Chapter 4: Cultivation of selected fungal isolates from koala faeces in liquid media for further enzyme characterisation

4.1 Introduction

The ability of filamentous fungi to grow and secrete enzymes into a liquid medium has been exploited in research and industry for many decades (Kavanagh, 2005; Section 1.2). Whereas small scale laboratory cultures provide an effective tool for characterising the enzyme activities of new fungal species or strains, large fermenter cultures of industrially established fungi have been used widely for the production of commercial enzymes. The secreted enzymes of the fungi are contained in the culture supernatants, which can be easily separated from the fungal mycelia and studied using biochemical and protein analysis techniques (Section 1.7.2 and 1.7.3).

In the work described in Chapter 3, seven fungal isolates from koala faeces were selected for further characterisation of their enzyme activities (Section 3.5). The hydrolase activities of the fungi were of particular interest due to the wide utilisation of hydrolases in industry (Kirk et al., 2002; Section 1.2). Therefore, in the work presented in this chapter and Publication 2 (Section 4.2), the selected fungi were grown in a hydrolase-inducing liquid medium to further characterise their xylanase, mannanase, endoglucanase, β-glucosidase, amylase, protease and lipase activities. Temperature and pH profiles of the enzymes were established by subjecting the culture supernatants to liquid enzyme assays, revealing attributes that could make the enzymes good candidates for development for industrial applications. Furthermore, the number and molecular weights of the enzymes and/or enzyme isoforms were determined by protein gel electrophoresis and zymography. Two strains of *Trichoderma reesei* were also included in the analysis. *T. reesei* RUT-C30 provided a good example of a high secreting fungal strain of which the cellulase production has been improved by random mutagenesis and screening (Montenecourt and Eveleigh, 1979). The wild-type *T. reesei* QM6a (Mandels and Reese, 1957) was included to demonstrate the natural protein secretion and enzyme

activity of the *T. reesei* species before human intervention (Publication 2). This provided a measure for assessment of the natural capacity of the fungal isolates to secrete enzymes and gave some indication if a future mutagenesis program could result in high-producing strains.

This chapter also includes some unpublished work. Zymography was used to detect the secretion of carbohydrate esterases (Sections 4.3.1 and 4.4.1), enzymes that can remove side chains from xylan or pectin, or break linkages between hemicelluloses and lignin (Aurilia et al., 2000). In addition, the ability of the selected fungi to secrete lignin-degrading enzymes (Section 3.4.8.1) was explored further using liquid media specifically designed for the induction of lignin-degrading oxidases (Sections 4.3.2 and 4.4.2). Of the major lignin-degrading oxidases (lignin peroxidase, manganese peroxidase, and laccase; Section 1.8.5), laccase is the most commonly exhibited by fungi from the phylum Ascomycota (Baldrian, 2006). Therefore, as all the selected isolates were ascomycetous species (Section 3.5), a screening assay was used to detect laccase activity (Sections 4.3.3 and 4.4.2).

In the final sections of this chapter, the value of the plate assays in predicting enzyme activities in liquid media is assessed (Section 4.4.3), and an overview is presented of the characterised enzyme activities of the seven fungal isolates from koala faeces. Finally, the selection of one isolate for secretome analysis (Chapter 5) is described (Section 4.5).

4.2 Publication 2: Peterson, R., Grinyer, J., Nevalainen, H., 2011. Extracellular hydrolase profiles of fungi isolated from koala faeces invite biotechnological interest. Mycological Progress. 10, 207-218.

In the work described in the following publication, seven fungal isolates from koala faeces were grown in a hydrolase-inducing liquid medium and the enzyme activities in the culture supernatants were comprehensively characterised using liquid enzyme assays and zymography. All laboratory work and manuscript preparation were carried out by myself, under the guidance and correction of the other listed authors.

Graphs depicting the temperature and pH profiles of the enzyme activities of the fungi and a table outlining the approximate molecular weights of bands on the zymograms formed online supplementary material for Publication 2 and are referred to frequently within the text. For ease of viewing, the supplementary material is presented in this chapter in printed form (pp. 122 - 138).

Due to copyright reasons, the selected article has been omitted from this thesis (the article appears from page 110-121):

Peterson, R., Grinyer, J., Nevalainen, H., 2011. Extracellular hydrolase profiles of fungi isolated from koala faeces invite biotechnological interest. Mycological Progress. 10, 207-218.

Supplementary material for Publication 2

The following fifteen pages contain a printed form of the supplementary material that was provided online for Publication 2.

Figures S1 - S14 contain the temperature and pH profiles of the enzyme activities of the fungal isolates from koala faeces and comparison strains *T. reesei* RUT-C30 and wild-type *T. reesei* QM6a, displayed in the following order:

Figure S1 and S2: xylanase

Figure S3 and S4: mannanase

Figure S5 and S6: endoglucanase

Figure S7 and S8: β-glucosidase

Figure S9 and S10: amylase

Figure S11 and S12: lipase

Figure S13 and S14: protease

Substrates and other details of enzyme assays can be found in Materials and methods of Publication 2. The top graphs in Figures S1 - S12 depict the enzyme activities of all strains and the bottom graphs focus on the strains with lower enzyme activities. Error bars indicate one standard deviation above and below the mean based on the results from triplicate assays using supernatants from duplicate cultures. The isolates *M. camptospora* A11 and *C. peruviana* P7 are not included in the xylanase, mannanase, endoglucanase, β-glucosidase and protease activity profiles due to undetectable levels of enzyme activity.

Supplementary Table S1 (presented after Figures S1 - S14 in this thesis) contains the approximate molecular weights of bands formed on the xylanase, mannanase, endoglucanase, β-glucosidase, amylase, lipase and protease zymograms that were produced from the fungal supernatants, with and without boiling prior to electrophoresis, as described in Publication 2.

