Shotgun Analysis of the Secretome of *Fusarium graminearum*

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Abstract *Fusarium* head blight, caused predominately by *Fusarium graminearum*, is one of the most destructive diseases of wheat (*Triticum aestivum* L.) worldwide. To characterize the profile of proteins secreted by *F. graminearum*, the extracellular proteins were collectively obtained from *F. graminearum* culture supernatants and evaluated using one-dimensional SDS-PAGE and liquid chromatography-tandem mass spectrometry. A total of 87 proteins have been identified, of which 63 were predicted as secretory proteins including those with known functions. Meanwhile, 20 proteins that are not homologous to genomic sequences with known functions have also been detected. Some of the identified proteins are possible virulence factors and may play extracellular roles during *F. graminearum* infection. This study provides a valuable dataset of *F. graminearum* extracellular proteins, and a better understanding of the virulence mechanisms of the pathogen.

Keywords *Fusarium graminearum* · Secretome · *Triticum aestivum* L. · LC-MS/MS · Virulence

Introduction

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a serious and devastating spike disease in wheat (*Triticum aestivum* L.) [1]. This disease has become a serious threat to the production and quality of wheat and a major concern in many parts of the world [2]. Although some progress related to pathogen identification and epidemiology has been made, the precise mechanism of pathogenesis is not well understood. To establish an infection in a host plant, *F. graminearum* must either evade or suppress plant defense responses, and acquire nutrients in a compartment by redirecting the metabolism of the host to the site of infection. It has been reported that the fungal invasion of healthy plant cells and organ tissues is facilitated through fungal secretion, including peptides, proteins and fungal lytic enzymes, resulting in changes in normal host cell wall structures and cellular functions, and ultimately disabling normal plant defense systems [3]. Recently, based on *F. graminearum* genomic information and bioinformatics, more than 600 extracellular proteins have been postulated [4]. Yet, such computerized assumption can only provide a potential protein profile, and does not represent the reality as many postulated genes do not have transcriptional or translational functions [5–7]. Proteomic analysis, however, has been proven to be the most powerful method for the identification of proteins in complex mixtures and is suitable for studying the modification of protein expression in an organism due to genetic and/or environmental variations [2]. The exoproteome of the fungus *F. graminearum* grown on different media were investigated [7–9], and more than three hundreds proteins secreted were identified. Comparing these studies, it can be drawn that the actual compositions of the secretome of *F. graminearum* grown on different media were different, and no experiment is saturating in regard to defining all the proteins secreted by the

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fungus at any given time or under certain condition. Therefore, extensive knowledge of this very diverse *F. graminearum* exoproteome is important to understanding the interactions between the fungus and wheat.

In this study, the characterization of the expressed secretome of *F. graminearum* grown on the medium different with previous reports was reported to provide more information about the secretome of *F. graminearum*. We believe that our data will provide a better understanding of the functional mechanisms of *F. graminearum* during the infection processes and help detect new putative virulence factors.

Materials and Methods

Fungal Strain, Media and Culture Condition

Fusarium graminearum Schw (*Gibberella zeae*) was kindly donated by Dr. Wang of Nanjing Agricultural University. The fungus was inoculated and grown on minimal medium [10]. Cultures were constantly shaken (200 rpm) during their incubation period at 26 °C until the exponential growth phase was reached.

Protein Preparation and SDS-PAGE

The suspension of *F. graminearum* at its logarithmic growth phase was collected and the supernatants were obtained by centrifugation at $3,000 \times g$ for 15 min at 4 °C and filtration through an Φ 0.20 μ m cellulose acetate membrane (GE Healthcare, USA) filter and used to extract secreted proteins as described [11]. The electrophoresis was run on the Ettan DALTII unit (GE Healthcare, Uppsala, Sweden) and the gels were visualized by Coomassie staining.

LC-MS/MS Shotgun Analysis of Secreted Proteins

Based on Coomassie staining visualization, the whole gel lanes of *F. graminearum* secreted protein were cut into ten pieces and subjected to in-gel tryptic digestion as described by Shevchenko [12]. The generated tryptic peptides were separated on an Agilent Zorbax SB-C18 column (0.075 μ m $\times$ 100 mm) using a Proxeon easy-nLC system (Odense, Denmark) at 300 nL min⁻¹. Mobile phase A (0.1 % formic acid in water) and the mobile phase B (0.1 % formic acid, 98 % acetonitrile in water) were selected. The tryptic peptide mixtures were eluted using a gradient of 5 % B for 10 min, 5–45 % B 70 min, 45–80 % B 5 min and maintained at 80 % B 15 min, 80–5 % B 5 min, respectively, and finally were maintained at 5 % B for 15 min. Flow from the column was directed to a Bruker MicroTOF-Q (Q-TOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) operating in positive ion.

