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Supplementary Material

Identification of Proteases: Carboxypeptidase and Aminopeptidase as Putative Virulence Factors of *Fusarium solani* Species Complex

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Abstract:

Background:

Fusarium keratitis accounts for around 50% of mycotic keratitis cases. Major virulence factors produced by keratopathogenic fungi are proteases.

Objective:

The aim of the current study was to identify proteases contributing to corneal pathogenicity of *Fusarium* species.

Methods:

Culture filtrates from fourteen *Fusarium solani* species complex (FSSC) isolates and three *F. delphinoides* isolates were evaluated for protease activity and gelatine zymography. Mass spectroscopy was carried out using a partially purified enzyme and total extracellular extract. Protease gene expression in an *in-vitro* condition and an *ex-vivo* goat corneal infection model was measured using qRT-PCR. Specific activity was observed in a wide range and at a broad pH range; and isolates Cs1 (maximum) and Cc50 (minimum) were selected for the infection model.

Results:

Gene expression in *in-vitro* condition showed the highest fold change for proteases (C7YY94, C7Z7U2 and C7Z6W1) while in an *ex-vivo* infection highest fold change was seen for proteases (C7Z6W1, C7YQJ2 and C7Z7U2); in decreasing order, respectively. Expression of aminopeptidase (C7Z6W1) was 50-fold higher in the infected cornea in both isolates (Cs1 and Cc50); while expression of carboxypeptidase (C7YVF3) was 15-fold higher only in isolate Cs1. Corneal histology showed less penetration of Cc50 than Cs1 into the stroma. Mass spectrometry showed the presence of carboxypeptidase (C7YVF3) and tripeptidyl amino peptidase.

Conclusion:

It can be concluded that clinical isolates of FSSC produce varying amounts of proteases and differ in specific activity and gene expression in both conditions (*in vitro* and *ex vivo*). Carboxypeptidase and aminopeptidase contribute to the pathogenic potential of *Fusarium solani* species complex.

Keywords: Mycotic keratitis, *Fusarium* spp., Protease, *Ex-vivo* infection model, Carboxypeptidase, Aminopeptidase.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Specific activity of extracted proteases at different pH using Azocasein assay.

	pH 3.0	pH 5.0	pH 7.4	pH 8.0	pH 10
			***Fusarium solani* species complex isolates**		
CSH1	123.29 ± 2.13	132.17 ± 1.77	163.93 ± 4.70	53.96 ± 2.37	126.71 ± 7.69
CSH2	115.83 ± 2.01	113.48 ± 3.81	166.19 ± 4.62	39.28 ± 5.23	109.45 ± 1.16
CSH3	165.50 ± 9.06	125.63 ± 5.32	199.68 ± 12.49	100.17 ± 10.84	149.09 ± 5.72
CSH5	146.81 ± 3.23	151.98 ± 9.30	215.04 ± 8.95	43.42 ± 7.11	147.32 ± 3.10

(Table 1) contd.....

CSH6	107.06 ± 3.45	112.72 ± 4.59	165.82 ± 2.61	18.28 ± 1.31	110.11 ± 3.99
CSH7	134.24 ± 12.94	123.39 ± 10.85	185.77 ± 22.20	22.15 ± 3.41	124.30 ± 2.07
CSH8	140.40 ± 2.41	133.80 ± 4.70	164.38 ± 9.63	131.02 ± 0.00	131.02 ± 4.70
CSH9	51.22 ± 1.89	53.94 ± 0.00	95.35 ± 8.06	49.04 ± 1.63	40.86 ± 3.27
CSH10	91.58 ± 1.53	94.23 ± 6.24	130.33 ± 5.34	84.54 ± 3.49	76.17 ± 2.02
CSH11	89.44 ± 4.24	90.09 ± 3.13	111.15 ± 3.93	82.31 ± 2.81	80.69 ± 5.91
CS1	199.86 ± 3.91	207.62 ± 9.99	223.68 ± 18.83	192.09 ± 3.23	154.29 ± 0.90
Cs2	154.76 ± 2.44	166.54 ± 4.92	176.29 ± 3.72	151.10 ± 3.22	154.76 ± 2.44
Cc50	38.34 ± 7.32	32.12 ± 0.73	30.05 ± 0.73	27.98 ± 2.19	29.01 ± 3.66
Cc240	95.87 ± 0.0	94.66 ± 4.08	93.94 ± 1.02	92.74 ± 0.68	92.01 ± 1.70
***F. delphinoides* isolates**					
Cc26	20.46 ± 1.15	19.64 ± 0.38	37.65 ± 1.92	26.19 ± 0.38	20.19 ± 1.15
Cc119	15.84 ± 2.16	17.03 ± 0.72	42.24 ± 0.24	13.28 ± 0.24	14.30 ± 0.24
CSH4	120.70±5.47	126.06±3.50	154.15±0.80	55.67±3.24	114.82±2.12

Supplementary Table 2. Relative gene expression or fold change of proteases genes in FSSC isolates in an *in vitro* condition.

Isolate	**Fold change**						
	C7Z7U2	**C7ZNV5**	**C7YQJ2**	**C7YVF3**	**C7YY94**	**C7Z436**	**C7Z6W1**
CSH1	15.370±0.397	0.241±0.024	0.201±0.006	0.125±0.009	30.304±0.726	0.282±0.002	3.387±0.145
CSH2	7.544±0.270	0.125±0.001	0.097±0.014	0.098±0.014	30.422±1.490	0.027±0.002	1.821±0.127
CSH3	6.268±0.474	0.526±0.051	0.496±0.015	0.023±0.018	11.832±0.704	0.428±0.166	1.928±0.074
CSH5	9.036±1.172	0.204±0.009	0.178±0.003	0.263±0.010	80.851±15.369	0.255±0.008	3.376±0.472
CSH6	9.352±1.234	0.151±0.003	0.122±0.004	0.087±0.012	34.951±10.524	0.244±0.014	1.886±0.224
CSH7	9.413±0.370	0.124±0.003	0.123±0.006	0.104±0.006	24.000±9.497	0.148±0.005	1.746±0.287
CSH8	7.144±0.640	0.132±0.002	0.122±0.007	0.108±0.002	112.95±35.404	0.170±0.018	1.849±0.183
CSH10	16.068±0.191	0.372±0.062	0.211±0.016	0.088±0.070	153.55±39.810	0.020±0.004	2.439±1.692
CSH11	17.309±2.532	0.306±0.033	0.167±0.018	0.077±0.030	161.70±77.518	0.067±0.000	1.544±0.166
CS1	12.732±0.436	0.547±0.024	0.574±0.075	5.542±0.190	3.276±0.464	0.547±0.005	5.012±2.932
CS2	8.876±0.043	0.238±0.008	0.150±0.009	0.084±0.011	62.138±15.372	0.301±0.005	1.762±0.138
Cc50	0.357±0.001	0.445±0.012	0.468±0.036	3.042±0.406	27.050±1.168	0.351±0.049	1.190±0.254
Cc240	6.730±0.296	0.485±0.042	0.441±0.017	0.895±0.039	19.305±4.038	0.483±0.028	1.705±0.058