

Supplementary Materials

Chitinases as food allergens

Claudia Leoni¹, Mariateresa Volpicella^{2,*}, Maria C.G. Dileo³, Bruno A.R. Gattulli¹ and Luigi R. Ceci^{1,*}

¹ Institute of Bioenergetics, Biomembranes and Molecular Biotechnologies, Italian National Research Council, Via Amendola 165/a, 70126 Bari (Italy);

² Department of Biosciences, Biotechnologies and Biopharmaceutic Sciences and

³ Department of Biosciences University of Bari, Via Amendola 165/a, 70126 Bari (Italy).

* Authors for correspondence:

M. Volpicella, mariateresa.volpicella@uniba.it

L.R. Ceci, luigi.ceci@ibiom.cnr.it

Keywords: Chitinase; allergen; food allergy; epitope mapping

List of Supplementary Materials:

- Table S1. Percent identity matrix obtained by Clustal2.1 for chitinases of class I and class II.
- Figure S1. Multiple alignment of Class I chitinases.
- Figure S2. Multiple alignment of Class II chitinases.
- Figure S3. Multiple alignment of Class III chitinases.
- Figure S4. Multiple alignment of Class IV chitinases.

Table S1

1: Q7Y0S1	100.00	65.88	55.95	63.11	60.35	61.61	62.11	60.79
2: O24007	65.88	100.00	61.49	65.90	59.43	66.67	64.00	65.14
3: P36361	55.95	61.49	100.00	67.61	68.75	71.49	71.48	69.38
4: Q42428	63.11	65.90	67.61	100.00	70.04	71.78	75.00	72.18
5: Q05538	60.35	59.43	68.75	70.04	100.00	74.49	72.37	72.76
6: Q4Z818	61.61	66.67	71.49	71.78	74.49	100.00	75.72	73.66
7: Q8VXF1	62.11	64.00	71.48	75.00	72.37	75.72	100.00	78.68
8: P93680	60.79	65.14	69.38	72.18	72.76	73.66	78.68	100.00

Table S1. Percent identity matrix obtained by Clustal2.1 [1] for chitinases of class I and class II. Only sequences corresponding to the catalytic module were aligned. Names of sequences are as reported in Table 1. Sequences highlighted in green belong to class II.

Figure S1. Multiple alignment of Class I chitinases. Red bar corresponds to a conformational epitope described for the homologous protein hevein [2]. Alignment was produced by Clustal2.1 [1] and shown by using the MView option available at EMBL-EBI [3]

Reference sequence (1): sp|Q05538|CHIC_SOLLC

Identities normalised by aligned length.