Protein Identification

A forward protein database was extracted from UniProtKB (<http://www.uniprot.org/>) using keyword “Fusarium”. For each reported protein sequence in the forward Fusarium protein database, a reversed protein sequence was generated using in-house developed software. All reversed protein sequences were then attached to the end of the forward Fusarium protein database to construct an integrated Fusarium protein database, which was then used for protein identification. Proteins were identified as described [11]. For functional attributions of the identified proteins, the proteins were subjected to BlastP (<http://www.ncbi.nlm.nih.gov/BLAST/>) analysis. The nearest homologues suggested possible functions. If no functions were assigned to the nearest homologues, their homologues were analyzed by BlastP and their functions predicted by the FunCat program (<http://mips.gsf.de/projects/funcat>).

Bioinformatic Analysis of the Identified Proteins

To confirm that identified proteins were secretory proteins, all proteins identified were further analyzed using SignalP 2.0, SecretomeP 2.0 and TMHMM to predict the possibility of protein secretion through classical secretion pathways or nonclassical secretion pathways and the presence or absence of transmembrane domains in the protein sequence. The

Fig. 1 Separation of *F. graminearum* extracellular proteins by one-dimensional SDS-PAGE. Lane 1 shows the purified *F. graminearum* extracellular proteins, and lane 2 displays the molecular weight markers. Ten equally spaced sections were excised as the broken line showed and subsequently used for downstream analysis

Table 1 *F. graminearum* extracellular proteins identified

No.	Category/ protein name	Accession no.	Peptide identified	Mr	pI	C (%)	Score
Protease							
1	Hypothetical protein FG05797.1	gil46122839	K.SLAPAQDGVLR.V R.LSEAGIVGQWGYER.G R.GLTYEYVQLAGHELPGYSAGAGYR.V	63,567	5.09	18	198
2	Hypothetical protein FG10677.1	gil46138325	K.AVSSMMISPQNYFSGDISR.R + 2 Oxidation (M)	89,781	5.51	2	52
3	Hypothetical protein FG04022.1	gil46116360	UK.AALSEWADM.R.S + Oxidation (M) R.NALVGKPTVGLTSR.A R.DATLVLDIAIYGLDER.D R.AGVIPSEHQDSVGTFR.T K.GHPAFASGQDGFSLASLETG.G K.GHPAFASGQDGFSLASLETGGER.D K.GERDETYWQALDFCQSSTR.K	63,694	5.54	42	192
4	Hypothetical protein FG02107.1*	gil46110451	DAPTILIASVFDPESTVTNAEGLR	80,500	5.86	3	66
5	Hypothetical protein FG02204.1	gil46110645	GGPVILLASGETSGEDR QAQQDIIDFTK YYGTSFPVPDLKPENMR VAFIDGEYDPWR	59,526	5.16	11	104
6	Hypothetical protein FG04546.1	gil46117408	K.TSGHMVPQYAPPAAFR.Q + Oxidation (M)	59,674	6.51	8	77
7	Hypothetical protein FG04527.1	gil46117370	K.TSGNLSWLR.V K.AIGAQSTYGECPEAAAYDK.F R.APSNDPFPSTYSTYLQSSVMK.A + Oxidation (M) K.AIGAQSTYGECPEAAAYDKFINSGR.G	52,260	5.54	13	111
8	Hypothetical protein FG04504.1*	gil46117324	R.FQDANNLQVDFIR.S K.APMYIISGGTGNIEGLSAVGTK.G + Oxidation (M)	55,188	5.41	14	101
9	Hypothetical protein FG02918.1	gil46113079	R.FGIYYAAGEDDSTGDK.A K.TGHDANIEYLGGTIIGPTVEDK.F K.TGHDANIEYLGGTIIGPTVEDKFSAGGLK.W	46,120	4.80	23	83
10	Hypothetical protein FG07775.1	gil46126795	VSVGGLVSSQAVESAQR K.VSVGGLVSSQAVESAQR.V	42,360	5.39	9	93
11	Hypothetical protein FG03072.1*	gil46114460	K.EVPAVVSTSYGDEEDSVPR.E R.EYATMTCNLIGLLGLR.G R.GFPDVAGHSVSPNYEVIYAGK.Q K.LKDLVLSF	65,043	5.79	10	89
Carbohydrate hydrolase							
12	Hypothetical protein FG00294.1*	gil46105332	WLIDVNMTNSR K.NAITNELWISIASI.S	67,032	6.25	4.66	65
13	Hypothetical protein FG03626.1	gil46115568	K.QIIDLPGVGENLQDHLR.I	63,908	8.86	10	58
14	Hypothetical protein FG09366.1	gil46135701	K.LTDLVIGVSVGSEDL.YR.N	47,730	4.73	5	38