Figure S1: Temperature profiles of xylanase activity at pH 5.5

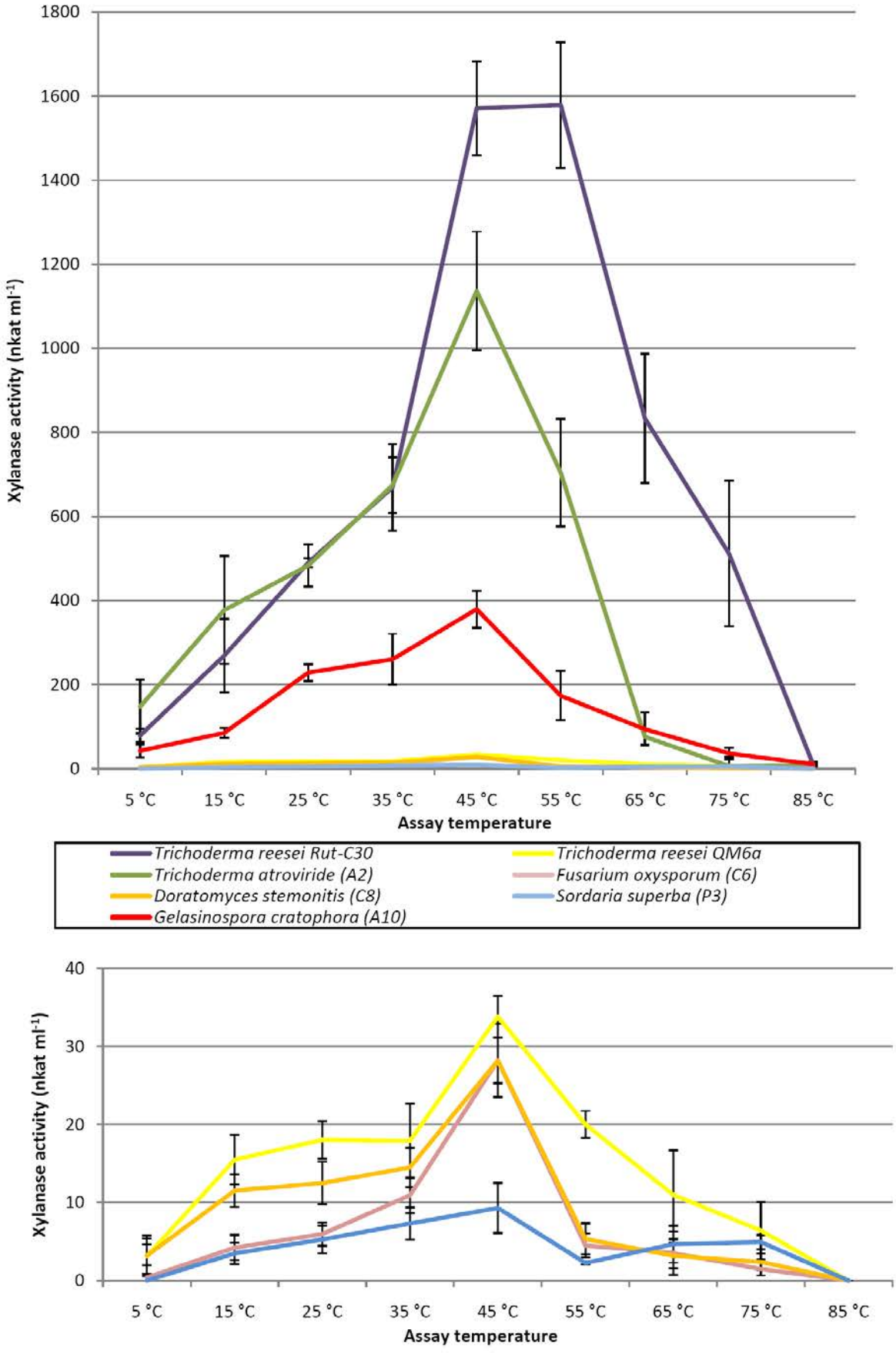


Figure S2: pH profiles of xylanase activity at 50 °C

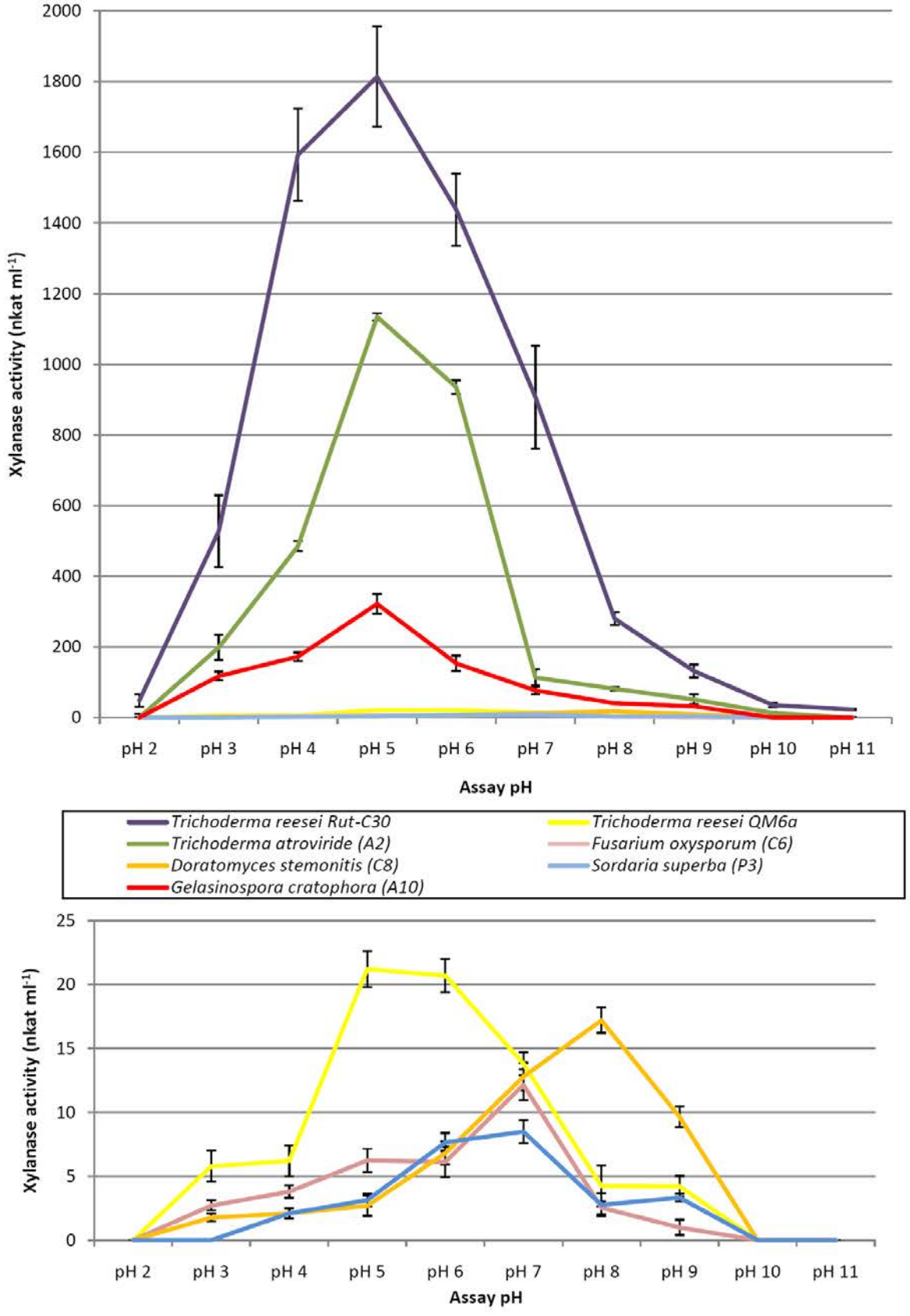


Figure S3: Temperature profiles of mannanase activity at pH 5.5

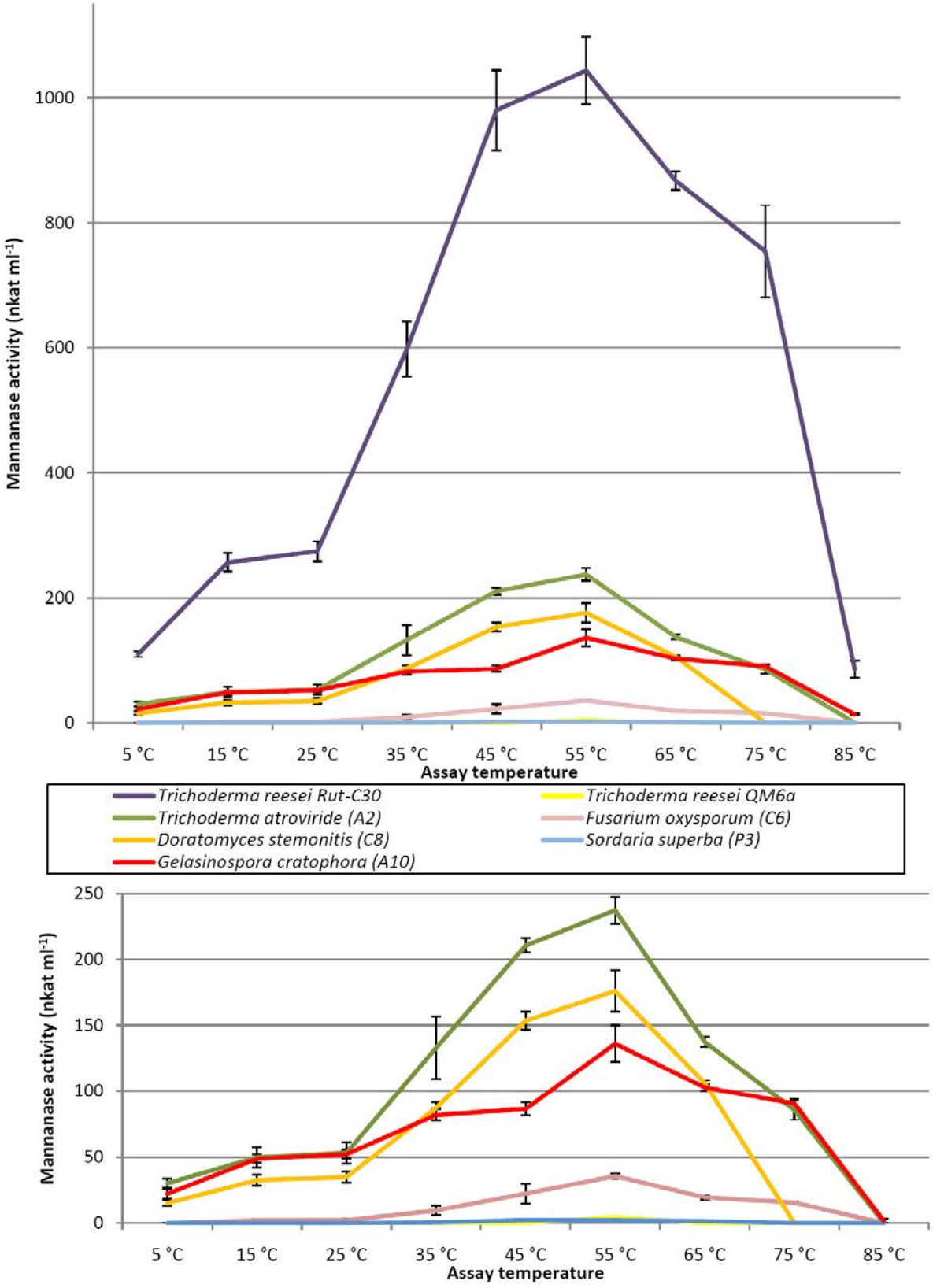


Figure S4: pH profiles of mannanase activity at 50 °C

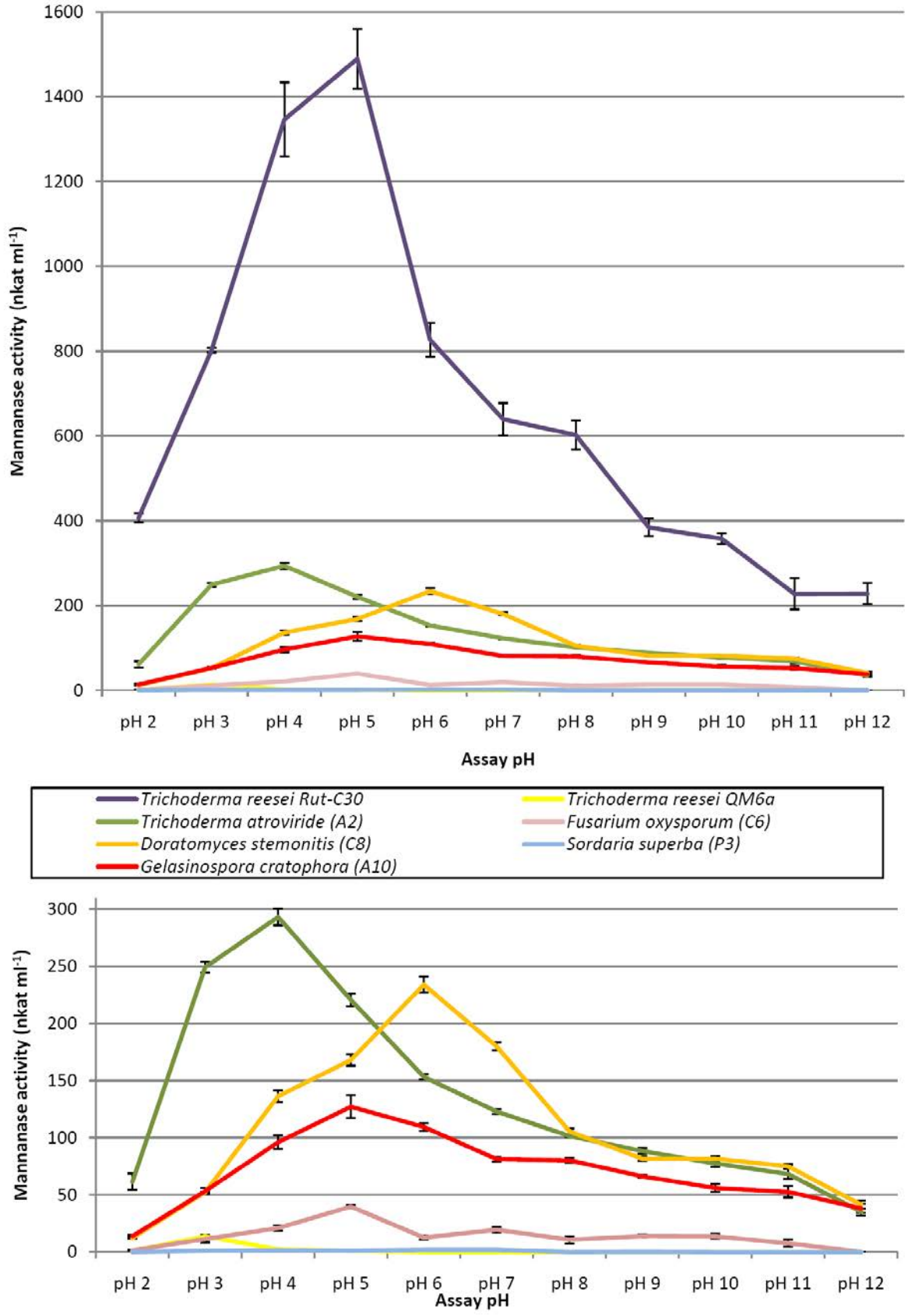


Figure S5: Temperature profiles of endoglucanase activity at pH 5