Colored by: identity

		cov	pid	1		80
1	sp Q05538 CHIC_SOLLC	100.0%	100.0%	-----	MR	SEFTTLFLFSVLLISA
2	sp P36361 CHI5_PHAVU	97.2%	61.6%	-----MKK-----NRMMI-----	MICS	VGVMMLVGGGS
3	tr Q42428 Q42428_CASSA	95.0%	66.2%	-----	MKLF	SLLFLAFLIGT
4	tr Q6T484 Q6T484_WHEAT	96.0%	69.1%	-----	MR	GVVVVAMAAAFVSA
5	tr P93680 P93680_PERA	99.1%	68.4%	-----	MVYCTASLPLLL	LLVGLAGEA
6	tr M0SI55 M0SI55_MUSAM	84.2%	49.9%	MRSRIQHAICRLGHRTSYPKTRLCD	FPHSLVHRKLHSGQESISLYK	HRIPPTPPPPPLRGMKALLIVIFTLASSLGA
7	tr O22318 O22318_MUSAC	35.7%	55.2%	-----	-----	VIFTLASSLGA
8	tr Q8VXF1 Q8VXF1_MUSAC	98.1%	68.8%	-----	MKALL	IVIFTLASSLGA
9	tr C3VD22 C3VD22_MUSPR	95.3%	70.4%	-----	MKALL	IVIFTLASSLGA
10	tr B6UYK6 B6UYK6_MUSPR	95.7%	68.9%	-----	MKALL	IVIFTLASSLGA
11	tr M0SI56 M0SI56_MUSAM	95.7%	69.5%	-----	MKALL	IVIFTLASSLGA
	consensus/100%				hhhh	.h.hts
	consensus/90%				.h.	hlhhhh.huu
	consensus/80%				hhhhhlhlhhhs	.sluA
	consensus/70%				hhlhl11lhshA	.uLGA
				81		160
1	sp Q05538 CHIC_SOLLC	100.0%	100.0%	SAEQCGSOAGGALCASGLCCSKFGWCNTNEYCGPGNCOSQCPGGF-----	GESGDLGGVI	SNSMFDQMLNHRND
2	sp P36361 CHI5_PHAVU	97.2%	61.6%	YGEQCGROAGGALCPGNCSSQFGWCCSTTDYCGKD-COSQCGGPS---PA-----	PTD	SALTSRSTFDQVLKHRND
3	tr Q42428 Q42428_CASSA	95.0%	66.2%	SAEQCGROAGGAACANNLCCSQFGWCNTAEYCGAG-COSQCSSPTTTTSSPTASS	GGGDVGS	LSIASLFDQMLKVRND
4	tr Q6T484 Q6T484_WHEAT	96.0%	69.1%	HAEQCGSOAGGATCPNCLCCSKFGFCSTSDYCGTG-COSQNGCSGGTPVPVPTP-SGGGVSSI	SQS	SLFDQMLLHRND
5	tr P93680 P93680_PERA	99.1%	68.4%	FTEQCGROAGGALCPGGLCCSQFGWCCSTSDYCGFT-COSQCGGVT---P-----	S-FGGGVASLI	SQSVENQMLKHRND
6	tr M0SI55 M0SI55_MUSAM	84.2%	49.9%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGKD-COSQCGSG---G-----	-----	-----
7	tr O22318 O22318_MUSAC	35.7%	55.2%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGQG-CXSQCTGST---PSPSTPS	GGGSVGSII	SSSLFEQMLKHRND
8	tr Q8VXF1 Q8VXF1_MUSAC	98.1%	68.8%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGQG-COSQCGSG---G-----	S-GGGSVASII	SSSLFEQMLKHRND
9	tr C3VD22 C3VD22_MUSPR	95.3%	70.4%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGEG-COSQCGGS-----	SGGSVASII	SSSLFEQMLKHRND
10	tr B6UYK6 B6UYK6_MUSPR	95.7%	68.9%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGQG-COSQCTGST---PSPSTPS	GGGSVGSII	SSSLFEQMLKHRND
11	tr M0SI56 M0SI56_MUSAM	95.7%	69.5%	FTEQCGROAGGALCPGGLCCSQYGWCCNTDPYCGQG-COSQCTGST---PSPSTPS	GGGSVGSII	SSSLFEQMLKHRND
	consensus/100%			.UEQCGSOAGGAhCsss.CCSpaGacGstS.YCG.s.C.SQCsus.....	sssluulI	StShFp.hL.aRND
	consensus/90%			.AEQCGSOAGGAhCsssLCCSpaGwCGstS.YCG.s.CSQCsGss.....	suGsluulI	SpShF-QMLpHRND
	consensus/80%			aAEQCGROAGGALCPuGLCCSpaGwCGstSssYCGts.CSQCsGss...s.....	suGsluulI	SpShF-QMLpHRND
	consensus/70%			aAEQCGROAGGALCPGGLCCSpaGwCCNTssYCGpC.CSQCsGss...s.....	SGGSVuSlI	SpSlF-QMLKHRND
				161		240
1	sp Q05538 CHIC_SOLLC	100.0%	100.0%	NACQCKNNFYSYNAFVTAAGSEPGFGTTGDI	TARKREIAAFLAQTSHETTGCWPTAF	DGPYAWGYCLREQSPGDYCTP
2	sp P36361 CHI5_PHAVU	97.2%	61.6%	GACPAK-GFYTYNAFIAAAKAYESFGNTGDTATRKREIAAFLAQTSHETTGCWATAFD	DGPYAWGYCFVRERNP-SAMCSA	
3	tr Q42428 Q42428_CASSA	95.0%	66.2%	PRCKSN-GFYTYNAFIAAARSNGFGTTGDDVTTRKREIAAFLAQTSHETTGCWATAFD	DGPYAWGYCFVMENNK-QTYCT-	
4	tr Q6T484 Q6T484_WHEAT	96.0%	69.1%	AACLAK-GFYNYGAFVAAANSFSGFATTGSTDVKKREIAAFLAQTSHETTGCWPTAF	DGPYAWGYCFNQRGATSDYCTP	
5	tr P93680 P93680_PERA	99.1%	68.4%	AAEQAK-GFYTYNAFIAAANSNGFASVGDITARKREIAAFLAQTSHETTGCWATAFD	DGPYAWGYCLKEQCNFPDYCFV	
6	tr M0SI55 M0SI55_MUSAM	84.2%	49.9%	-----GS-GFYTYNAFIAAANSFSGFGTTGDDTARKREIAAFLAQTSHETTGCWATAFD	DGPYAWGYCFVQEQNPSPDYCVA	
7	tr O22318 O22318_MUSAC	35.7%	55.2%	AAQCPK-GFYTYNAFIAAANSFSGFGTTGDKATXXRE-----	-----	
8	tr Q8VXF1 Q8VXF1_MUSAC	98.1%	68.8%	AAQCPK-GFYTYNAFIAAANSFSGFGTTGDDAKKKREIAAFLAQTSHETTGCWATAFD	DGPYAWGYCFVQEQNPSPDYCVA	

