRIDTLPENCSSYSFSVIEGEALTD-IASIS 99
Que ac 1-051 .....-..... 99
Que ac 1-129 ....R.I....G.HF.Q....T.V....FT.....D..F.KLEK.. 100
Que ac 1-128 .....I.....F.....-..... 99
Que ac 1-124 .....-..... 99
Que ac 1-146 .....-..... 99
Que ac 1-043 .....-..... 99
Que ac 1-143 .....I.....F.....-..... 99
Que ac 1-132 .....-..... 99
Que ac 1-006 .....-..... 99
Que ac 1-016 .....L.....-..... 99
Que ac 1-025 ....R.I....G.HF.Q.....-...V. 99
Que ac 1-029 .....-..... 99
Que ac 1-030 .....I.....F.....-...V. 99
Que ac 1-038 .....-..... 99
Que ac 1-069 .....I.....F.....-...V. 99
Que ac 1-071 .....-..... 99
Que ac 1-088 .....I.....F.....-...V. 99
Que ac 1-180 .....I.....F.....-..... 99
Que ac 1-183 .....I.....F.....-...V. 99
Que ac 1-205 .....I.....F.....-..... 99
Que ac 1-234 .....I.....F.....-...V. 99
****.*:*****.*:.* ***** *.***** :*:*****.* * :.:*

Que ac 1-134 TEVKFVASPDGGSIMKSTTKYQTKGGFQLKEEQIQAAVEKGTGLFKAVEA 149
Que ac 1-051 .....D..... 149
Que ac 1-129 ..T.I.....V.T.S.....DI..PD...REGK.QASR..R.... 150
Que ac 1-128 .....D..... 149
Que ac 1-124 .....D.....TI..... 149
Que ac 1-146 .....R.....D.....T..... 149
Que ac 1-043 .....D..... 149
Que ac 1-143 .....D..... 149
Que ac 1-132 .....D.....TI..... 149
Que ac 1-006 .....D..... 149
Que ac 1-016 .....D..... 149
Que ac 1-025 .....D.....TI..... 149
Que ac 1-029 .....T.....D..... 149
Que ac 1-030 .....D..... 149
Que ac 1-038 .....D..... 149
Que ac 1-069 .....D.....R.TI..... 149

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Que ac 1-071 .....D..... 149
Que ac 1-088 .....D.....TI..... 149
Que ac 1-180 .....D..... 149
Que ac 1-183 .....D.....R.TI..... 149
Que ac 1-205 .....D.....TI..... 149
Que ac 1-234 .....D.....TI..... 149
**.*:*****::*:*****:***:***:*.::*:****

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Que ac 1-134 YLLAHPDLYN 159 (13.3%, 10/75) [100.0%, 159/159] Que ac 1.0101
Que ac 1-051 .....A.. 159 (9.3%, 7/75) [98.1%, 156/159] Que ac 1.0102
Que ac 1-129 ..V....P.. 160 (9.3%, 7/75) [72.5%, 116/160] Que ac 1.0201
Que ac 1-128 .....A.. 159 (8.0%, 6/75) [95.0%, 151/159] Que ac 1.0103
Que ac 1-124 ....P.... 159 (6.7%, 5/75) [95.6%, 152/159] Que ac 1.0104
Que ac 1-146 .....V.. 159 (6.7%, 5/75) [96.2%, 153/159] Que ac 1.0105
Que ac 1-043 ..... 159 (4.0%, 3/75) [98.7%, 157/159] Que ac 1.0106
Que ac 1-143 .....S.P.. 159 (4.0%, 3/75) [95.0%, 151/159] Que ac 1.0107
Que ac 1-132 .....P.. 159 (4.0%, 3/75) [96.9%, 154/159] Que ac 1.0108
Que ac 1-006 .....P.. 159 (2.7%, 2/75) [98.1%, 156/159] Que ac 1.0109
Que ac 1-016 .....P.. 159 (2.7%, 2/75) [96.9%, 154/159] Que ac 1.0110
Que ac 1-025 .....R..A.. 159 (2.7%, 2/75) [90.6%, 144/159] Que ac 1.0301
Que ac 1-029 .....V.. 159 (2.7%, 2/75) [96.9%, 154/159] Que ac 1.0111
Que ac 1-030 ..... 159 (2.7%, 2/75) [96.2%, 153/159] Que ac 1.0112
Que ac 1-038 .....P.. 159 (2.7%, 2/75) [98.1%, 156/159] Que ac 1.0113
Que ac 1-069 .....V.. 159 (2.7%, 2/75) [93.7%, 149/159] Que ac 1.0114
Que ac 1-071 .....P.. 159 (2.7%, 2/75) [97.5%, 155/159] Que ac 1.0115
Que ac 1-088 .....P.. 159 (2.7%, 2/75) [92.5%, 147/159] Que ac 1.0116
Que ac 1-180 .....P.. 159 (2.7%, 2/75) [95.0%, 151/159] Que ac 1.0117
Que ac 1-183 ..... 159 (2.7%, 2/75) [93.1%, 148/159] Que ac 1.0118
Que ac 1-205 .....A.. 159 (2.7%, 2/75) [93.7%, 149/159] Que ac 1.0119
Que ac 1-234 .....P.. 159 (2.7%, 2/75) [92.5%, 147/159] Que ac 1.0120
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(B)