Table 1 continued

No.	Category/ protein name	Accession no.	Peptide identified	Mr	<i>pI</i>	C (%)	Score
15	Hypothetical protein FG06278.1*	gil46123801	DLVSGISTGTYASDSATFK AVALSASDYTSSNPVWK	61,941	5.42	6	65
16	Hypothetical protein FG11032.1*	gil46139035	K.TTQNVNGLSMLPR.Q + Oxidation (M) R.VYHSISLLLPDGR.V K.TSLYDSSSDSWIPGPDMPQVAR.G + Oxidation (M) R.NDAFGGSPGGITLTSSWDPSTGIVSDR.T R.GIPFEDSTPVFTPEIYVPEQDTFYK.Q	73,177	8.30	21	100
17	Hypothetical protein FG11326.1	gil46139623	K.VGAITVDDMSLSFFK.D + Oxidation (M) K.TSSGEVTWLSDPNNR.A K.LQGTTNPSGSPESGGLGEPK.F	63,799	8.71	13	75
18	Hypothetical protein FG02658.1	gil:46111553	K.NADNPDFTFEQVQCPK.E	39,459	8.92	4	64
19	Hypothetical protein FG03017.1	gil46114350	R.TLAYPSGGVNYPTPMR.I + Oxidation (M) K.SCDPDVGLAASSYTVDFTK.G K.VMVQNLNPADSYTYGDETGSYK.S + Oxidation (M)	41,546	4.38	20	86
20	Hypothetical protein FG03529.1*	gil46115374	K.YFGVANQDR.K K.LAQQIYDVR.G K.ATVWVGETGWPTK.G K.ILVGIWSTDDAHFGR.D K.VGHTDTWTAWVDGTNDVVT.K.A K.DASGFWFTAFTPAQTTEVEK.Y	33,723	9.36	33	148
21	Hypothetical protein FG00251.1*	gil 46105246	R.ASAPIGSAISR.N K.TTQNVNGLSMLPR.Q K.TWTSLPNAK.V R.ITISTDSSISK.A	117,079	8.30	6	113
Lipase							
22	Hypothetical protein FG03875.1*	gil46116066	R.ALMNGAGFISAADSR.N + Oxidation (M) K.DADTPFPILVSDGR.A R.LADGLSDQEEAWVR.R K.GGPAYTFSSIADTSNFK.D K.GVLTDLDANDEDIADYSPNPFYK.W	71,061	4.73	18	183
23	Hypothetical protein FG03687.1	gil46115690	K.VTVWGESAGAR.S K.ALDEAWTGHR.D R.SASFAGDFQQHSGR.R K.TGCDASSNSLDCLR.E K.DAVATATELYPDDPAK.G K.KTGCDASSNSLDCLR.E R.SLGMQLVAYDGQHDGLFR.A + Oxidation (M) K.FAHVPLLMGNNFDEGTAYGK.K + Oxidation (M)	62,942	5.26	33	167
24	Hypothetical protein FG07678.1	gil46126601	K.QYVAPNLDLEHTFSGR.K IAIVYFENENYEK R.QNHILGILLGDAVPK.H	47,778	6.57	12	89
25	Hypothetical protein FG03333.1*	gil46114982	K.IFEGQVELYNR.L K.RPILAGGDTPSDSDGPMIFR.G + Oxidation (M)	41,223	7.66	18	56