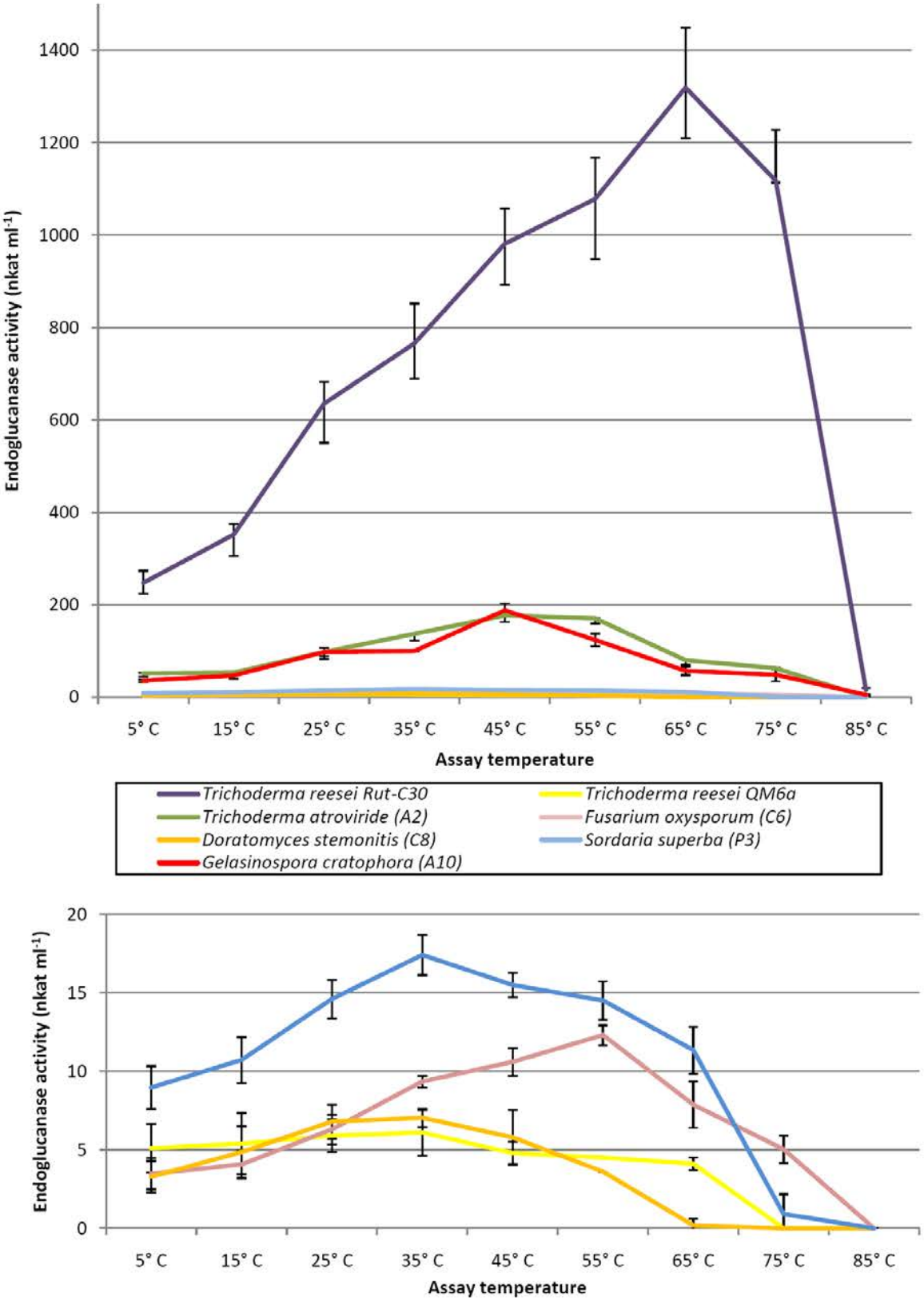


Figure S6: pH profiles of endoglucanase activity at 50 °C

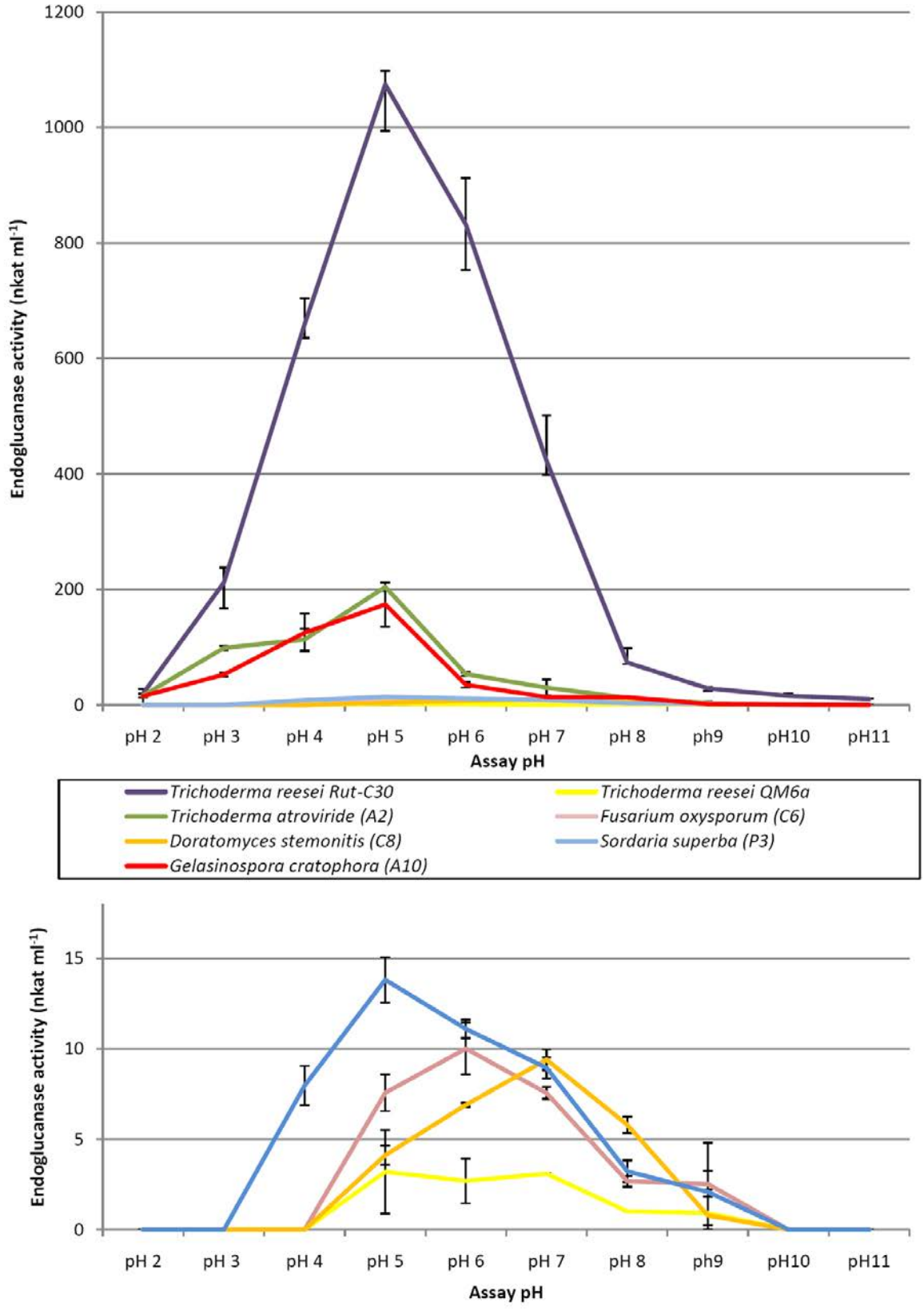


Figure S7: Temperature profiles of β-glucosidase activity at pH 5

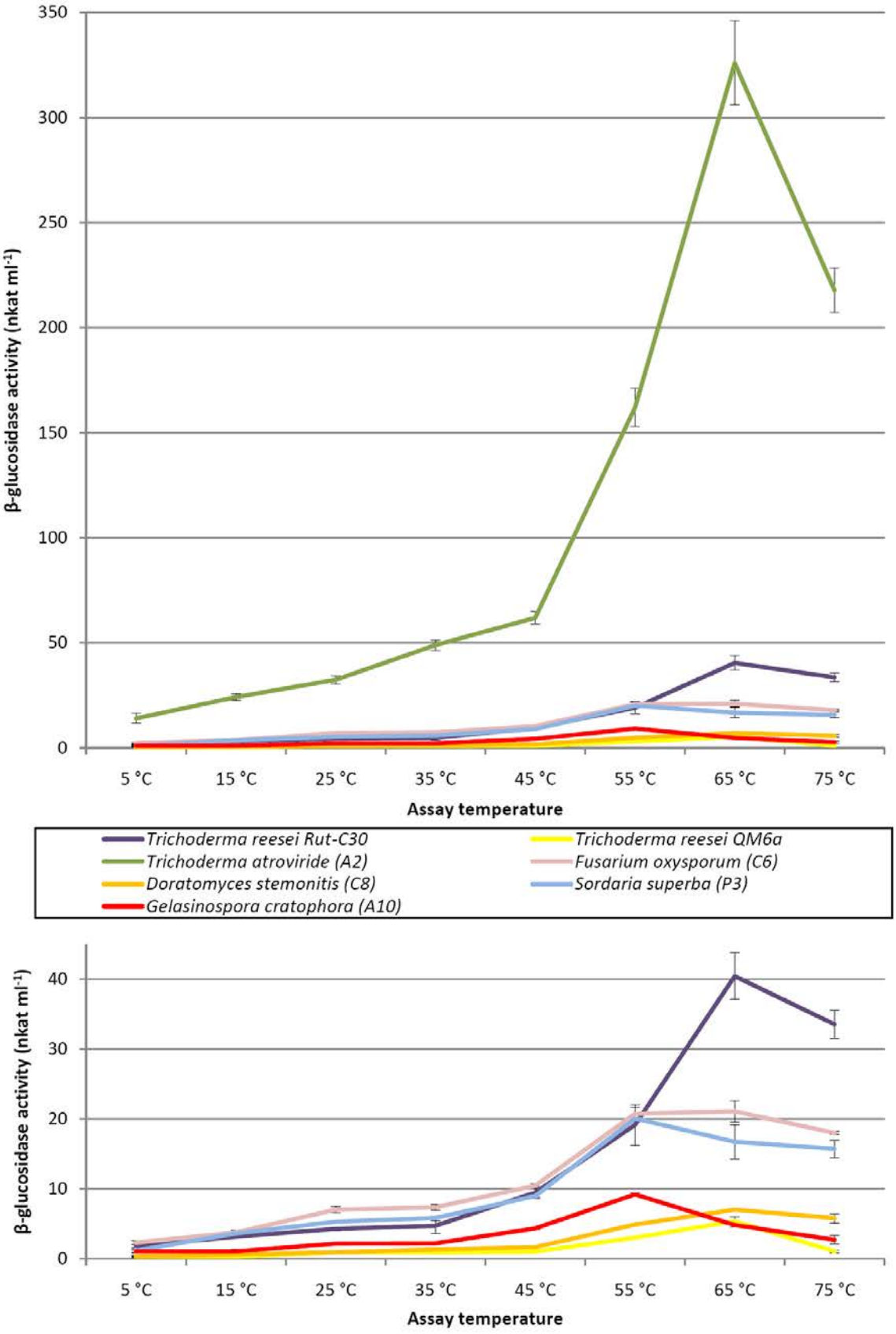


Figure S8: pH profiles of β-glucosidase activity at 60 °C

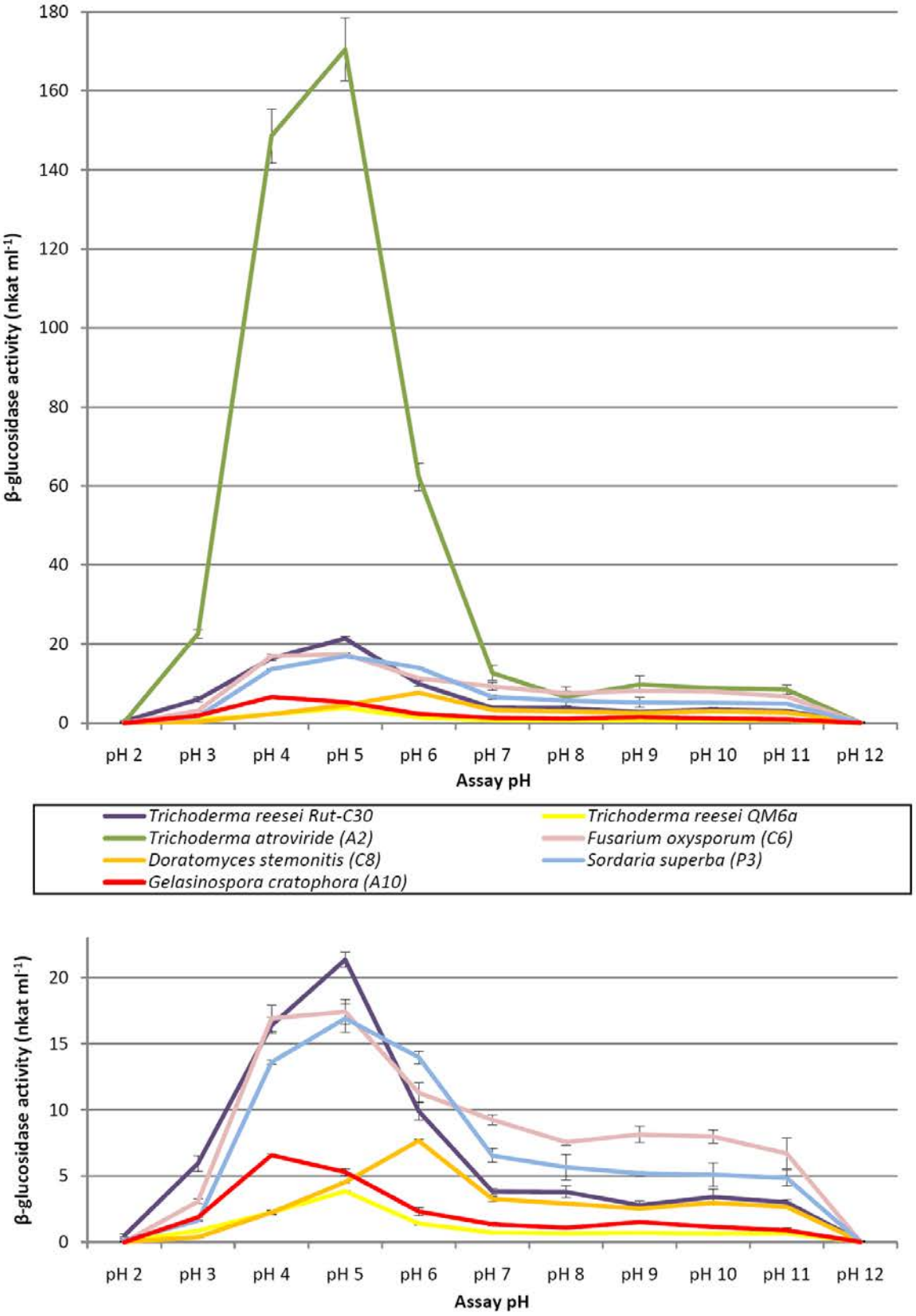


Figure S9: Temperature profiles of amylase activity at pH 5

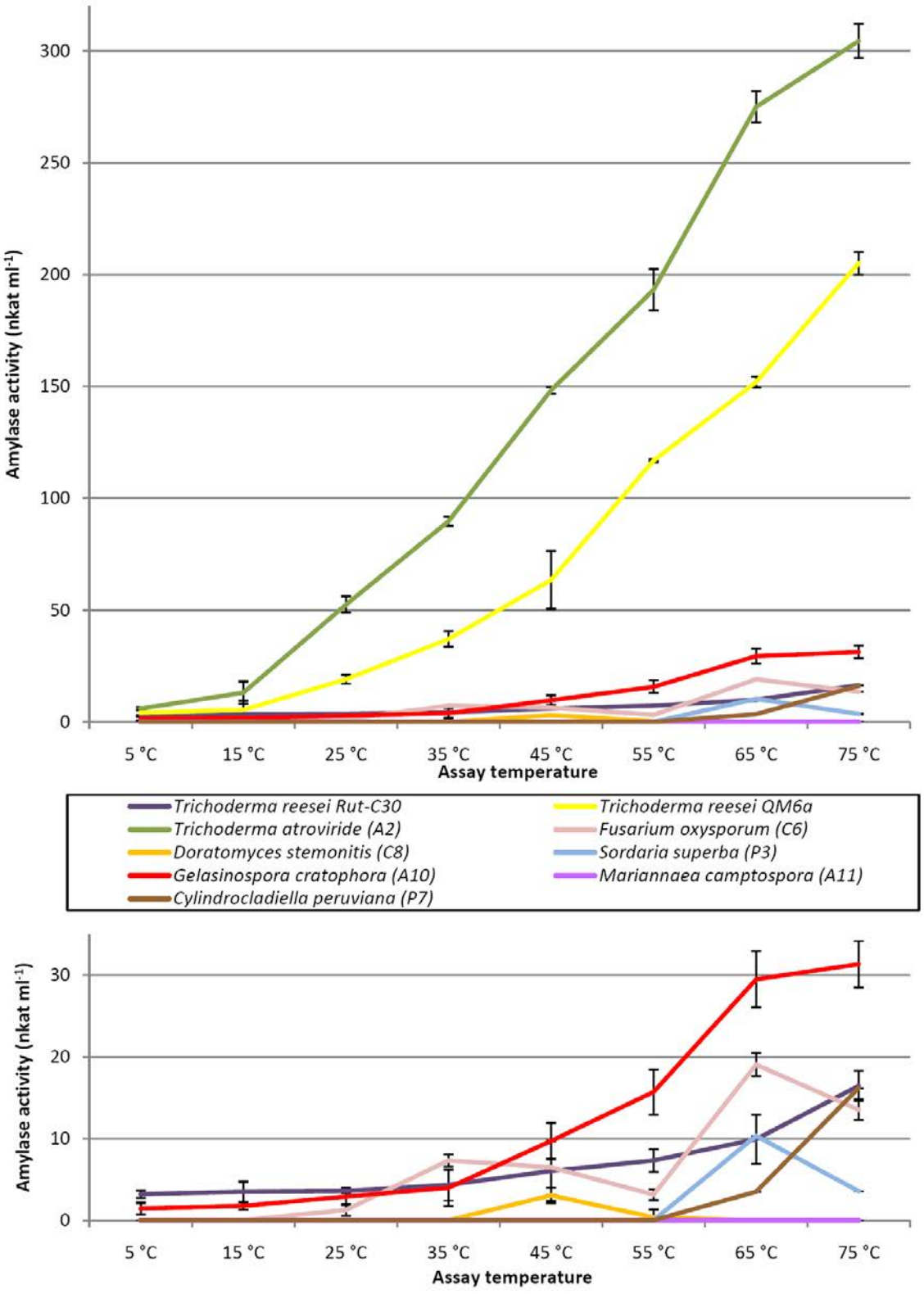


Figure S10: pH profiles of amylase activity at 65 °C

