

Isolation, identification, and stability of Ficin 1c isoform from fig latex

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Supplementary Table S1. Ficin isoforms characteristics based on Uniprot data.

Transcriptome-derived protein sequence	#N amino acids	pI	Mw (Da)
Ficin 1a (A0A2Z6DRL4)	217	6.89	23642.55
Ficin 1b (A0A2Z6DRT1)	217	8.42	23571.56
Ficin 1c (A0A2Z6DRL5)	216	8.64	23586.62
Ficin 2a (A0A2Z6DRL9)	216	5.39	23276.18
Ficin 2b (A0A2Z6DRP9)	216	5.39	23248.17
Ficin 2c (A0A2Z6DRM5)	216	5.39	23248.17
Ficin 3 (A0A2Z6DRN1)	217	9.50	23703.88
Ficin 4 (A0A2Z6DRL6)	215	8.88	23287.30
Ficin 5 (A0A2Z6DRM9)	217	8.85	23810.96
Ficin 6a (A0A2Z6DRW8)	217	7.70	24053.24
Ficin 6b (A0A2Z6DRN0)	217	7.70	24053.24
Protein sequence with known 3D structure	#N amino acids	pI	Mw (Da)
Ficin isoform A (A0A182DW06)	217	8.94	23532.64
Ficin isoform B (A0A182DW08)	217	7.69	24114.15
Ficin C (A0A182DW09)	217	8.10	24173.32
Ficin D (A0A182DW11)	217	4.87	23657.57

Supplementary Table S2. Peptide-mass fingerprint of the isolated ficin isoform.

1. Name: Ficin1c **MW:**
23587 Da **pI:** 8.6

m/z Submitted	MH ⁺ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
679.1800	679.3232	100.0	-0.143	67	72	0	(R) <u>GGWMTK</u> (A)
750.0800	750.3451	100.0	-0.265	96	102	0	(K) <u>GECNVTK</u> (A)
750.0800	750.3781	100.0	-0.298	113	118	0	(R) <u>YESVPR</u> (N)
785.3100	785.4152	100.0	-0.105	198	205	0	(R) <u>NVAEPAGK</u> (C)
878.8000	878.4400	100.0	0.360	95	102	1	(R) <u>KGECNVTK</u> (A)
885.2000	885.4716	100.0	-0.272	73	79	0	(K) <u>AYDYIHK</u> (N)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-) <u>LPETVDWR</u> (I)
1061.4300	1061.5586	100.0	-0.129	103	112	0	(K) <u>ASQTVATIDR</u> (Y)
1105.6600	1105.4810	100.0	0.179	182	190	0	(R) <u>NSWGNWGER</u> (G)
1178.3000	1178.5333	100.0	-0.233	206	216	0	(K) <u>CGIAMHSTYPV</u> (-)
1568.7200	1568.8391	100.0	-0.119	127	142	0	(K) <u>AVANQPVSVTIEAGGR</u> (A)
1628.7500	1628.7663	100.0	-0.0163	80	94	0	(K) <u>NGGITSQSNYPYTAR</u> (K)
1696.5900	1696.9341	100.0	-0.344	126	142	1	(K) <u>KAVANQPVSVTIEAGGR</u> (A)
1757.1600	1756.8613	100.0	0.299	80	95	1	(K) <u>NGGITSQSNYPYTARK</u> (G)

The matched peptides cover **48.6%** (105/216AA's) of the protein.

2. Name: Ficin1a (**8% of sequence difference between Ficin 1a and Ficin 1c**)
MW: 23643 Da **pI:** 6.9

m/z Submitted	MH ⁺ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
885.2000	885.4716	100.0	-0.272	73	79	0	(K) <u>AYDYIHK</u> (N)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-) <u>LPETVDWR</u> (I)
1628.7500	1628.7663	100.0	-0.0163	80	94	0	(K) <u>NGGITSQSNYPYTAR</u> (K)
1757.1600	1756.8613	100.0	0.299	80	95	1	(K) <u>NGGITSQSNYPYTARK</u> (G)

The matched peptides cover **14.3%** (31/217AA's) of the protein.