Table 1 continued

No.	Category/ protein name	Accession no.	Peptide identified	Mr	<i>pI</i>	C (%)	Score
Degradation of lignin							
26	Hypothetical protein FG02951.1*	gil46114091	WVEEGVAPDTITGMR	124,479	8.48	1	47
Degradation of chitosan							
27	Hypothetical protein FG10998.1*	gil46138967	K.TENSDLFWGIR.G R.LGHVATELWNQGKR.A K.NGYSVDGAYIGDEASLR.K K.NGYSVDGAYIGDEASLRK.A K.VPTDAQGSQSWKEDGTAYNLR.L	52,863	8.11	19	195
28	Hypothetical protein FG03212.1	gil46114740	K.GLGGFATYEAGGDYNNILIDAVR.A R.QGLGCNTINENDTANFLEFLK.E	45,184	5.18	26	103
29	Hypothetical protein FG01283.1	gil46108802	R.AAVMGEVLWSGR.T + Oxidation (M) K.DVVVQSWLGGGAIK.T	76,278	5.49	9	63
Toxin synthesis related proteins							
30	Hypothetical protein FG10212.1	gil46137393	R.VNAQVYHADPSACGLK.K K.SRPMTAVSCSDGSNGLITK.Y + Oxidation (M) K.INVLAIIDHAASGFNIGLDAMNALTGGQATALGR.V + Oxidation (M)	14,717	9.10	49	76
31	Hypothetical protein FG11205.1	gil46139381	V DATATQVAVSNCGLK 1632.8141 R.AMTAVSCSDGTNGLITR.Y + Oxidation (M)	14,857	9.08	24	107
32	Hypothetical protein FG07807.1*	gil46126859	R.AQSTDYTLPIKDGK.C K.DFTASCIAHSTFCDYEFK.V	16,015	5.75	22	62
33	Hypothetical protein FG07654.1*	gil46126553	AVAESLPR841.4657	111,321	6.58		45
34	Hypothetical protein FG06779.1	gil46124803	TYFDVSAIVDPNDKDNVK	17,406	4.32	11	69
35	Hypothetical protein FG06497.1*	gil46124239	K.GDPVNEIQVFLEIAALK.A R.DIPFCGDNCYSTASVGGK.L K.LPSLLDFSK.Q	39,950	4.82	11	95
Second metabolite process							
36	Hypothetical protein FG00100.1	gil46103667	INDQFIAGTTTGK	55,065	4.69	2	59
37	Hypothetical protein FG09980.1	gil46136929	K.ALDDAGIYLVLDVNNPK.Y K.IPVGYSAADVASNR.M R.SDFFAFNDYSWCNSDFK.T K.IKGNTVTPEDFDLFLK.S	48,923	4.95	14	150
38	Hypothetical protein FG04664.1*	gil46117876	VLDLMKDDVLESTVVSRR	92,106	6.16	2	86
39	Hypothetical protein FG04793.1*	gil46119756	K.SSGVAFDEFDPAFGYDYNPK.T	50,445	8.95	4	63
40	Hypothetical protein FG06612.1*	gil46124469	LGVYLDFFDDVDVPQPMR FSEDETFLVSETFLR	53,592	5.01	7	102

Table 1 continued

No.	Category/ protein name	Accession no.	Peptide identified	Mr	<i>pI</i>	C (%)	Score
41	Hypothetical protein FG09141.1*	gil46134003	QVNSGGFST 895.4036	62,432	5.26	1	55
42	Hypothetical protein FG04491.1*	gil46117298	LADEHKTMHFAVQHDALR2118.0429	232,358	5.78	1	53
43	Hypothetical protein FG03379.1	gil46115074	K.TFTASEIEDALASQHGAR.V K.HGTCISTLEPSCYSNYQTGEEAADYVKK.T K.WLAEAGIEPSTDKTFTASEIEDALASQHGAR.V K.TFTASEIEDALASQHGAR.V	25,084	4.77	39	230
Unknown							
44	Hypothetical protein FG07988.1	gil46127221	K.LGYATIADK.G K.DGELNLYTFQNSQVAYVDR.S K.DGELNLYTFQNSQVAYVDR.S	18,933	9.13	21	101
45	Hypothetical protein FG02560.1	gil46111357	K.AGEAFEAIIVVR.E	14,571	5.43	15	92
46	Hypothetical protein FG04741.1	gil46118923	K.GDTPTGDAQAYVGPK.D	15,273	6.71	10	50
47	Hypothetical protein FG03894.1*	gil46116104	K.HNILSPGAGCK.L	16,773	9.49	7	72
48	Hypothetical protein FG03035.1	gil46114386	K.LSIHSVGSPVGTSIK.S	15,742	5.85	11	71
49	Hypothetical protein FG04735.1	gil46118840	NYSCPNPVTLYAK	16,007	8.88	10	74
50	Hypothetical protein FG02077.1*	gil46110391	K.ATSCVIDECGTDVAINEVLPATENLCK.N AIASETSCDKTDLACVCK	18,733	4.62	38	98
51	Hypothetical protein FG11466.1*	gil46139903	APDANMSFCAAK	48,693	5.94	3	45
52	Hypothetical protein FG00209.1*	gil46105162	SLFYIGDDNVLR	54,023	4.72	3	45
53	Hypothetical protein FG00492.1*	gil46107218	CQSYCITVPSNEQDIEK	19,935	4.31	11	77
54	Hypothetical protein FG01179.1	gil46108594	TYKDSLDMTIDPNSVDLTTR	20,835	4.29	11	86
55	Hypothetical protein FG09742.1*	gil46136453	K.VGDGGLKFDPAETTA.T	21,693	5.00	12	69
56	Hypothetical protein FG09403.1*	gil46135775	K.SLTYGCICGDGK.Q	22,132	4.28	7	89