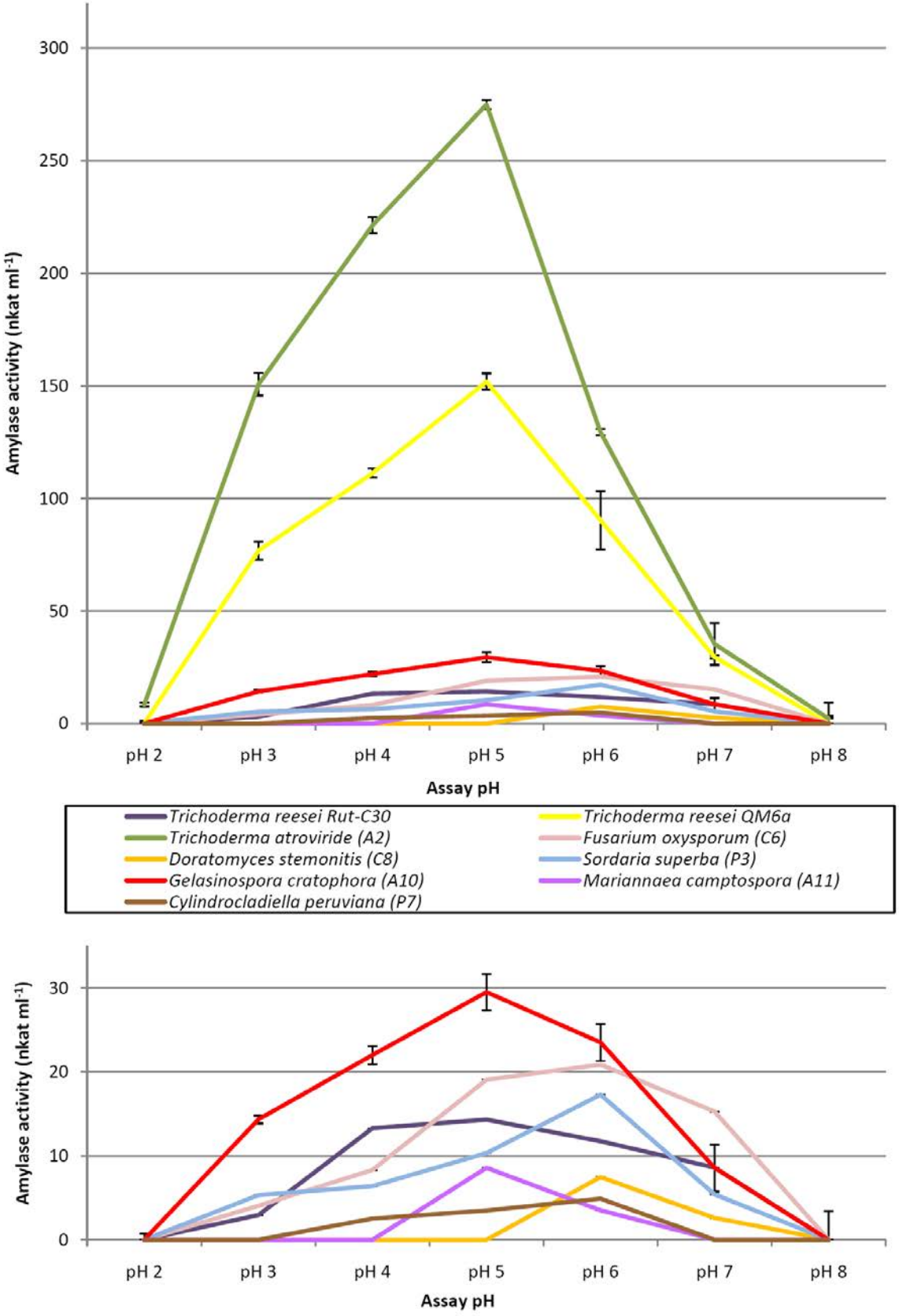


Figure S11: Temperature profiles of lipase activity at pH 8

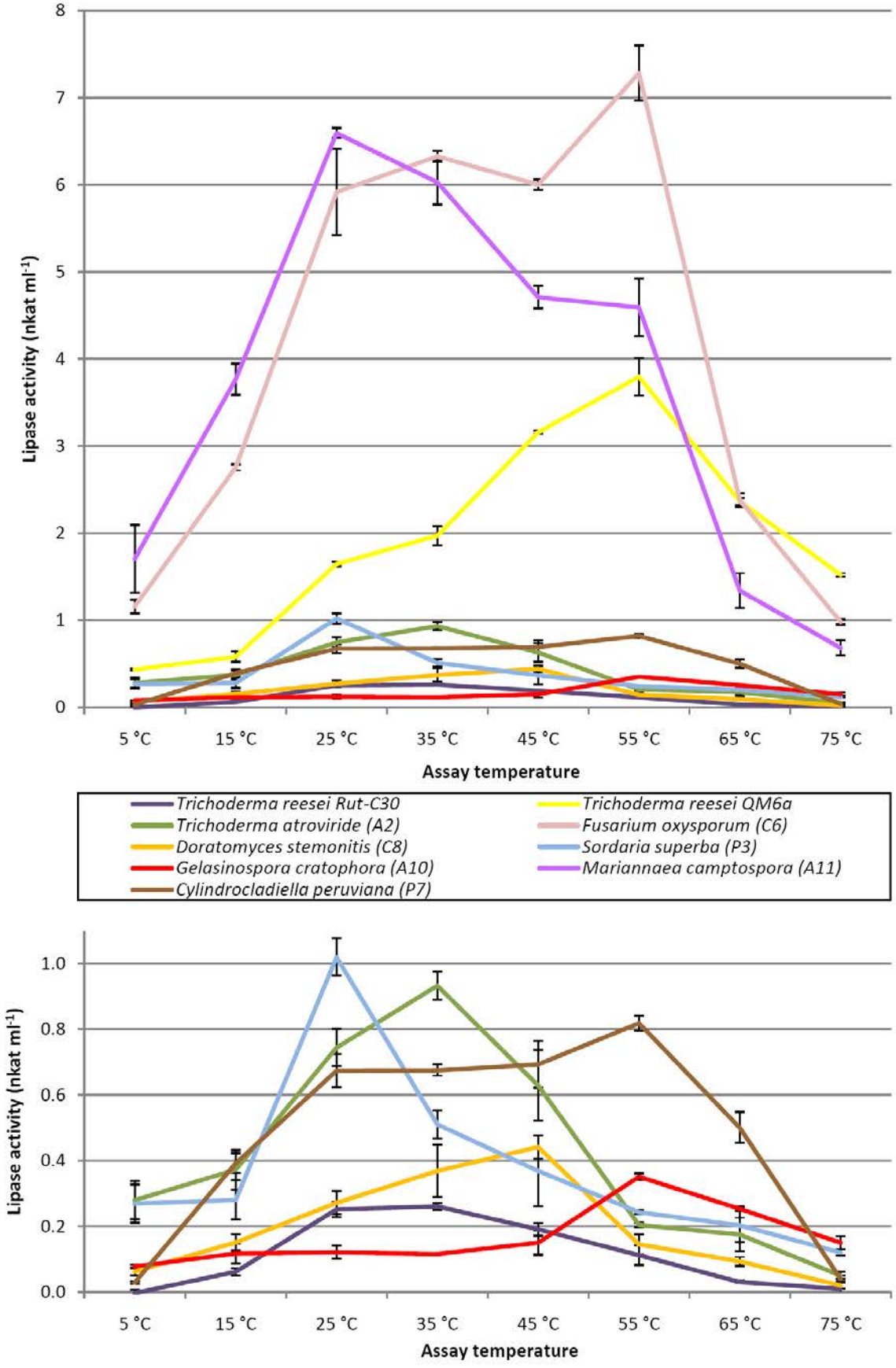
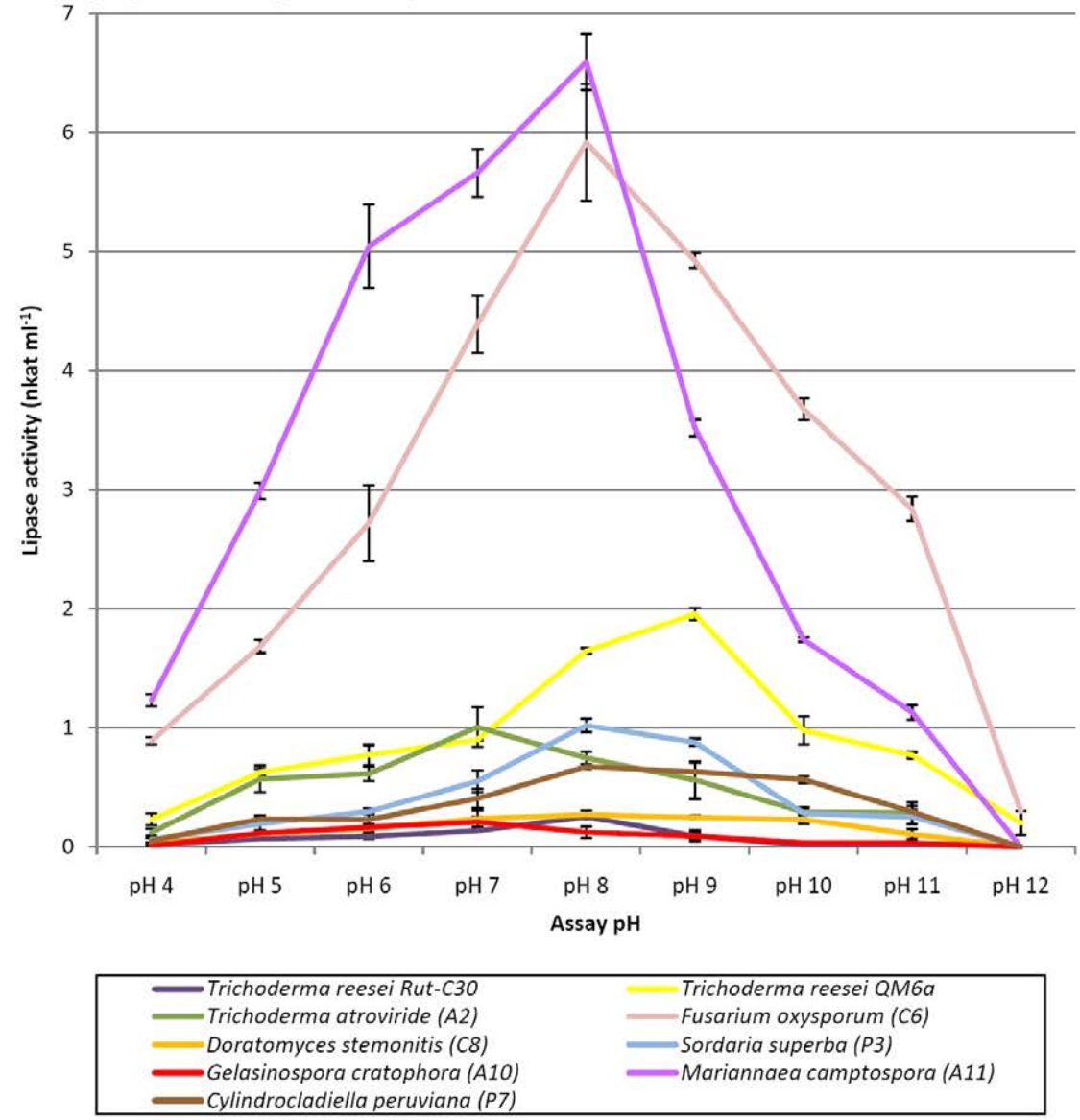


Figure S12: pH profiles of lipase activity at 25 °C



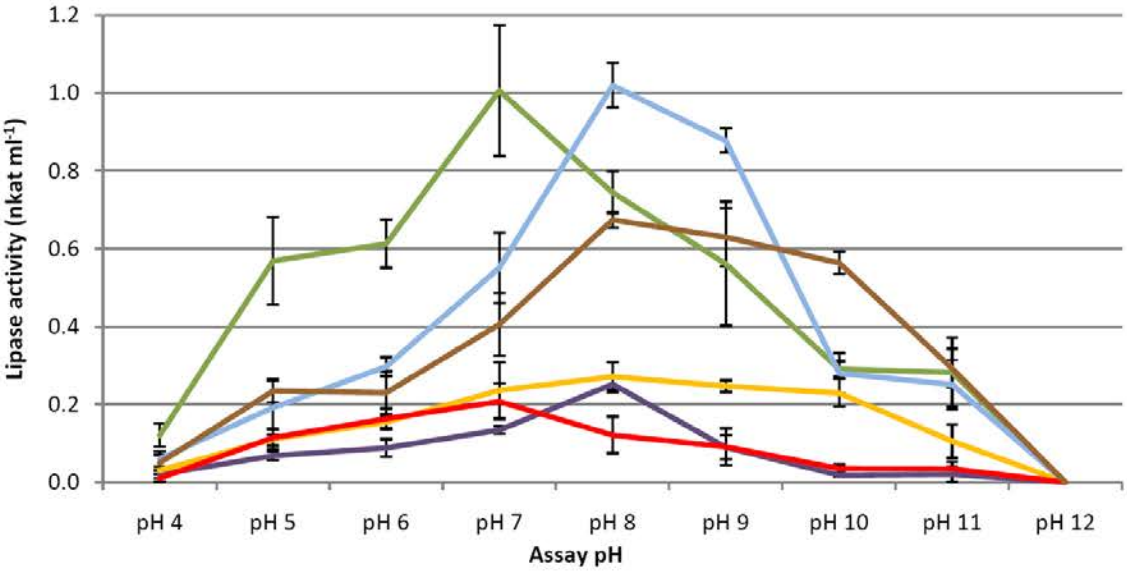


Figure S13: Temperature profiles of protease activity at pH 7.5

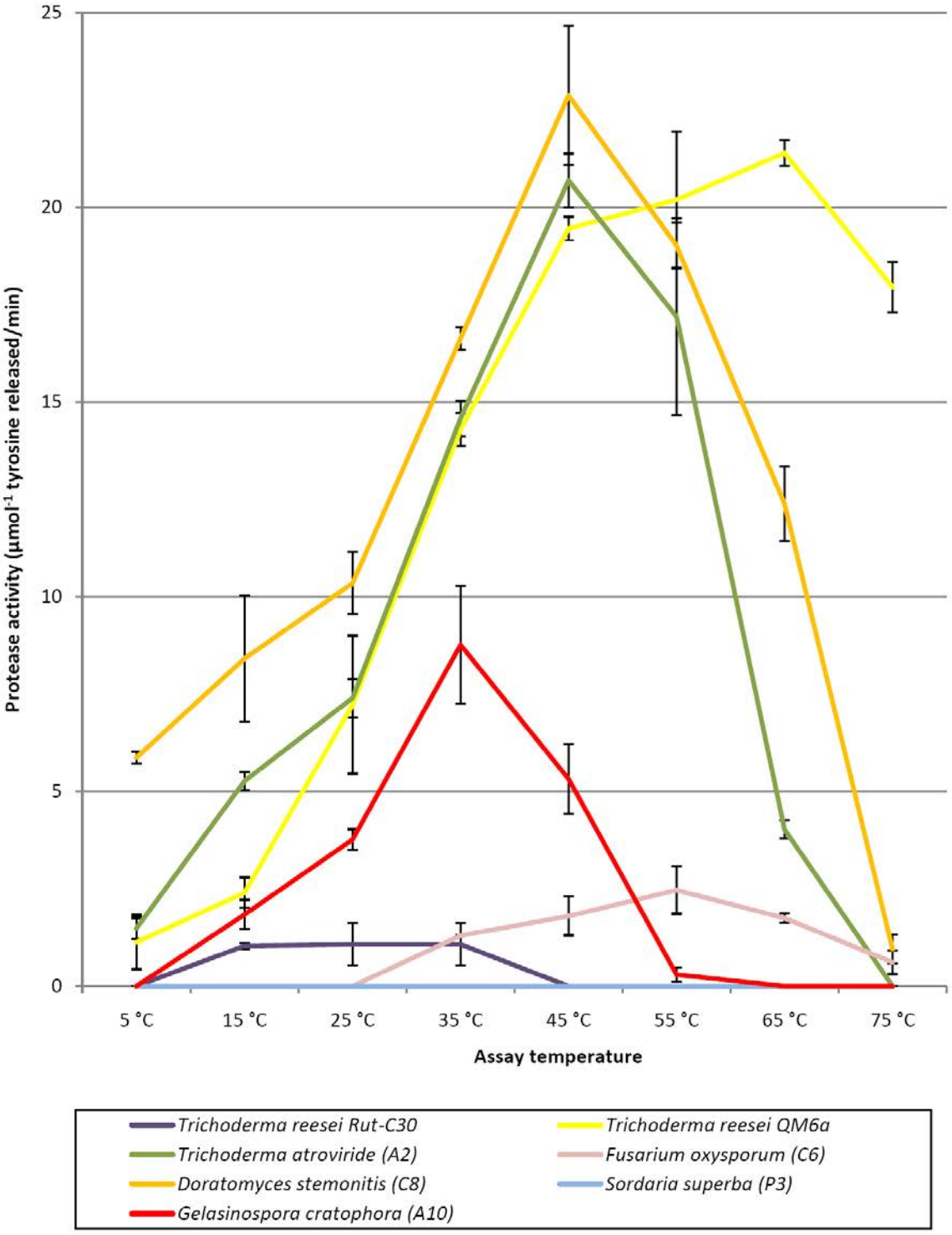


Figure S14a: pH profiles of protease activity at 45 °C (pH 3-6; haemoglobin substrate)