3. Name: Ficin3 (**18% of sequence difference between Ficin 1a and Ficin 1c**) **MW:** 23704 Da **pI:** 9.5

m/z Submitted	MH ⁺ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
885.2000	885.4716	100.0	-0.272	73	79	0	(R) <u>AYDYIHK</u> (N)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-) <u>LPETVDWR</u> (S)
1061.4300	1061.5586	100.0	-0.129	103	112	0	(K) <u>ASQTVATIDR</u> (Y)
1586.5400	1586.7784	100.0	-0.238	67	79	1	(K) <u>GGWMNRAYDYIHK</u> (N)

The matched peptides cover **14.3%** (31/217AA's) of the protein.

1. Name: FicinC (12% of sequence difference between Ficin C and Ficin 1c) MW: 24174 Da pI: 8.1

m/z Submitted	MH+ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
885.2000	885.4716	100.0	-0.272	73	79	0	(K) <u>AYDYIIK</u> (N)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-)LPETVDWR(I)
1297.5400	1297.6279	100.0	-0.0879	207	218	0	(K) <u>CGIAMQSTYPVK</u> (K)
1425.9400	1425.7229	100.0	0.217	207	219	1	(K) <u>CGIAMQSTYPVKK</u> (T)
1568.7200	1568.8391	100.0	-0.119	127	142	0	(K) <u>AVANQPVSVTIEAGGR</u> (A)
1696.5900	1696.9341	100.0	-0.344	126	142	1	(K) <u>KAVANQPVSVTIEAGGR</u> (A)

The matched peptides cover **20.3%** (45/222AA's) of the protein.

2. Name: FicinD (30% of sequence difference between Ficin D and Ficin 1c) MW: 23658 Da pI: 4.9

m/z Submitted	MH+ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
885.2000	885.4716	100.0	-0.272	73	79	0	(K) <u>AYDYIIK</u> (D)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-)LPETVDWR(S)
1730.5200	1730.7980	100.0	-0.278	80	94	1	(K) <u>DRGITSESDYPY TAR</u> (K)

The matched peptides cover **13.6%** (30/220AA's) of the protein.