9	tr C3VD22 C3VD22_MUSPR	95.3%	70.4%	AAC PCK -GFYTYNAFIAAAS S S S SGFGTTGD D ATKK K REIAAF L AQTSHETTGCWATA F DGPYAWGYCFVQEQNPSSDY C VA
10	tr B6UYK6 B6UYK6_MUSPR	95.7%	68.9%	AAC P GN-GFYTYNAFIAAAN S S S SGFGTTGD D ATKK K REIAAF L AQTSHETTGCWATA F DGPYAWGYCFVQEQNPSSDY C VA
11	tr M0SI56 M0SI56_MUSAM	95.7%	69.5%	AAC PCK -GFYMYNAFIAAAN S S S SGFGTTGD D ATKK K REIAAF L AQTSHETTGCWATA F DGPYAWGYCFVQEQNP S SY C VA
consensus/100%				up.sFY.YsAFIsAA ^t .asuFussGs.sh..RE.....
consensus/90%				st C .up.GFYsYsAFI AAA pu S S S Gfu C TGD.ss+KREIAAF L AQTSHETTGCW S TAFDGPYAWGYCF..Eps...sY C s.
consensus/80%				s AC .up.GFY O YsAFIAAAS S S S SGFGTTGD S SS+KREIAAF L AQTSHETTGCW S TAFDGPYAWGYCF l pe ps s.ssY C ss
consensus/70%				u AC .u K .GFYTYNAFIAAAS S S S SGFGTTGD S SS+KREIAAF L AQTSHETTGCWATA F DGPYAWGYCF l pe ps ssuDY C ss