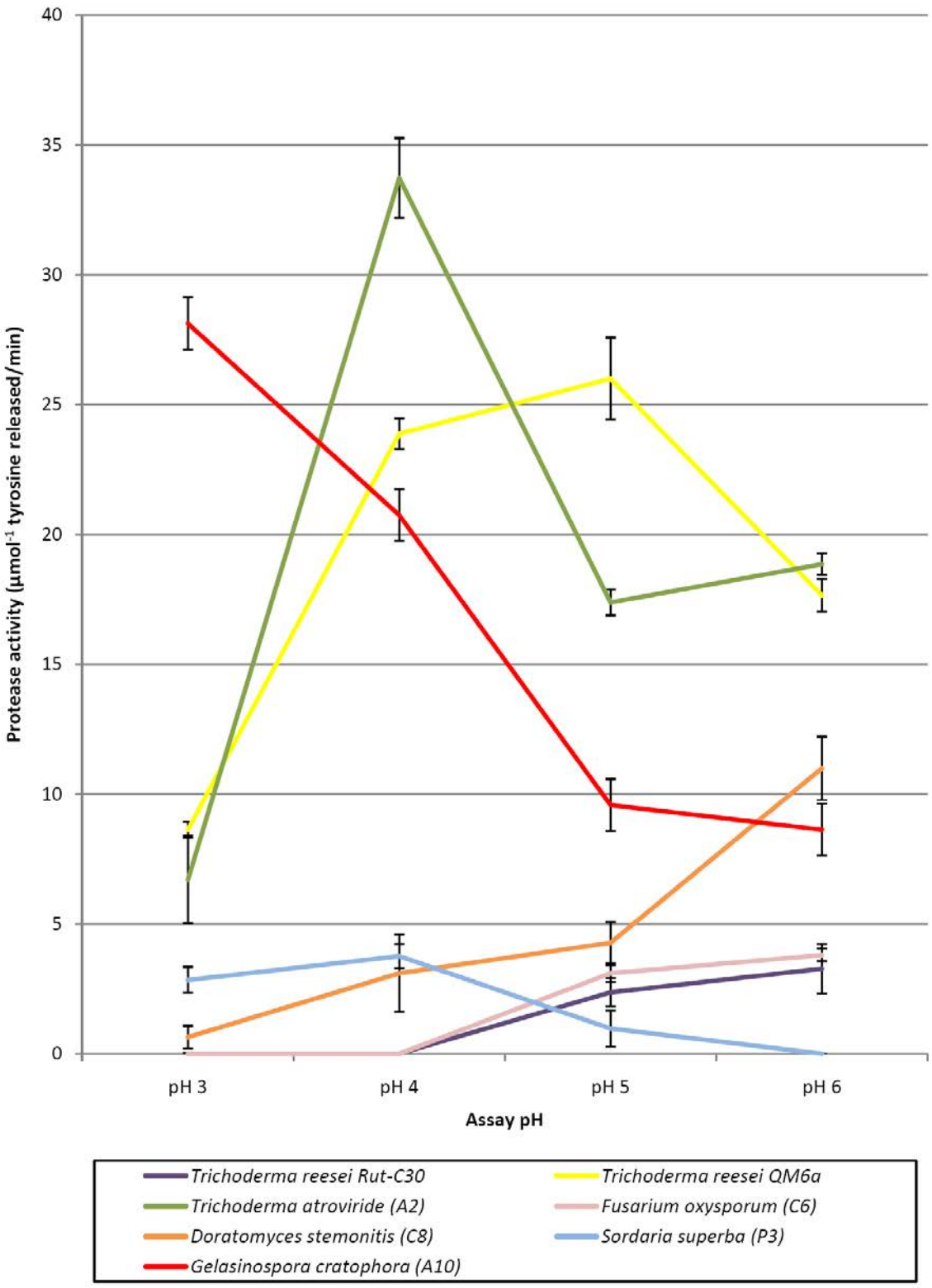


Figure S14b: pH profiles of protease activity at 45 °C (pH 5-11; casein substrate)

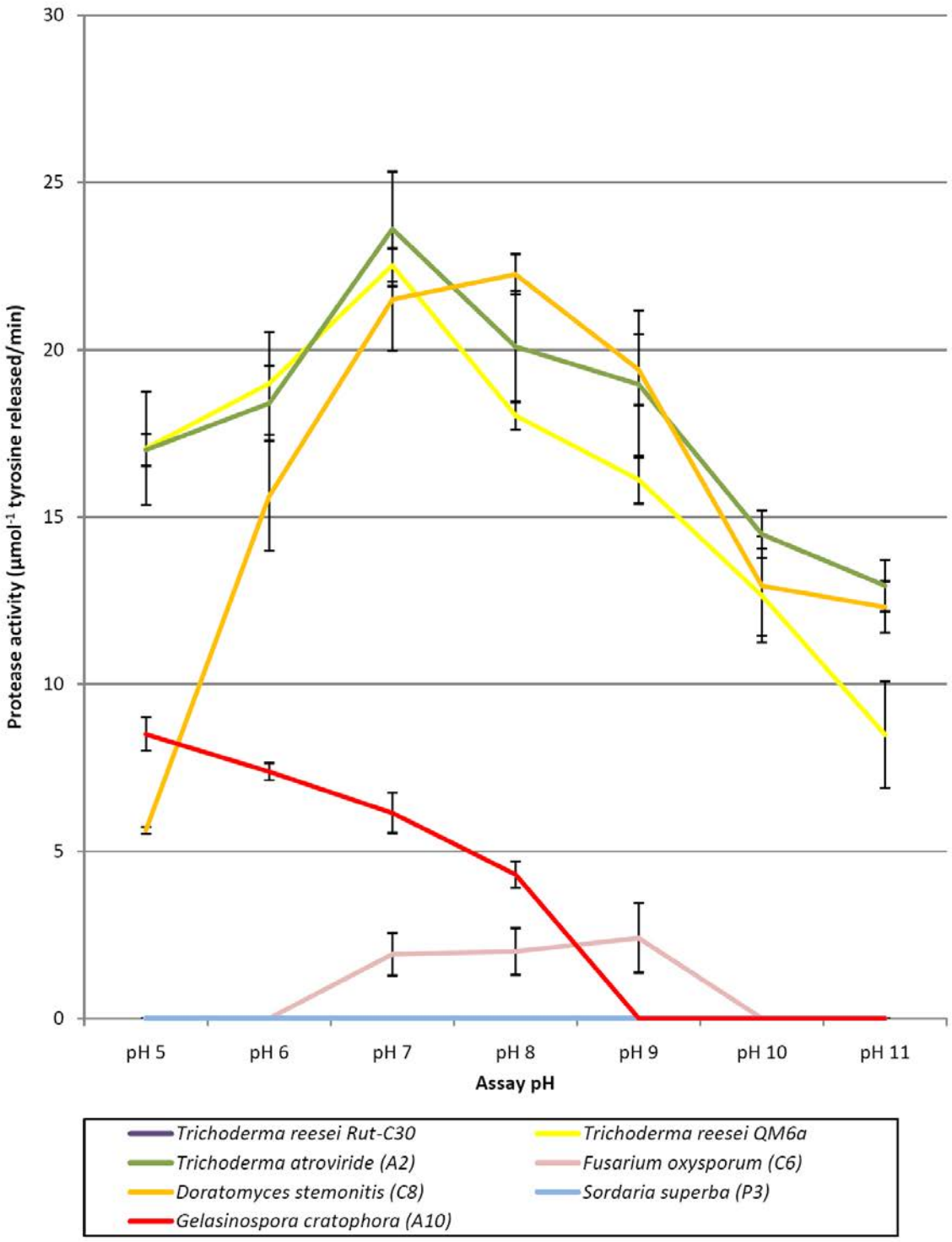


Table S1: The approximate molecular weight (kDa) of proteins displaying xylanase, mannanase, endoglucanase, β-glucosidase, amylase, lipase and protease activities on zymograms produced using the supernatants from cultures of the indicated fungal strains, as described in Materials and Methods. Zymogram bands are indicated for samples that were boiled and samples that were not boiled prior to electrophoresis.

	Approx. MW (kDa) of zymogram bands													
Enzyme activities detected*:	**Xylanase**		**Mannanase**		**Endoglucanase**		**β-glucosidase**		**Amylase**		**Lipase**		**Protease**	
Sample preparation:	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**	**Boiled**	**Not boiled**
Trichoderma reesei RUT-C30	25, 46, 47, 50-86, 95	25, 35, 40, 50-80, 95	47	100-130	26, 28, 30, 33, 34, 35, 40, 43, 45, 48, 50-120	28, 35-130	N	130	N	N	N	N	26†	27, 86
Trichoderma reesei QM6a	25	90	46	42	26, 35, 45	35, 48-80, 90-130	N	48, 130	48, 100, 110	33, 47	N	22, 25	26	45, 48, 86
Trichoderma atroviride (A2)	25, 36, 80, 84	25	46, 48, 52	28, 30, 40, 80, 88	26, 33, 35, 37, 40, 44, 46, 52, 70	26, 28, 36-130	N	80	47, 92	48, 55, 68	N	30, 32, 45, 85	26	26, 120
Gelasinospora cratophora (A10)	26, 30, 38, 42, 60, 86	26, 36, 48-120	46, 52, 55, 60, 84	75-130	27, 34, 38, 40, 46, 50, 78, 90	30, 35, 37, 45-130	78	135	80, 100, 110	65, 110, 130	N	N	N	130
Fusarium oxysporum (C6)	26, 30	40, 120	48, 60, 64	46, 130	22, 24, 26, 46-60	50-130	N	125, 135	85, 86, 105	65, 70, 120	N	32, 34	N	120
Doratomyces stemonitis (C8)	30, 45, 60	27, 33-38	33, 37, 39, 48	33, 35, 38, 44, 47	20, 34, 55	22, 29, 40	N	40, 120, 130	N	N	N	N	N	26, 34-38
Sordaria superba (P3)	65	80-86	49	120	22, 24, 32, 46, 60	26, 28, 36-47	N	70, 130	N	65	N	N	26†	60†, 130†
Cylindrocladiella peruviana (P7)	28	N	N	55, 75	N	N	N	130, 135	N	68	N	N	N	80, 120
Mariannaea camptospora (A11)	N	N	N	N	N	N	N	60, 130	N	65	N	22-28‡	N	N

* Substrates are described in Materials and Methods and Table 1.
† Bands of the indicated molecular weight were detected on the gelatin gel but not on the casein gel (see Results)
‡ No defined bands. Activity was detected as a smear across all molecular weights, concentrated more in the 22-28 kDa region
"N" indicates that no clearing bands were visible on the zymogram.
A range (eg. 50-80) indicates clearing of substrate between the given molecular weights on the gel, due to merging of individual bands or insufficient separation of proteins (particularly in samples that were not boiled).

4.3 Methods additional to those contained in Publication 2

The following sections contain methods used in the work described in Chapter 4 that are not included in Publication 2. A zymography method to detect carbohydrate esterases (enzymes that can release side chains of xylan and pectin, or linkages between hemicelluloses and lignin; Section 4.1) is presented in Section 4.3.1. Cultivation of the fungi in liquid media designed for the induction of lignin-degrading oxidases (oxidase-inducing media) is described in Section 4.3.2. A simple assay to screen for laccase (the major lignin-degrading oxidase exhibited by ascomycetous fungi; Section 4.1) is presented in Section 4.3.3.

4.3.1 Carbohydrate esterase zymogram

Following growth in the hydrolase-inducing medium (Publication 2), the supernatants from the seven selected fungi from koala faeces and *T. reesei* QM6a and RUT-30 were screened for carbohydrate esterase activity using zymography (adapted from Aurilia et al., 2000). For each fungal strain, 30 µl supernatant was combined with loading buffer (without boiling), loaded onto a 12.5 % (w/v) SDS-PAGE gel without substrate, and subjected to electrophoresis as described in Publication 2. Following electrophoresis, the gel was soaked for one hour in 2.5 % (v/v) Triton X-100, rinsed in MQ water, and incubated for 30 min at RT in Universal buffer pH 8. The gel was then soaked in a solution of 0.5 % (w/v) Fast Blue BB and 0.1 % (w/v) α-naphthyl acetate (Sigma-Aldrich, St. Louis, USA) in Universal buffer pH 8 for approximately 15 min until bands became visible. The zymogram was then fixed in 1 % (v/v) acetic acid.

4.3.2 Cultivation of fungal strains in oxidase-inducing liquid media

Attempts were made to grow each of the selected fungal strains in each of the following oxidase-inducing liquid media, with and without 0.1 % (w/v) alkali (kraft) lignin (Sigma-Aldrich):

1) 0.02 % (w/v) ammonium tartrate, 0.2 % (w/v) $MgSO_4.7H_20$, 0.01 % (w/v) $CaCl_2$, trace elements, pH 4 (Tien and Kirk, 1984).

2) Tien and Kirk (1984) medium, pH 5.5, with the addition of 0.05 % (w/v) malt extract, 0.05 % (w/v) yeast extract or 0.05 % (w/v) peptone (Arora and Gill, 2005).

3) 0.5 % (w/v) ammonimum tartrate, 0.02 % (w/v) KH_2PO_4, 0.001 % (w/v) $CaCl_2$, 0.05 % (w/v) glucose, 0.01 % (w/v) thiamine, 0.01 % (v/v) Tween 80, trace elements, pH 5.5 (adapted from Lopez et al., 2007).

4) 1 % (w/v) glucose, 0.2 % (w/v) ammonium tartrate, 0.1 % (w/v) yeast extract, 0.05 % (w/v) $MgSO_4.7H_20$, 0.05 % (w/v) KCl and 0.5 ml of mineral salts, pH 5.5 (Pelaez et al., 1995).

In each case, the fungal isolates were inoculated by mycelial plugs (4 × 0.5 cm) into 250 ml shake flasks containing 50 ml of the liquid medium. Incubation was for at least 7 days at 28 ºC on a shaker at 250 rpm and was continued for a total of 14 days for fungal cultures that appeared to be sustaining growth (samples of the supernatant were also taken at 7 days for the 14 day cultures). At the end of the incubation period the cultures were subjected to centrifugation at 4,000 × *g* for 15 min. Protein in the supernatant was quantified using the Bradford Assay (Bradford, 1976) and screening for laccase activity was carried out as described below (Section 4.3.3).