3. Name: FicinB (11% of sequence difference between Ficin B and Ficin 1c) MW: 24157 Da pI: 7.7

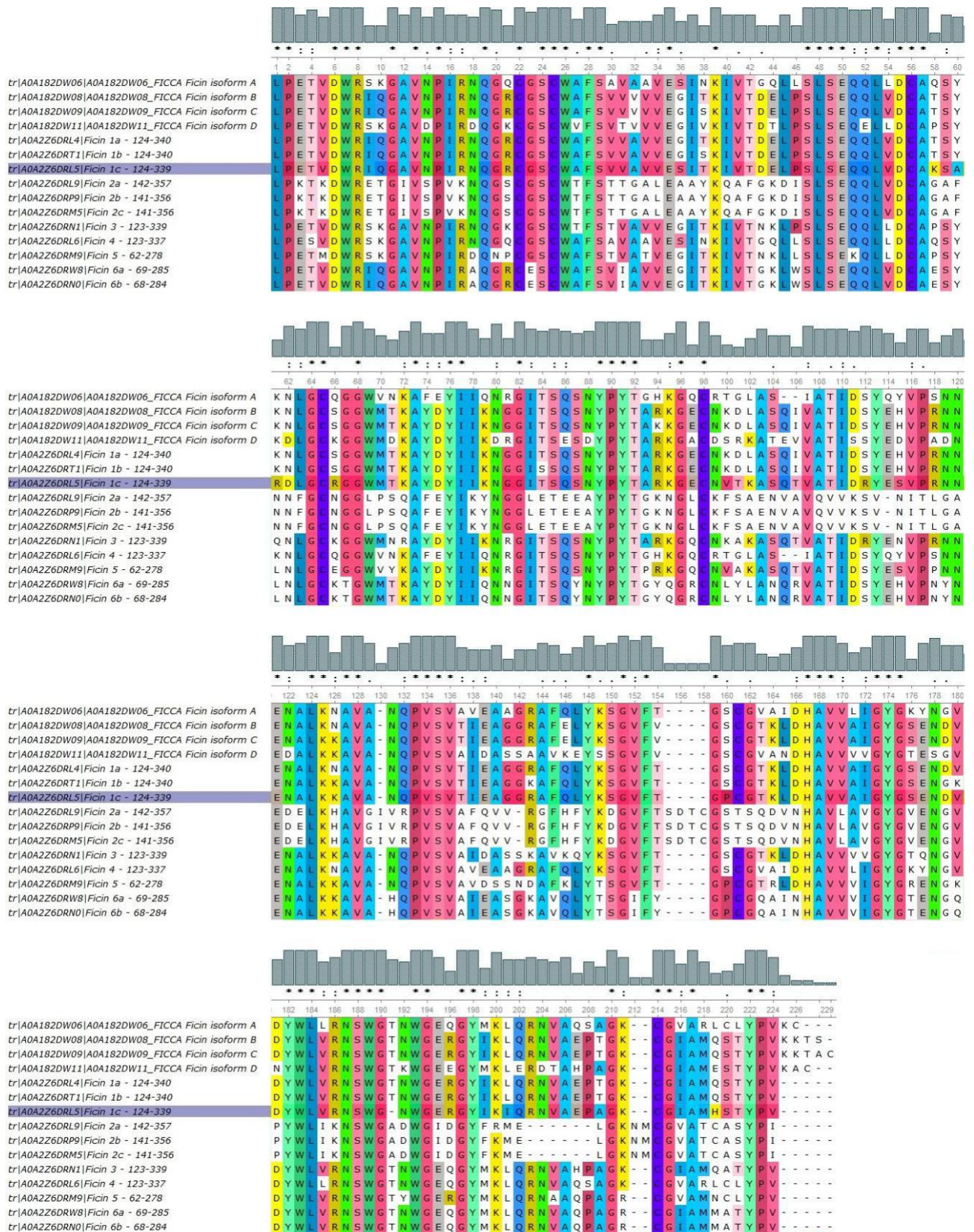
m/z Submitted	MH+ Matched	Intensity	Delta Da	Start	End	Missed Cleavages	Sequence
885.2000	885.4716	100.0	-0.272	73	79	0	(K) <u>AYDYIIK</u> (N)
1015.3100	1015.5207	100.0	-0.211	1	8	0	(-)LPETVDWR(I)
1297.5400	1297.6279	100.0	-0.0879	207	218	0	(K) <u>CGIAMQSTYPVK</u> (K)
1425.9400	1425.7229	100.0	0.217	207	219	1	(K) <u>CGIAMQSTYPVKK</u> (T)

The matched peptides cover **12.6%** (28/222AA's) of the protein.

Supplementary Table S3. Sequence coverage of Ficin 1c by templates and confidence values of the match to the template in the Phyre2 model.

Template PDB code	Protein name and function	Alignment coverage (%)	Confidence (%)	Identity (%)
1PCI	Pro Caricain, Cystein protease from <i>Carica papaya</i>	99	100	53
5EGW	Amb a 11, Cystein protease allergen from <i>Ambrosia artemisiifolia</i> (2.70 Å)	99	100	48
5EF4	Amb a 11, Cystein protease allergen from <i>Ambrosia artemisiifolia</i> (2.05 Å)	99	100	48
6U7D	Recombinant stem bromelain precursor, cystein protease from <i>Ananas comosus</i>	99	100	53
1AEC	Actinidin-E-64 komplex, cystein protease from <i>Actinidia chinensis</i>	99	100	58
4YYW	Ficin D2, hydrolase form <i>Ficus carica</i>	99	100	70
7PCK	Wild type human Procathepsin K, lysosomal cysteine protease from <i>Homo sapiens</i>	99	100	41
3TNX	Thermostable variant of papain (2.60 Å), hydrolase from <i>Carica papaya</i>	99	100	50
1S4V	KDEL-tailed cystein endopeptidase functioning in programmed cell death, from <i>Ricinus communis endosperm</i>	99	100	54
2C0Y	Cys25Ala mutant of human procathepsyn S, hydrolase from <i>Homo sapiens</i>	99	100	42
1PPO	Papaya protease omega, cystein protease from <i>Carica papaya</i>	99	100	53
4YYQ	Ficin A, hydrolase from <i>Ficus carica</i>	99	100	73

Supplementary Figure S1. Sequence alignment of mature forms of ficin isoforms.



Supplementary figure S3. Deconvoluted Amide I region of Ficin 1c FTIR spectrum.