		cov	pid	241	:	3	:	320
1	sp Q05538 CHIC_SOLLC	100.0%	100.0%	SSQWPCAPGRK Y GRG P IQIS H NYNYG C GR A IGVD L INN P DLVAT D PV I SFK S AIWFWMT T QSP K SCHD V ITGRW P PS				
2	sp P36361 CHI5_PHAVU	97.2%	61.6%	TPQ E PCAPGQQ Y YGRG P IQIS H NYNYG C GR A IGVD L INN P DLVAT D SV I SFK S AIWFWMT T QSP K SSH D VITSRW T PS				
3	tr Q42428 Q42428_CASSA	95.0%	66.2%	SK S WPCVFGK Q Y Y GRG P IQL T HNYNYG Q AG K AIGAD L INN P DLVAT N ET I SFK T AIWFWMT T Q A N K SSH D VITGNWR P PS				
4	tr Q6T484 Q6T484_WHEAT	96.0%	69.1%	SSQWPCAPGKK Y GRG P IQIS H NYNYG Q AG Q AIG T D L INN P DLVAT S AT V SFK T AIWFWMT T QSP K SSH D VITGRW S PS				
5	tr P93680 P93680_PERA	99.1%	68.4%	TAQWPCAPGKK Y GRG P IQIS H NYNYG Q AG R AIG D LINN P DAVAT D PV I SFK T AIWFWMT T QSP K SCH N VITGRW T PS				
6	tr M0SI55 M0SI55_MUSAM	84.2%	49.9%	SSQWPC A AGKK Y GRG P IQIS H NYNYG Q AG R AIG S D L INN P DLVAT D AT I SFK T AIWFWMT T QSP K -----				
7	tr O22318 O22318_MUSAC	35.7%	55.2%	-----				
8	tr Q8VXF1 Q8VXF1_MUSAC	98.1%	68.8%	SSQWPC A AGKK Y GRG P IQIS H NYNYG Q AG R AIG S D L INN P DLVAT D AT I SFK T AIWFWMT T QSP K SCH D VITGSW T PS				
9	tr C3VD22 C3VD22_MUSPR	95.3%	70.4%	SSQWPC A AGKK Y GRG P IQIS H NYNYG Q AG R AIG S D L INN P DLVAT D AT I SFK T AIWFWMT T QSP K SCH N VITGGW T PS				
10	tr B6UYK6 B6UYK6_MUSPR	95.7%	68.9%	SSQWPC A AGKK Y GRG P IQIS H NYNYG Q AG R AIG S D L INN P DLVAT D AT I SFK T AIWFWMT T QSP K SCH N VITGGW T PS				
11	tr M0SI56 M0SI56_MUSAM	95.7%	69.5%	SSQWPC A AGKK Y GRG P IQIS H NYNYG Q AG R AIG S D L INN P DLVAT D AT I SFK T AIWFWMT T QSP K SCH D VITGSW T PS				
consensus/100%								
consensus/90%				o.pa P Cs.Gpp Y aGRG P IQ l o a NYNYG.sG p AIG.D l l n p D hVA o sssl S F K o Al WFWMT S Qus K				
consensus/80%				o S QWPC a sG p Y a GRG P IQ I S a NYNYG.sG + AIG S D L INN P DLVAT D ss I S F K o Al W FWMT T QSP K S s H s V I h u t w p S				
consensus/70%				S u QWPC a sG K K Y GRG P IQ I S a NYNYG P AG R AIG S D L INN P DLVAT D ss I S F K T AIWFWMT T QSP K S s H s V I T G t w p S				