4.3.3 Assay for laccase activity

To screen for laccase activity in the fungal supernatant, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) was used as a substrate (Nagai et al., 2003). The reaction mixture contained 50 µl 2M ABTS in McIlvaine buffer (citrate/ phosphate buffer, pH 3) and 50 µl supernatant, and incubation was carried out at 25 ºC for 30 min. The reaction was stopped by adding 100 µl of 5 % trichloroacetic acid, and absorbance at 420 nm was measured on a Fluostar microplate reader (BMG, Durham, USA) and compared to a sample blank, to which TCA was added prior to the addition of ABTS. Laccase activity was demonstrated by the formation of blue/ green colour in the sample and an increase in absorbance at 420 nm.

4.4 Results and Discussion

In the following sections, the results of work not included in Publication 2 are presented and discussed (Section 4.4.1 - 4.4.2). In addition, comparisons are made between the enzyme activities displayed by the fungi in the agar plate assays and liquid assays (Section 4.4.3).

4.4.1 Carbohydrate esterase activities assessed by zymogram analysis

Carbohydrate esterases play an important role in plant cell wall degradation by removing the side chains of polysaccharides such as xylan or pectin and/or by breaking the ester linkages between the side chains and the phenolic units of lignin, thereby opening up access for hydrolase attack (Section 1.8.4; Aurilia et al., 2000). Zymography was used to detect the carbohydrate esterase activities of the fungal isolates following growth in the hydrolase-inducing liquid medium (Section 4.3.1). The zymogram technique utilised (adapted from Aurilia et al., 2000; Section 4.3.1) was sensitive to a broad range of carbohydrate esterases including acetyl xylan esterases, feruloyl esterases and methyl esterases.

The result of the zymogram analysis for carbohydrate esterase activity is shown in Figure 4.1. The protein bands exhibiting the greatest activity were from *Gelasinospora cratophora* A10, *Doratomyces stemonitis* C8 and *Sordaria superba* P3 supernatants. Fainter bands representing proteins with weaker carbohydrate esterase activity were displayed by *Fusarium oxysporum* C6, *Cylindrocladiella peruviana* P7 and *Trichoderma reesei* RUT-C30 and *T. reesei* QM6a (Fig. 4.1).

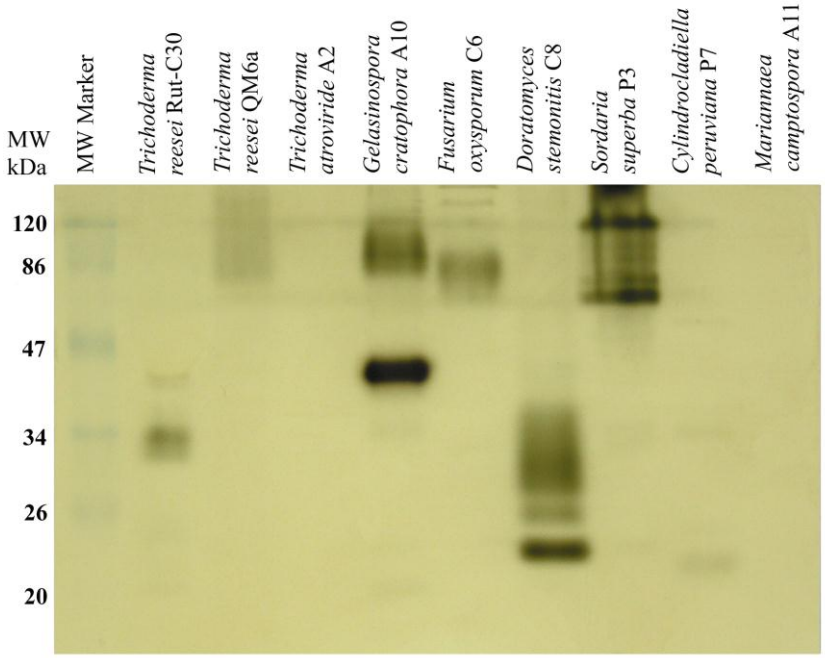


Figure 4.1: Zymogram showing carbohydrate esterase activity in the supernatants of *T. reesei* RUT-C30, *T. reesei* QM6a, and the selected fungal isolates from koala faeces (lanes 4 - 10). Lane 1: Molecular weight marker. The zymogram gel was stained with 0.5 % (w/v) Fast Blue BB and 0.1 % (w/v) α-naphthyl acetate as described in Section 4.3.1.

Carbohydrate esterases have been reported previously from *T. reesei* (Li et al., 2008), *F. oxysporum* (Christakopoulos et al., 1999; Topakas and Christakopoulos, 2004) and *Neurospora crassa* (Crepin et al., 2004), a closely related species to *G. cratophora* A10 (Garcia et al., 2004). However, the carbohydrate esterase activity of *T. atroviride*, *G. cratophora*, *D. stemonitis*, *S. superba*, *C. peruviana* or *M. camptospora* has not been investigated previously to our knowledge. Although further exploration of the carbohydrate esterase activities of the fungi isolated from koala faeces was not undertaken in this work, the zymogram analysis suggested that carbohydrate esterases could play a role in lignocellulose degradation for the coprophilous fungi from koala faeces, particularly in the strains *G. cratophora* A10, *D. stemonitis* C8 and *S. superba* P3.

4.4.2 Growth of fungi in oxidase-inducing media and screening for laccase activity

All of the seven isolates from koala faeces that were selected for liquid cultivation (Section 3.5) had displayed "ligninolytic phenoloxidase" activity (the ability to breakdown the phenolic units of lignin; Section 1.8.5) in the agar plate assays (Fig. 1, Publication 1; Fig.

3.16, Section 3.4.8.3). The phenol degradation on the lignin-agar plates could have been caused by any of the major lignin-degrading oxidases (lignin peroxidases, manganese peroxidases or laccases), which can break down both phenolic and non-phenolic units of lignin (Section 1.8.5); alternatively, the phenol degradation could have been caused by mild oxidants such as GMC oxidoreductases, which are only capable of breaking down phenolic units and not the whole lignin polymer (Section 3.4.8.1). As all of the seven selected isolates were from the phylum Ascomycota (Section 3.5), it seemed likely that the phenol degradation they had displayed in the agar plate assay was due to the secretion of mild oxidants or laccases, since peroxidase activities are rarely observed in ascomycetous species (Baldrian, 2006). However, this could not be determined on the basis of the agar plate assays alone (Section 3.4.8.3).

Further investigation of the lignin-degrading activities of the seven selected fungal isolates was clearly warranted. The hydrolase-inducing liquid medum (Publication 2) was not suitable for the induction of lignin-degrading enzymes (oxidases; Section 1.8.5) due to the presence of carbon sources (cellulose, lactose, soybean flour) that would be more readily broken down by the hydrolase activities of the fungi. Therefore, numerous attempts were made to cultivate the fungi in liquid media designed specifically for the induction of lignin-degrading oxidases (oxidase-inducing media; Section 4.3.2). However, in most of the oxidase-inducing media the fungal mycelia did not extend beyond the small PDA plugs used for inoculation and the fungi died within 2 - 4 days (only a few brown and disintegrating hyphae remaining).

The only medium that supported the growth of just three fungal strains (*G. cratophora* A10, *F. oxysporum* C6 and *D. stemonitis* C8) was medium 4, both with and without 0.1 % lignin (Section 4.3.2; Pelaez et al., 1995). Medium 4 contained a higher glucose and yeast extract content than any of the other media used, which may have supported the growth of the fungal species more in the first days of cultivation, enabling them to become strong enough to begin secreting oxidative enzymes that could access the other available substrates. However, the

small amount of mycelia produced by *F. oxysporum* C6 and *D. stemonitis* C8 began to die and disintegrate after seven days and protein secretion was not detectable. In contrast, *G. cratophora* A10 grew more strongly and after seven days had secreted 0.12 mg/ml protein in the medium containing 0.1 % (w/v) lignin, and 0.08 mg/ml in the medium without lignin. After fourteen days, total secreted protein in the supernatant of the *G. cratophora* A10 cultures, with and without lignin, had increased to 0.2 and 0.15 mg/ml respectively.

As *G. cratophora* A10 is an ascomycetous fungus it seemed more likely that the isolate would be secreting laccases or mild oxidants to survive in the oxidase-inducing liquid medium, rather than the peroxidases more typically secreted by white-rot basidiomycetous species (Shary et al., 2007). Consequently, a screening assay was used to detect laccase activity in the *G. cratophora* A10 culture supernatant (Section 4.3.3) and positive results were obtained. The highest laccase activity (detected by a deeper blue colour change and a greater increase in absorbance at 420 nm) was achieved in the medium containing 0.1 % (w/v) lignin, and an increase in laccase activity was observed from day seven to day fourteen. No laccase activity was detected in the supernatants of *F. oxysporum* C6 and *D. stemonitis* C8 cultures (measured on day 7), nor in the liquid cultures in which the other selected fungal isolates (*T. atroviride* A2, *M. camptospora* A11, *S. superba* P3, *C. peruviana* P7) had died (measured on day 4).

The inability of most of the selected fungi to grow in oxidase-inducing liquid media (Section 4.3.2) suggested that their oxidase activities alone were insufficient to access enough nutrition needed to sustain life under liquid cultivation conditions. This is not unusual amongst ascomycetous fungi, as hydrolases typically form the major part of their enzyme systems (Griffin, 1994). However, all of the selected fungi had secreted oxidases on solid media, demonstrated by the degradation of the phenolic units of lignin in the agar plate assays (Chapter 3; Fig. 3.16e). It is possible that the ability to secrete oxidases simply may not have transferred well to cultivation in liquid media, a phenomenon that has been reported previously for numerous fungal strains in past research (Maltseva et al., 1989; Téllez-Jurado

et al., 2005; Rodríguez Couto and Sanromán, 2005). In addition, many of the isolates exhibiting activity in the agar plate assay may have only secreted mild oxidants such as GMC (glucose/ methanol/ choline) oxidoreductases that can only oxidise the phenolic units of lignin, not fully degrade the lignin polymer (Lopez et al., 2007). The mild oxidants may not have enabled the fungi to access sufficient nutrition to sustain growth in the oxidase-inducing liquid media; however, the mild oxidants could play an important role in the survival of the fungi on koala faeces by opening up the lignin polymer sufficiently to increase the access of secreted hydrolases to polysaccharides in the lignocellulosic biomass (Espagne et al., 2008).

4.4.3 The value of the plate assays as a predictor of enzyme activities in the hydrolase-inducing liquid medium

Most of the hydrolase activities of the fungal isolates that were displayed in the agar plate assays (Fig 3.16) were also displayed in liquid assays carried out on the supernatants of the fungi grown in the hydrolase-inducing liquid medium (Supplementary Figures S1 - S14, Publication 2). Furthermore, the isolates that could grow and sustain hydrolase activity at high temperatures (*G. cratophora* A10, *T. atroviride* A2) or low temperatures (*F. oxysporum* C6 and *M. camptospora* A11) on the agar plate assays also displayed optimal activities of the corresponding enzymes at higher or lower temperatures, respectively, in comparison to the other isolates in the liquid enzyme assays. However, some discrepancies were also observed. *C. peruviana* P9 and *M. camptospora* A11 had displayed high xylanase, mannanase and endoglucanase activities in the plate assays (Fig. 3.16), but the two isolates grew very poorly in the hydrolase-inducing medium (Publication 2). Consequently, their poor growth also resulted in very low protein secretion and low enzyme activities in the liquid enzyme assays (with the exception of lipase activity from *M. camptospora* A11; Supplementary Fig. S11 - S12, Publication 2).

There were also discrepancies between plate assay results and liquid assay results for just one or two individual enzyme activities of the fungal isolates that did grow well in the hydrolase-inducing medium. For example, mannanase activity was not displayed by *G. cratophora* A10 in the agar plate assay (Fig 3.16b), yet the isolate was ranked third in comparison to the other fungal isolates for mannanase activity in the liquid assays (Supplementary Fig. S3 - S4, Publication 2). This may have been an indication that the mannanase activity of *G. cratophora* A10 could not be induced by locust bean gum in the agar plate medium (Publication 1) but was induced by cellulose or other substrates provided in the liquid hydrolase-inducing medium (lactose, soybean flour; Publication 2). It is also possible that the mannanase activity displayed by *G. cratophora* A10 could be caused by an endoglucanase, induced by cellulose but with mannan degrading abilities, as has been previously reported for endoglucanase II of *T. reesei* (Macarrón et al., 1996).

In contrast, *D. stemonitis* C8 displayed the greatest amylase activity of all the fungal isolates from koala faeces in the plate assays (Fig. 3.16h), but amylase activity was very low or undetectable in most of the liquid amylase assays following growth in the hydrolase-inducing medium (Supplementary Fig. S9 - S10, Publication 2). This suggested that the *D. stemonitis* C8 amylase was predominantly an inducible enzyme secreted in the presence of starch, a substrate that was included in the agar plate medium (Publication 1) but not in the hydrolase-inducing liquid medium (Publication 2). The secretion of amylases is substrate-induced in many filamentous fungi, but a low level of constitutive amylase activity is also maintained in most species (Radford, 2004).

Overall, there was only a small number of discrepancies between the hydrolase activities of the fungi in the agar plate assays and those displayed in the enzyme assays, so it could be concluded that the agar plate assay technique had generally served as a good screening tool and predictor of hydrolase activities of the fungi produced in liquid medium.

4.5 Summary and selection of a fungal isolate for secretome analysis

In the work described in Publication 2, the hydrolase activities of seven selected fungal isolates from koala faeces were comprehensively characterised following growth in a hydrolase-inducing liquid medium. Liquid assays were used to establish temperature and pH profiles of the enzymes in the culture supernatant and zymography was employed to determine the number and molecular weight of the enzymes or enzyme isoforms. Enzymes that could be of interest for development for industrial applications were revealed: heat tolerant xylanase(s), mannanase(s) and endoglucanase(s) secreted by *T. atroviride* A2 and *G. cratophora* A10; heat tolerant β-glucosidase(s) secreted by *T. atroviride* A2; cold tolerant lipase(s) secreted by *M. camptospora* A11; and xylanase(s), mannanase(s), endoglucanase(s), β-glucosidase(s) and protease(s) with neutral to alkaline pH optima, secreted by *D. stemonitis* C8 (Publication 2). The enzymes may have potential for development for industrial applications such as the production of paper, textiles, food products or detergents (Section 1.2; Demain et al., 2005). Furthermore, the isolates *G. cratophora* A10 and *T. atroviride* A2 displayed high levels of protein secretion that could make them possible candidates for future strain development by classical mutagenesis and screening or genetic engineering (Chapter 6).