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Que ac 1 MGVDYNYESQETSVIPPARLFKAFVLDSNLIKVLPHAVKSTEIEGNGGPGTIKKFTFG 60
Que m 1 ...VA...EDA.....P..V.Q.L.....I... 60
Que a 1 ...FT...EDA.....P..V.Q.L.....I... 60
Que s 1 ...FT...EN..A.....V.A...P..A.Q.I.....I... 60
Que r 1 ...FTH..E...A.....L.....Q....Q.I.....I... 60
Bet v 1 ...F...TET....A.....I..G...FP..A.Q.IS.V.N.....IS.P 60
Cas s 1 ...FTH.....P.....Q.I.....I... 60
Fag s 1 ...FT...ET.T..T.....A...P..A.Q.I..S.....S.....I... 60
The c 1 ...VT..MEVATS....KM.....P..V.Q.I..S.LL..D....S...I... 60
Cor a 1 ...F...TET.....R.....P..A.K.I..I.....IC.D 60
Mal d 1 ...F...TET....AP.....I..G...P..IA.Q.I.....D..V....V... 60
Pru av 1 ...FT...EF..E...P.....A...VP..IA.Q.I.HS..L..D.....I... 60
Pru ar 1 ...FT...TEF.....EK.....I..A...P..A.T...G...L..D..V....V... 60
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Que ac 1 EASKVKYAKHRIDTLDPENCSYSFSVIEGEALTD-IASISTEVKFVASPDGGSIMKSTTK 119
Que m 1 .G.HL.H.....VI....FTF.....D..F.KLENV...T.I.....V...C. 120
Que a 1 .G.HL.H.....VI....FT.....D..F.KLENV...T.I.....V...S. 120
Que s 1 .G.QF..V...V.EVNH.HFTFAY....D..S.MLEK..Y.T.I.....L...S. 120
Que r 1 ....Y.....AP.....T.....D.....-...V...M.....N.. 119
Bet v 1 .GFPF..V.D.V.EV.HT.K.NY....GPIG.TLEK..N.I.I..T.....L.ISN. 120
Cas s 1 ....Y..S.....A.....T.....DV...-..ENV...T.....T..... 119
Fag s 1 .G.QFN.M.....EI.NA.FT.AYTL...D.ISETLEK.AY.I.L.....L...S. 120
The c 1 .G.QF..V..KVEGI.K..F...Y.V...D..MNTLEK..Y.T...G....VC...S. 120
Cor a 1 .G.PFN.I.QKVEEI.QA.F..RY.V...D..S.KLEK.NY.I.I...H....L..IS. 120
Mal d 1 .G.QYG.V.Q.VNGI.KD.FT..Y.M...DT.S.KLEK.TY.T.LI.....I.T.SH 120
Pru av 1 .G.QYG.V..K..SI.K..Y...YTL...D..G.TLEK..Y.T.LV...S....I...SH 120
Pru ar 1 .G.QYA.V...V.GI.KD.L...YTL...D..S.V.EN.AYDI.LV.....V.T.SH 120
* . : * : : : : : : : : * * * * : : : : : * : * * * : * :

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Que ac 1 YQTKGGFQLKEEQIQAAVEKGTGLFKAVEAYLLAHPDLYN 159 [159/159, 100.0%]
Que m 1 .....D...TD.L.RGGK..AS.V.....V....E.Y 160 [116/160, 72.5%]
Que a 1 .....D...TD.L.RGGK..ASEV.....V.....K 160 [117/160, 73.1%]
Que s 1 .H...DHEI....VK.GK..AS.....G..... 160 [110/160, 68.8%]
Que r 1 .....D.....V..TID.AA.H.....P.. 159 [132/159, 83.0%]
Bet v 1 .H...DHEV.A..VK.SK.M.ET.LR...S.....S.A.. 160 [93/160, 58.1%]
Cas s 1 .....D.....V...I..A.....N....K 159 [135/159, 84.9%]

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Figure S1. Sequence alignment of Que ac 1 isoforms obtained by RT-PCR (A) and Que ac 1 and homologous allergens (B). Que ac 1 (*Quercus acutissima*, sawtooth oak), Que m 1 (*Quercus mongolica*, Mongolian oak), Que a 1 (*Quercus alba*, white oak, EU283863), Que s 1 (*Quercus suber*, cork oak, JQ290115), Que r 1 (*Quercus rubra*, northern red oak, JQ290112), Bet v 1.0101 (*Betula verrucosa*, European white birch, Z80099), Cas s 1 (*Castanea sativa*, European chestnut, FJ390843), Fag s 1 (*Fagus sylvatica*, European beech, FJ390847), The c 1 (*Theobroma cacao*, cacao, XM_007035635), Cor a 1 (*Corylus avellana*, European hazelnut, CAA96549), Mal d 1 (*Malus domestica*, apple, CAA96535), Pru av 1 (*Prunus avium*, sweet cherry, O24348), Pru ar 1 (*Prunus armeniaca*, apricot, O50001). The sequences matched with Edman degradation was blue-colored and the sequences identified by tandem MS analysis were red-colored.