		cov	pid	321	:	1	396
1	sp Q05538 CHIC_SOLLC	100.0%	100.0%	GADQAA N RVPG F GV T TNI I NGGLE C CG S DSR V QDR I GFYRR Y CGILGV S PC E N L D C GN Q RS F CGNGL L VD I M----			
2	sp P36361 CHI5_PHAVU	97.2%	61.6%	SADVAARR L PGY G TV T TNI I NGGLE C CG R QDSR V QDR I GF F K R YCDLLGV S YGN L D C YSQ T PEGN S L F LS D LV T S Q			
3	tr Q42428 Q42428_CASSA	95.0%	66.2%	AAD T SA G RV P S Y GV T TNI I NGGLE C CG S DD R VA N R I GFY K R Y CD T LG V S Y GN L D C YN Q K P EA-----			
4	tr Q6T484 Q6T484_WHEAT	96.0%	69.1%	GADQAA G RV P GV T TNI I NGGLE C CG R QD A R V AD R IGFY K R Y CD L LG V S Y GN L D C YN Q R E FA-----			
5	tr P93680 P93680_PERA	99.1%	68.4%	AAD R AA G RL P GY G V T TNI I NGGLE C CG K GF N DK V AD R IGFY K R Y CD L LG V S Y GN L D C YN Q RS F GV S T N PLA S S-----			
6	tr M0SI55 M0SI55_MUSAM	84.2%	49.9%	-----RPGY G V T TNI I NGGLE C CGY D AR V AD R IGFY K R Y CD L LG V S Y GN L D C YN Q R E ASTAAT A T F ----			
7	tr O22318 O22318_MUSAC	35.7%	55.2%	-----			
8	tr Q8VXF1 Q8VXF1_MUSAC	98.1%	68.8%	NADQAA G RL P GY G V T TNI I NGGLE C CGY D AR V AD R IGFY K R Y CD L LG V S Y GN L D C YN Q R E ASTAAT A T F ----			
9	tr C3VD22 C3VD22_MUSPR	95.3%	70.4%	NAD R AA G RL P GY G V T TNI I NGGLE C CG S DA R VAD R IGFY K R Y CD L LG V S Y GN L D C YN Q SP E T-----			
10	tr B6UYK6 B6UYK6_MUSPR	95.7%	68.9%	NAD R AA G RL P GY G V T TNI I NGGLE C CG S DA R VAD R IGFY K R Y CD L LG V S Y GN L D C YN Q SP E T-----			
11	tr M0SI56 M0SI56_MUSAM	95.7%	69.5%	NAD R AA G RL P GY G V T TNI I NGGLE C CG S DA R VAD R IGFY K R Y CD L LG V S Y GN L D C YS Q SP E T-----			
consensus/100%							
consensus/90%				hPuaG s h T N I NGGLE C CG + G.ss+V t s R IG Fa + RY C s h L GVu..G p N L D C h s Q p s S			
consensus/80%				s AD .u a SR L PGY G V h TNI I NGGLE C CG + G.D S R V t D R I GFY K R Y CD L LG V S Y GN L D C Y s Q p s S			
consensus/70%				s AD p AA s R L P GY G V h TNI I NGGLE C CG + G.D S R V AD R IGFY K R Y CD L LG V S Y GN L D C Y N Q p s S			

Figure S2. Multiple alignment of Class II chitinases. Red bars correspond to conformational epitopes described for the rice chitinase [4]. Alignment was produced by Clustal2.1 [1] and shown by using the MView option available at EMBL-EBI [3].