The additional work described in this chapter, but not included in Publication 2, provided a broader understanding about the range of enzymes that the selected isolates could employ to degrade lignocellulosic biomass. The zymogram analysis (Section 4.3.1 and 4.4.1) revealed that most of the isolates secreted carbohydrate esterases, which could increase the access of secreted hydrolases to their target substrates by removing side chains of hemicellulose and pectin polymers, or by breaking the links between hemicellulose and lignin (Section 4.4.1). Only one isolate, *G. cratophora* A10, was able to sustain growth in a liquid medium designed for the induction of lignin-degrading oxidases. Laccase activity was detected in the culture supernatant, an enzyme that *G. cratophora* A10 could employ to degrade lignin in koala faeces.

In the work described thus far (Chapter 3; Chapter 4), the fungi from koala faeces were investigated for particular enzyme activities of interest using agar plate and liquid assays. The assays detected the activities of the main enzymes known to be involved in plant cell wall degradation (cellulases, hemicellulases and ligninases), and enzymes that can degrade protein, starch and lipids (proteases, amylases and lipases, respectively). However, the enzymes had not been identified at this stage. Furthermore, the ability of the fungi to secrete other enzymes, not yet assessed by enzyme assay, remained unknown. In order to obtain a broader understanding of the full array of enzymes that the coprophilous fungi could secrete to break down plant biomass and other substances in koala faeces, one isolate was selected for analysis of an entire secretome by mass spectrometry (Chapter 5). *D. stemonitis* C8 was selected for the secretome analysis because a wide range of enzyme activities had been exhibited by the isolate throughout the enzyme assays (Publication 1; Publication 2). Furthermore, the *D. stemonitis* C8 xylanase(s), mannanase(s), endoglucanase(s) and β-glucosidase(s) with neutral to alkaline pH optima were of special interest for industrial applications.

Chapter 5: The secretome of *Doratomyces stemonitis* C8, isolated from koala faeces

5.1 Introduction

Fungi secrete a wide range of enzymes to obtain nutrition from their environments. The secreted enzymes have evolved to work together, synergistically, to degrade the available substrates in the most efficient way possible (Webster and Weber, 2007; Section 1.1). Identifying the enzymes in a fungal secretome can reveal how the fungus can subsist in its own natural habitat. Furthermore, enzymes and enzyme combinations can be discovered that could be suitable for increasing the efficiency of industrial processes (Teter and Cherry, 2005; Section 1.9.1). Enzymes that work synergistically to degrade plant biomass have been of particular interest to industry for the production of biofuels (Herpoël-Gimbert et al., 2008; Vanden Wymelenberg et al., 2010) and for application in the textile, paper and detergent industries (Aehle, 2007).

In the work described in this chapter, mass spectrometry was employed to identify an array of proteins in the secretome of one fungal isolate from koala faeces, *Doratomyces stemonitis* C8. The work is of particular significance because it represents the first time, to our knowledge, that the secretome of a coprophilous fungus has been explored by proteomic analysis. *D. stemonitis* C8 was selected for the secretome study because a broad range of enzyme activities were displayed by the isolate in the agar plate assays (Publication 1), liquid assays and zymograms (Publication 2), indicating the secretion of numerous enzymes that could degrade plant biomass and protein in the faeces. Furthermore, *D. stemonitis* C8 secreted xylanase(s), mannanase(s), endoglucanase(s) and β-glucosidase(s) with optimal activities under neutral to alkaline conditions (Publication 2), a feature that made these enzymes of particular interest to industry. The proteomic analysis of the *D. stemonitis* C8 secretome described here resulted in the identification of enzymes likely to be responsible for activities

displayed in the enzyme assays and zymograms. In addition, other enzymes that could work synergistically to increase the efficiency of lignocellulose degradation were revealed.

Gel electrophoresis and mass spectrometry constituted the backbone of the *D. stemonitis* C8 secretome study, commonly employed techniques to obtain protein identifications from the secretomes of numerous fungal species in the past (Vinzant et al., 2001; Bouws et al., 2008; Mueller et al., 2008; Nagendran et al., 2009; Section 1.9.1). However, as the genome of *D. stemonitis* C8 has not been sequenced, identification of the *D. stemonitis* C8 proteins presented a particular challenge requiring cross-species identification (Liska and Shevchenko, 2003; Section 1.9.3.3). *De novo* sequencing (Seidler et al., 2010; Section 1.9.3.3) and manual database searches formed an integral part of the analysis, and zymography and enzyme assay provided valuable support for the identifications made. Furthermore, a new technique was developed to identify proteins from the *D. stemonitis* C8 supernatant directly from bands exhibiting enzyme activity in zymograms. The method is described in Publication 3 (Section 5.2) and was subsequently employed as part of the full analysis of *D. stemonitis* C8 secretome, described in Publication 4 (Section 5.3). Further discussion about the secretome of *D. stemonitis* C8 is presented in Section 5.4, including comparisons between the secretome of *D. stemonitis* C8 and the secretomes of other fungal species that have been analysed in previous research.

5.2 Publication 3: Peterson, R., Grinyer, J., Joss, J., Khan, A., Nevalainen, H., 2009. Fungal proteins with mannanase activity identified directly from a Congo Red stained zymogram by mass spectrometry. Journal of Microbiological Methods. 79, 374-377.

The following publication contains a description of a method which employs mass spectrometry to identify proteins directly from bands excised from zymogram gels. The effect of zymogram substrates or stains on mass spectrometric analysis had not been described previously in any published work, to our knowledge. Therefore, Publication 3 contains valuable information for future projects involving the identification of proteins with enzyme activity. *D. stemonitis* C8 was included in the work described in Publication 3 and consequently three proteins were identified from the *D. stemonitis* C8 secretome. Moreover, the technique was subsequently used as part of the full analysis of the *D. stemonitis* C8 secretome, described in Publication 4 (Section 5.3).

All of the listed authors were involved in the conception and development of the method described in Publication 3. Most of the laboratory work was carried out by myself, with the exception of the MALDI TOF/TOF MS/MS, which was conducted by the Australian Proteomics Analysis Facility (APAF). I analysed the mass spectrometry data using the search engine Mascot, and *de novo* sequencing was carried out by Janice Joss (APAF). The manuscript was written by myself, under the guidance and correction of the other authors.

Due to copyright reasons, the selected article has been omitted from this thesis (the article appears from page 152-155):

Peterson, R., Grinyer, J., Joss, J., Khan, A., Nevalainen, H., 2009. Fungal proteins with mannanase activity identified directly from a Congo Red stained zymogram by mass spectrometry. Journal of Microbiological Methods. 79, 374-377

The work described in Publication 3 established that mass spectrometry could be used to identify proteins directly from bands excised from mannanase zymogram gels. To our knowledge, this was the first time in a published work in which the substrate locust bean gum or the stain Congo Red were shown not to interfere with mass spectrometric analysis. Whether mass spectrometry could also be used to identify proteins from zymograms specific for other enzyme activities, using other substrates and stains, was explored further during the full secretome analysis of *D. stemonitis* C8 described in Publication 4 (Section 5.3).

Another outcome of the method development work (Publication 3) was the identification of three proteins in the secretome of *D. stemonitis* C8 (Table 1, Publication 3):

- a cellobiohydrolase (band 7 from the SDS-PAGE gel, Fig. 1, Table 1)
- a glycosyl hydrolase family 10 protein (band 11 from the zymogram, Fig. 1, Table 1)
- an endo-1,4-β-mannosidase (band 14 from the zymogram, Fig. 1, Table 1)

The only one of the above proteins that was likely to have mannanase activity was the endo-1,4-β-mannosidase (also known as mannan endo-1,4-β-mannosidase or endo-1,4-β-mannanase; Section 1.8.2). Cellobiohydrolases are not known to have mannanase activity and proteins from glycosyl hydrolase family 10 are predominantly xylanases (Henrissat, 1991; www.cazy.org). It seemed that the cellobiohydrolase and GH10 protein had a similar molecular weight to unidentified mannanases and were therefore present in the zymogram gel slices that were subjected to mass spectrometric analysis. Furthermore, it appeared likely that the cellobiohydrolase and GH10 xylanase were relatively abundant proteins in the *D. stemonitis* C8 secretome, hence masking the presence of the mannanases by dominating the MALDI TOF/TOF MS/MS spectra. These possibilities were explored further during the full secretome analysis of *D. stemonitis* C8, described in Publication 4 (Section 5.3). In addition, as part of the full secretome analysis, further attempts were made to identify the *D. stemonitis* C8 mannanases using a 2D mannanase zymogram and quadropole time-of-flight liquid chromatography tandem mass spectrometry (Q-TOF LC MS/MS; Publication 4).

5.3 Publication 4: Peterson R, Grinyer J, Nevalainen H. 2011. Secretome of the coprophilous fungus *Doratomyces stemonitis* C8, isolated from koala faeces. Applied and Environmental Microbiology. 77, 3793-3801.

In the work described in Publication 4, proteins in the secretome of *D. stemonitis* C8 were identified using gel electrophoresis, zymography and mass spectrometry. The laboratory work described in the manuscript was carried out by myself, with the advice and practical assistance of Jasmine Grinyer. The MALDI TOF/TOF MS/MS and Q-TOF LC MS/MS, was carried out by the Australian Proteome Analysis Facility (APAF). All data analysis (Mascot-based searches and *de novo* sequencing) and manuscript preparation was carried out by myself under the guidance and correction of the other listed authors.

A printed version of the supplementary material, which is accessible online with Publication 4, is provided in Appendix 1. The supplementary material consists of the following:

1) Figure S1 is an image of the 2D gel produced from the electrophoresis of protein in the *D. stemonitis* C8 supernatant. The image is the same as Fig. 1 in Publication 4 except that the spots that were excised and subjected to mass spectrometric analysis are numbered from 1 – 120. The protein spots are referred to by their numbers in the supplementary peptide tables (Tables S1 – S2).

2) Tables S1 - S4 contain the details of the peptide sequences that were derived by mass spectrometric analysis of proteins in the 2D gel, 1D gel and 2D mannanase zymogram produced from the *D. stemonitis* C8 supernatant (Table S1 MALDI TOF/TOF MS/MS data from 2D gel; Table S2 Q-TOF LC MS/MS data from 2D gel; Table S3: Q-TOF LC MS/MS data from 1D gel; Table S4: Q-TOF LC MS/MS data from 2D mannanase zymogram).

Due to copyright reasons, the selected article has been omitted from this thesis (the article appears from page 158-166):

Peterson R, Grinyer J, Nevalainen H. 2011. Secretome of the coprophilous fungus Doratomyces stemonitis C8, isolated from koala faeces. Applied and Environmental Microbiology. 77, 3793-3801

5.4 Further discussion of the work described in Publication 4

In the following sections, the work described in the Publication 4 is discussed in greater depth. In Section 5.4.1, the enzymes that were identified in the *D. stemonitis* C8 secretome (Table 1, Publication 4) are discussed in relation to the enzyme activities *D. stemonitis* C8 had displayed in the agar plate assays (Publication 1) and liquid enzyme assays (Publication 2). The value of each of the techniques used in the proteomic analysis of the *D. stemonitis* C8 secretome is assessed in Section 5.4.2. The secretome of *D. stemonitis* C8 is compared to the secretomes of other fungal species in Section 5.4.3, and the potential of the identified *D. stemonitis* C8 enzymes for industrial application is discussed in Section 5.4.4.

5.4.1 Proteins identified in the *D. stemonitis* C8 secretome and their relationship to assayed enzyme activities

Many of the proteins that were identified in the *D. stemonitis* C8 secretome using mass spectrometry (Table 1, Publication 4) could be directly related to enzyme activities that *D. stemonitis* C8 had displayed in the agar plate assays (Publication 1) and liquid enzyme assays (Publication 2). The identified GH7 cellobiohydrolases, GH5 and GH74 endoglucanases, GH3 β-glucosidases, GH10 endo-1,4-β-xylanases, and GH5 endo-1,4-β-mannanases (mannan endo-1,4-β-mannosidases; Table 1, Publication 4) are likely to be responsible for the displayed cellobiohydrolase, endoglucanase, β-glucosidase, xylanase and mannanase activities of *D. stemonitis* C8, respectively (Fig. 1, Publication 1; Supplementary Fig. S1 - S8, Publication 2). Furthermore, the identified M36 metalloprotease, S8 subtilisin protease and aminopeptidase Y-like proteases (Table 1, Publication 4) are probably responsible for the displayed protease activity of *D. stemonitis* C8 (Fig. 1, Publication 1; Supplementary Fig. S13 - 14, Publication 2). In addition, numerous GMC (Glucose- Methanol- Choline) oxidoreductases, enzymes that modify the phenolic units of lignin, were identified in the *D. stemonitis* C8 secretome. The GMC oxidoreductases could be responsible for the phenol degradation displayed on the lignin agar plates by *D. stemonitis* C8 (Fig. 1, Publication 1).

5.4.2 Evaluation of the methods used to identify proteins in the *D. stemonitis* C8 secretome

Numerous techniques were employed to identify proteins in the secretome of *D. stemonitis* C8. The outcomes achieved from each of the techniques provide valuable information for future secretome studies, particularly for microorganisms without sequenced genomes. Since the genome of *D. stemonitis* has not been sequenced, identification of proteins in the *D. stemonitis* C8 secretome required cross-species identification (Section 1.9.3.3), based on similarity to proteins from other fungal species in the NCBI database. The amino acid sequences of proteins can differ slightly between different fungal species (Liska and Shevchenko, 2003). Therefore, it was often the case that only one unique peptide match was made between a *D. stemonitis* C8 protein and a protein in the database (Table 1, Publication 4). The results of the enzyme assays (Publication 1; Publication 2) and zymograms (Fig. 2, Publication 2; Fig. 2, Publication 4) therefore became extremely valuable in supporting some of the identifications made. In many cases, zymogram bands and spots could be aligned with identified enzymes from a protein band or spot of a similar molecular weight on the 1D SDS-PAGE gel (Supplementary Table S3, Publication 4, Appendix 1) or 2D gel (Fig. 1, Publication 4).

Two forms of mass spectrometric analysis were used to identify proteins in the *D. stemonitis* C8 secretome: MALDI TOF/TOF MS/MS and Q-TOF LC MS/MS (Section 1.9.3.2). MALDI TOF/TOF MS/MS is a cheaper and faster analysis technique and was used to analyse the 120 protein spots on the 2D gel. However, only 10 % of the protein spots could be identified using this method. The increased number and resolution of mass spectra generated by Q-TOF LC MS/MS (Section 1.9.3.2) facilitated a greater number of peptide matches and hence more protein identifications were made from the 1D and 2D gels (Table 1, Publication 4). Nevertheless, as Q-TOF LC MS/MS process is a more time consuming and expensive form of

analysis it was best suited to the smaller number of samples generated from bands on the 1D gel or selected spots on the 2D gel.