Table 1 continued

No.	Category/ protein name	Accession no.	Peptide identified	Mr	<i>pI</i>	C (%)	Score
57	Hypothetical protein FG01748.1	gil46109732	R.FQSTLSNYLTTR.K K.ELPGAVLSGSPMADSPNLR.N R.EPDLTIPDFATLR.G K.EQNPANSVYYLAQLER.Y K.MGNPVDVGTAVVTYSSDK.L	47,911	6.92	20	365
58	Hypothetical protein FG03526.1	gil46115368	DVGESGLLIQESR	37,082	9.08	4	56
59	Hypothetical protein FG08238.1	gil46127721	VVKPDQSYAPANLPN QIVWPAYTDK 1219.6237	15,346	9.30	18	125
60	Hypothetical protein FG06130.1	gil46123505	LVPQCDKLESLPK K.FVVIEQAR.I	26,020	8.74	10	76
61	Hypothetical protein FG04213.1	gil46116742	K.ASFTVPVLEMR.A K.ALFDGKTDYTCK.V K.TDYTCKVPNGADSATFK.Y	20,933	8.69	31	125
62	Hypothetical protein FG08122.1	gil46127489	K.TFGLVALR.S K.KEDFATFR.I K.EQQIAYTDR.S K.GKEQQIAYTDR.S K.KPVSCIYSQYSN.- R.SGMGQGVLYQTGQK.N + Oxidation (M) K.DGNLVFGSNNAGFMACPLK.S K.STDPWSIWVATGTDHPGNSEK.E K.VDKDGNLVFGSNNAGFMACPLK.S + Oxidation (M)	21,010	9.02	67	359
63	Hypothetical protein FG10089.1	gil46137147	R.LGELNFGTEGVTK.I K.LDQVSGSVVVSSTTDIEEFCK.Y	42,529	4.66	11	105

Score was determined from protein blast against the nonredundant protein database

Mr and *pI* the theoretical *Mr* and *pI*, *C* sequence coverage

* The proteins have not been found in early reports [6, 7]

classical secretory proteins were predicted as described [13]. Non-classical secretory proteins were predicted by SecretomeP (<http://www.cbs.dtu.dk/services/SignalP/>). All proteins with N-terminal secretion signals were also identified by the prediction programs TargetP v1.1 (<http://www.cbs.dtu.dk/services/TargetP/>).

Results

Identification and Characterization of the *F. graminearum* Extracellular Proteins

The collected proteins present in the supernatants were separated by SDS-PAGE and the profile of extracellular proteins is shown in Fig. 1. The protein bands were cut into

ten pieces (Fig. 1) and used for subsequent mass spectrometric analysis, and a total of 87 proteins have been identified. Of the proteins identified, 63 were predicted as secretory proteins (Table 1), of which 58 appeared to be classical secretory proteins. Besides the 58 classical secretory proteins, five proteins were predicted by SecretomeP and identified as non-classical secretory proteins. All the proteins with N-terminal secretion signals were also identified based on the TargetP v1.1 prediction programs and were proved to be extracellular proteins.

Most of the 63 secretory proteins have relatively low molecular weights ranging from 10 to 70 kDa, and forty-three proteins showed *pI*s of 4–7 (Fig. 2). According to their functions, these secretory proteins can be classified into eight categories. The most abundant category was unknown proteins (31 %), which were predicted from nucleic acid

Fig. 2 Distribution of molecular weights (**a**) and *p*I's (**b**) of the 63 secreted proteins

sequences only and either had no homologues in the protein sequence databases, or was homologous to genomic sequences of unknown functions [14]. Other proteins have known functions or sequence similar to those with known functions, including those that are involved in protein hydrolysis (17 %) and carbohydrate hydrolysis (16 %). Other functional classes are related to lipase hydrolysis, lignin degradation, chitosan degradation, toxin synthesis, and secondary metabolism, respectively (Fig. 3; Table 1).