Reference sequence (1): tr|Q7Y0S1|Q7Y0S1_SOLLC

Identities normalised by aligned length. Colored by: identity

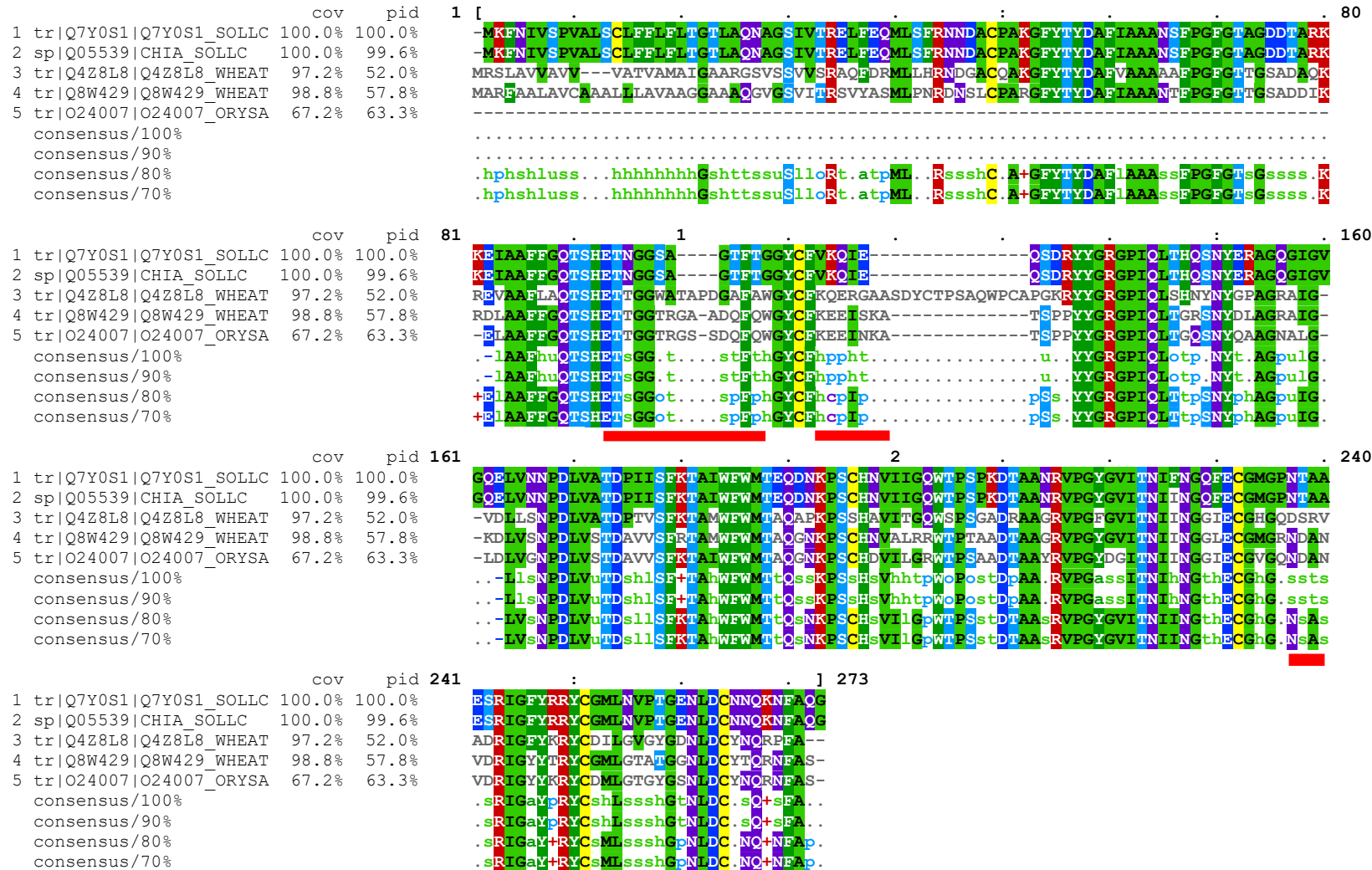
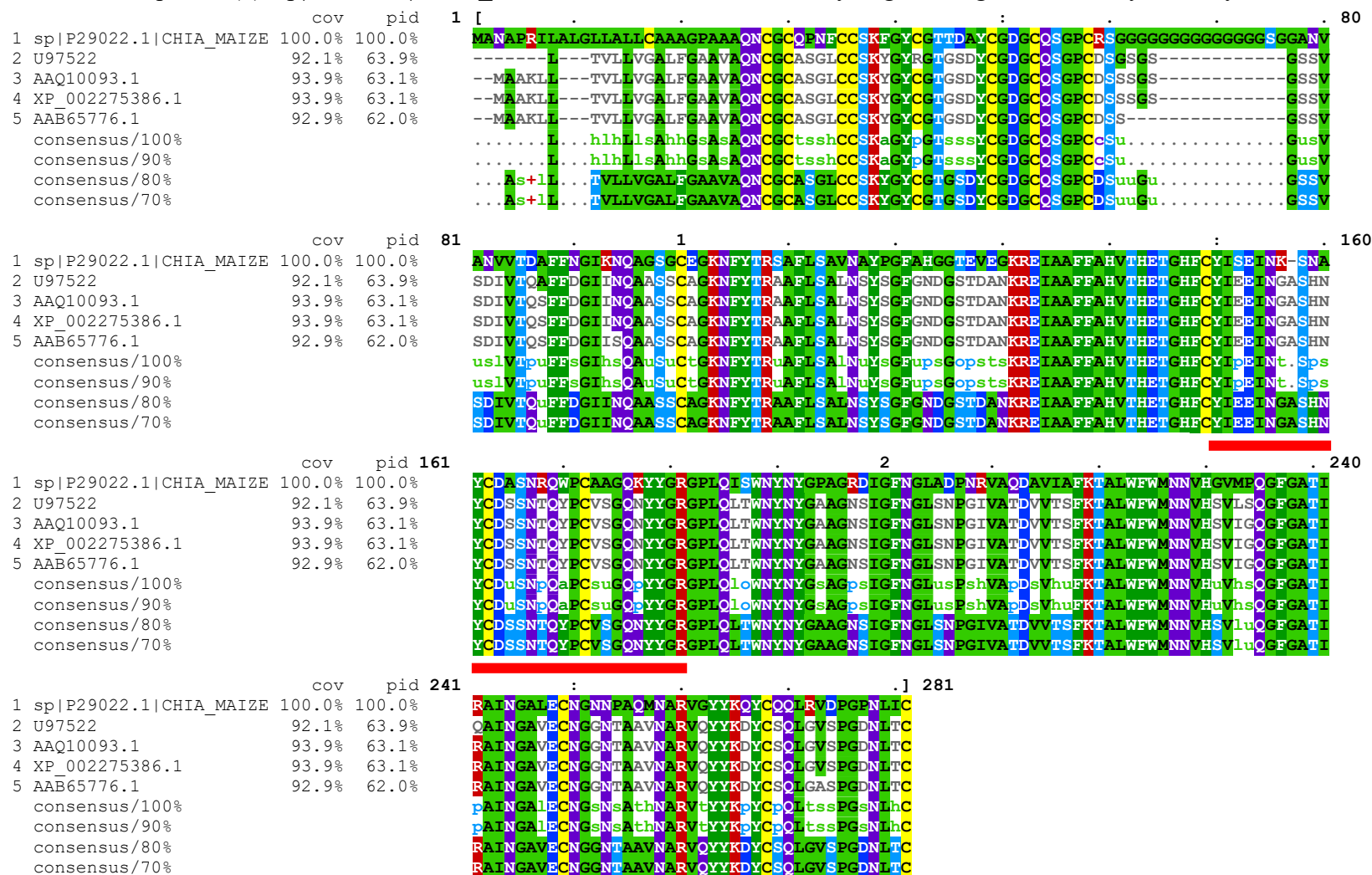


Figure S3. Multiple alignment of Class III chitinases. Red bar corresponds to a conformational epitope described for the pomegranate chitinase [5]. Alignment was produced by Clustal2.1 [1] and shown by using the MView option available at EMBL-EBI [3].

[illegible]

Figure S4. Multiple alignment of Class IV chitinases. Red bars correspond to a conformational epitope described for the rice chitinase [4]. Alignment was produced by Clustal Omega [1] and shown by using the MView option available at EMBL-EBI [3]. Reference sequence (1): sp|P29022.1|CHIA_MAIZE. Identities normalised by aligned length. Colored by: identity.



References

1. Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T.J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J.; et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **2011**, *7*, 539.
2. Alenius, H.; Kalkkinen, N.; Reunala, T.; Turjanmaa, K.; Palosuo, T. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, hevein. *J. Immunol.* **1996**, *156*, 1618–1625.
3. Chojnacki, S.; Cowley, A.; Lee, J.; Foix, A.; Lopez, R. Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Research* **2017**, *45*, W550–W553.
4. Mishra, A.; Gaur, S.N.; Lavasa, S.; Arora, N. In vitro assessment of allergenicity features and localization of probable IgE binding regions. *Food and Chemical Toxicology* **2015**, *84*, 181–187.
5. Tuppo, L.; Giangrieco, I.; Alessandri, C.; Ricciardi, T.; Rafaiani, C.; Ciancamerla, M.; Ferrara, R.; Zennaro, D.; Bernardi, M.L.; Tamburrini, M.; et al. Pomegranate chitinase III: Identification of a new allergen and analysis of sensitization patterns to chitinases. *Mol. Immunol.* **2018**, *103*, 89–95.