Discussion

Exoproteome of *F. graminearum*

The sequencing and reannotation of the genome of *F. graminearum* makes it particularly well suited for proteomics studies. In this study, we investigated the exoproteome of *F. graminearum* grown on minimal medium lacking organic nitrogen, and a total of 63 secreted proteins were identified. Among the proteins identified, 27 proteins were not by the earlier study (Table 1). The difference in the results might be because of differences in the media used, and the actual composition of the secretome might be depending on growth conditions. Therefore, no experiment

is saturating in regard to defining the entire secretome. Because many secreted proteins are virulence or virulence effectors and constitute the first contact between a pathogen and its host [8], all the secreted proteins identified in our study and earlier reports are promising candidates to have a role in pathogenesis, and might provide new insights into *Fusarium*/plants interactions [7, 8].

Virulence Factors of *F. graminearum*

To invade a host plant for its nutrition requirement, *F. graminearum* needs to secrete a number of enzymes in several families to degrade the plant cells [15]. During the infection process, host-cell proteins and lignin are degraded and used to satisfy the fungal basic metabolic requirements [16]. In this study, a number of proteins (No. 12–21) involved in xylan, cellulose, and hemicellulose hydrolysis process were identified. This indicates that these proteins may be associated with the degradation of host-cell proteins and lignin during the fungal infection process. In addition to the enzymes involved in cell-wall degradation, 11 proteins (No. 1–11) homologous to extracellular protease, including exopeptidase, peptidase, carboxypeptidase, aminopeptidase and other proteases were detected. These proteins might be required for hydrolyzing other host cell components. Some proteins (No. 26–29) with lignin-related degradation activities were also identified. These proteins might be involved in the degradation of lignin resulting in a more flexible damaged cell wall that is more easily penetrated by the fungal hyphae. A few other proteins that are homologous to lipases, including phospholipase and triglyceride lipase (No. 22–25) were also detected. These proteins may play roles in degrading plant lipids, cell membrane and intracellular components, and ultimately assisting in degradation of the host cells. Therefore, the described proteins likely provide nutrients that *F. graminearum* need for surviving on wheat or suppressing the wheat to combat the fungal pathogen and are possible virulence factors.

Phytopathogenic fungi produce several extra-cellular proteins, which may induce plant cell necrosis. In many cases, these proteins have phytotoxic activity and act directly against the hosts either by suppressing the plant defensive systems, or by modifying the structure of the plant cellular membrane. One of these proteins is ceratoplatenin, which elicits defense-related responses such as phytoalexin synthesis and cell death in both host and non-host plants, and is involved in the development of the plant disease [17]. In this study, two proteins (No. 30 and 31) that are homologous to ceratoplatenin had been detected. These two proteins might be involved in various stages of the host-fungus interaction and act as phytotoxins, and thus could be the causal agent of FHB in wheat. One protein

Fig. 3 The functional category distribution of the 63 identified proteins

(No. 32) homologous to the GT1 glycosyltransferase family was detected. It was reported that GT1 glycosyltransferase family proteins play a role in the biosynthesis of amylovoran, which acts as a virulence factor of the bacterial plant pathogen, *Erwinia amylovora*, responsible for the devastating disease known as fire blight in some Rosaceous plants [18]. The FHB was somewhat like the fire blight in the symptom of fire blight wilting, suggesting that this protein is very likely associated with the FHB disease development in wheat. Interestingly, the three proteins identified (No. 33–35) highly homologous to the mycotoxin metabolism-related proteins and two hypothetical proteins, FG06779.1 (No. 34) and FG06497.1 (No. 35) associated with sporulation and response to toxin-detoxification, were found in this study. It has been reported that some mycotoxins, exert phytotoxic effects and act as virulence factors, leading to the pathogenicity of *F. graminearum* [19]. Although further investigations on the roles of these proteins in mycotoxin biosynthesis are needed, it is speculated that these proteins may play important roles in the synthesis of mycotoxin and act as potential virulence factors.

We identified 20 proteins with unknown function and were unable to discern their roles in pathogenicity (No. 44–63, Table 1). These proteins are of interest as they might be candidates with some new biological functions, such as gene disruption and be responsible for virulence. Further studies on the roles of these proteins during the FHB pathogenicity using bioinformatics tools, gene expression and antibodies specific to these proteins would likely yield new scientific insights into the pathogenicity of this important plant pathogen.

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