De novo sequencing (Section 1.9.3.3) was an effective way of obtaining further protein identifications from the *D. stemonitis* C8 secretome based on similarities between *D. stemonitis* C8 peptide sequences (derived directly from the tandem mass spectra) and proteins in the NCBI database. However, as currently available *de novo* sequencing software programs such as Mascot Distiller only generate several possible amino acid sequences from the mass spectra, manual BLAST searching of the candidate *de novo* peptides against the NCBI database was still required. This involved visual assessment of over 8,000 spectra to select those spectra that were of good quality (clear peaks, minimal background noise), personal evaluation of each of the *de novo* solutions generated by Mascot Distiller, and manual BLAST searching of over 5,000 selected *de novo* solutions against the NCBI database. In real terms, over 400 hours were spent on the *de novo* sequencing process. Nevertheless, the results were substantial, as described below.

Protein identifications that could not be obtained using the search engine Mascot were achieved on the basis of two *de novo* peptides matching a protein in the database (Expect < 0.001; Table 1, Publication 4). In addition, over one hundred additional *de novo* peptides from *D. stemonitis* C8 proteins were found to bear similarity to amino acid sequences from fungal enzymes in the NCBI database (Expect > 0.001; Supplementary Tables S1- S4, Publication 4, Appendix 1), which had also been overlooked by automated Mascot searches. Some of the *de novo* peptides matched to enzymes of the same type as those identified by the Mascot searches but from different fungal species (for example, GH7 cellobiohydrolases, GH10 xylanases). Other *de novo* peptide matches revealed enzyme types not previously identified in the *D. stemonitis* C8 secretome, including GH45 endoglucanases, GH11 xylanases, Cip1 proteins, GH6 cellobiohydrolases (cellobiohydrolase II), α-mannosidases and polygalacturonases. All of these enzymes could be intrinsically involved in plant biomass

degradation. This work has validated the importance of *de novo* sequencing to reveal identities of proteins in the secretomes of novel organisms. The development of more advanced software programs, involving automated comparisons of *de novo* solutions to protein sequences in databases, may simplify and speed the process of *de novo* sequencing in the future, thus making *de novo* sequencing a more viable option for large scale, high throughput projects.

Attempts to use Q-TOF LC MS/MS to identify the *D. stemonitis* C8 enzymes directly from bands excised from the 1D zymogram gels (Fig. 2, Publication 4) were not very successful. The substrates and stains used in the xylanase, mannanase, endoglucanase and β-glucosidase zymograms (Table 1, Publication 2) did not appear to interfere with the mass spectrometric analysis. However, the only proteins that could be identified from the 1D zymogram bands were those that were most abundant at that molecular weight, namely the GH10 xylanase (gi|116179352) or GH7 cellobiohydrolases (gi|39971383, gi|4204214; Table 1, Publication 4). Peptides from the highly abundant proteins monopolised the mass spectra, the same phenomenon that had occurred when using MALDI-TOF/TOF MS/MS to identify proteins in the 1D mannanase zymogram bands (Publication 3; Section 5.2). It was hoped that the extra protein separation step provided in liquid chromatography (LC) and the greater number and resolution of the mass spectra produced by Q-TOF MS/MS analysis would reveal the identity of the target enzymes. Unfortunately this was not the outcome. However, combining the separating powers of 2D electrophoresis with zymography in the 2D mannanase zymogram, and subjecting the zymogram spots to Q-TOF LC MS/MS finally lead to the desired result and the *D. stemonitis* C8 mannanase could be confidently identified (Table 1, Publication 4).

5.4.3 Comparisons between the secretome of *D. stemonitis* C8 and the secretomes of other fungal species

This work has entailed the first proteomic analysis of the secretome of a coprophilous fungus reported to date, to my knowledge. Comparing the *D. stemonitis* C8 secretome to the secretomes of other fungi that have been explored in past research can begin to broaden understanding about how coprophilous fungi are adapted to their specific niche.

The secretome of *T. reesei* has been studied extensively over several decades (Section 1.9.2) due to the interest that hypercellulolytic and high secreting strains of the species hold for the industrial production of enzymes. The secretome of *D. stemonitis* C8 had some similarities to the secretome of the hypercellulolytic *T. reesei* RUT-C30, which has been studied recently by mass spectrometric analysis (Herpoël-Gimbert et al., 2008; Nagandren et al., 2009; Table 5, Section 1.9.2). The *T. reesei* RUT-C30 secretome was dominated by cellobiohydrolase I (GH7) and cellobiohydrolase II (GH6) when grown in cellulase-inducing medium (Herpoël-Gimbert et al., 2008; Nagandren et al., 2009; Section 1.9.2). When grown in the hydrolase-inducing medium in this work, the *D. stemonitis* C8 secretome also contained a high proportion of cellobiohydrolase I (GH7 cellobiohydrolase), identified from two of the largest spots on the 2D gel (Fig. 1, Publication 4), and *de novo* sequencing revealed that protein from another large spot on the 2D gel (spot 25, Supplementary Fig. S1, Publication 4, Appendix 1) had some similarity to cellobiohydrolase II (GH6; Spot 25, Supplementary Table S2, Publication 4, Appendix 1). Furthermore, both the *D. stemonitis* C8 and the *T. reesei* RUT-C30 secretomes contained GH5 and GH74 endoglucanases, GH10 endo-1,4-β-xylanases, GH5 endo-1,4-β-mannanases (mannan endo-1,4-β-mannosidases) and GH62 α-L-arabinofuranosidases to degrade cellulose and hemicellulose polymers (Table 1, Publication 4; Table 5, Section 1.9.2).

One feature of the *D. stemonitis* C8 secretome that distinguished it from the secretome of *T. reesei* was the presence of numerous enzymes involved in the degradation of pectin (Table 1,

Publication 4). The genome of the wild-type *T. reesei* QM6a (Martinez et al., 2008) contains very few genes encoding pectin degrading enzymes. This reflects the low levels of pectin contained in the natural habitat of *T. reesei*, consisting of dead and decaying wood and plant biomass (Jennings, 1995). In comparison, the greater degree of specialisation of the *D. stemonitis* C8 secretome for the degradation of pectin is well suited to the comparatively high levels of pectin in the koala faeces, originating from the koala's diet of Eucalyptus leaves (Voragen et al., 2009). Numerous pectin degrading enzymes have also been found in the secretomes of plant pathogenic fungi, such as the *Aspergillus* species, which also use plant foliage as a nutrient source (de Vries and Visser, 2001).

A GH10 endo-1,4-β-xylanase was one of the most abundant enzymes in the *D. stemonitis* C8 secretome, identified from a large spot on the 2D gel (pI 6.5 - 7; Fig. 1, Publication 1) and dominating a good proportion of the MS/MS spectra (Table 1, Publication 4; Supplementary Tables S1 - S4, Publication 4, Appendix 1). Xylanases typically constitute a small proportion of the secretomes of saprophytic fungi. For example, xylanases have only been identified from small spots on 2D gels produced from the culture supernatants of *T. reesei* strains following growth in cellulase-inducing media (Vinzant et al., 2001; Herpoël-Gimbert et al., 2008). However, the increased specialisation of the *D. stemonitis* C8 secretome to xylan degradation is also a feature of the predicted secretome of *Podospora anserina*, the only coprophilous fungus with a sequenced genome (Espagne et al., 2008). It has been speculated that *P. anserina* could have increased access to xylan in herbivore faeces due to the high number of genes in the *P. anserina* genome encoding carbohydrate esterases, which could release the xylan cross-linked to lignin by 4-O-methyl-glucuronic acid or ferulic acid (Espagne et al., 2008). Similarly, the *D. stemonitis* C8 secretome also contained carbohydrate esterases, as demonstrated in the carbohydrate esterase zymogram (Fig 4.1, Section 4.4.1) and by the identification of 4-O-methyl-glucuronoyl-methylesterase (Table 1, Publication 4).

Several proteases were identified in the secretome of *D. stemonitis* C8 (subtilisin/ proteinase K-like proteases, metalloproteases and aminopeptidase Y, ScAPY like proteins, Table 1, Publication 4), and high protease activity was displayed in the *D. stemonitis* C8 supernatant in the assays and zymograms (Publication 2). Proteases have been identified in the secretomes of many fungal species in the past, including saprophytic (Oda et al., 2006; Sato et al., 2007; Tsang et al., 2009), plant pathogenic (Mueller et al., 2008; Paper et al., 2007; Shah et al., 2009) and human pathogenic species (Monod et al., 2002; Karkowska-Kuleta et al., 2009). The high protease activity in the *D. stemonitis* C8 secretome could enable the coprophilous fungus to gain nutrition from protein in the koala faeces, which could be in the form of sloughed epithelial cells, fur or other microbial inhabitants (Osawa, 1991).

5.4.4 The identification of enzymes in the secretome of *D. stemonitis* C8 with potential for industrial application

Enzymes identified by mass spectrometric analysis of the *D. stemonitis* C8 secretome (Publication 4) may have potential for industrial applications. Some of the enzymes could be valuable supplements to the enzymes secreted by industrially-established fungi, such as *T. reesei*, to increase the efficiency of plant biomass degradation for the production of ethanol-based biofuels (Teter and Cherry, 2005). For example, the *D. stemonitis* C8 pectin esterase, pectate lyase, α-L-rhamnosidase and/or rhamnogalacturonan lyase (Table 1, Publication 4) could be added to *T. reesei* enzyme cocktails to increase pectin degrading ability (Pain and Hertz-Fowler, 2008). Furthermore, *D. stemonitis* C8 cellobiohydrolases, endoglucanases, β-glucosidases, xylanases, β-xylosidases, and mannanases (Table 1, Publication 4) could increase the efficiency of enzymatic lignocellulose degradation to fermentable sugars. For example, a GH5 endo-1,4-β-mannanase was identified in the *D. stemonitis* C8 secretome that had significant similarity to a GH5 endo-1,4-β-mannanase of the coprophilous fungus *Podospora anserina* (Table 1, Publication 4). The *P. anserina* GH5 endo-1,4-β-mannanase has recently been used as a supplement to enzymes secreted by

T. reesei strain CL847, improving the release of total sugars from lignocellulose by 28 %, and of glucose by 18 % (Couturier et al., 2011). In addition, enzymes were identified in the *D. stemonitis* C8 secretome that could increase the rate of lignocellulose degradation by releasing cellulose and hemicellulose from lignin (GMC oxidoreductases, 4-O-methyl-glucuronoyl-methylesterase; Table 1, Publication 4).

The xylanases of *D. stemonitis* C8 were of particular interest as they had displayed optimal activity in alkaline conditions (pH 8; Fig. 1a, Publication 2), a feature that could potentially make the *D. stemonitis* C8 xylanases suitable for industrial applications (Demain et al., 2005). Mass spectrometric analysis resulted in confident identification of the xylanase of *D. stemonitis* C8, with four unique peptides found to have significant similarity to a GH10 endo-1,4-β-xylanase of *Chaetomium globosum* (Table 1, Publication 4). Xylanases with activities in the neutral to alkaline range have been identified from *Chaetomium* species previously and found suitable for kraft bleaching for paper manufacture (Mäntylä et al., 2007). The *D. stemonitis* C8 GH10 xylanase could thus have potential for similar purposes.

Endoglucanases from the *D. stemonitis* C8 secretome displayed optimal activity at pH 7 (Fig. 1e, Publication 2). Neutral endoglucanases are highly sought after by the textile industry for bio-stoning and fabric softening because they cause less back staining and damage to the integrity of the fibre than the acidic endoglucanases currently used (Li et al., 2011). A GH5 endoglucanase and a GH74 endoglucanase were identified in the *D. stemonitis* C8 secretome that could be possible candidates for this application. Furthermore, *de novo* sequencing resulted in the possible identification of a low molecular weight GH45 endoglucanase in the *D. stemonitis* C8 secretome (Table S2, Appendix 1). Low molecular weight endoglucanases are particularly valued in the textile and biofuel industries for their ability to break up crystalline areas of cellulose polymers (Himmel et al., 2010).

Three kinds of proteases were identified in the *D. stemonitis* C8 secretome C8 (a subtilisin/ proteinase K-like protease, a metalloprotease and aminopeptidase Y/PA_ScAPY like proteins, Table 1, Publication 4). The subtilisin/ proteinase K-like protease could be similar to the Proteinase-K like keratinase of a related species *Doratomyces microsporus*, which has been found suitable for application in the leather industry (Friedrich and Kern, 2003). The metalloprotease might be of interest to the pharmaceutical, brewing or baking industries (Mansfeld, 2007), and the aminopeptidases may hold potential for the manufacture of protein hydrolysates to improve the flavour and nutritional value of soy, meat and milk products (Sanz, 2007).

5.5 Summary

In the work described in this chapter, the secretome of the coprophilous fungus *Doratomyces stemonitis* C8 from koala faeces was studied using mass spectrometry. As the genome of *D. stemonitis* has not been sequenced, protein identification in the *D. stemonitis* C8 secretome required cross-species identification and *de novo* sequencing. Enzyme assay and zymography provided further support for the protein identifications made. In addition, a new technique was developed to identify enzymes in the *D. stemonitis* C8 secretome directly from zymogram gels by mass spectrometry (Publication 3).

A broad array of enzymes involved in the degradation of plant biomass and protein were revealed in the *D. stemonitis* C8 secretome, dominated by GH7 cellobiohydrolase and GH10 endo-1,4-β-xylanase (Publication 4). In addition, numerous enzymes involved in the degradation of pectin were identified, distinguishing the *D. stemonitis* C8 secretome from the secretomes of some industrially exploited fungi such as *T. reesei*. As the first secretome analysis of a coprophilous fungus reported to date, the work has revealed new information about the range of enzymes coprophilous fungi could employ to survive on faeces. Furthermore, enzymes were identified that could have potential for industrial applications.

Chapter 6: Main findings and future prospects

A little tapped resource in unique natural niche has been explored in this work. As part of advancing knowledge about a community of coprophilous fungi, novel enzymes with potential for industrial applications were revealed. The significance of each of the main findings is discussed in this chapter and suggestions are made for future work.

6.1 Main findings

The following main outcomes have been attained:

1) **Broadening of knowledge about a community of coprophilous fungi.** In the past, very little research had been carried out on the coprophilous fungi from koala faeces, and the studies reported have only involved the morphological identification of species (Cribb, 1997; Bell, 2005). In the current work, thirty-seven fungal isolates from koala faeces were identified using ITS sequencing (Publication 1; Section 3.4.5), representing the first time worldwide that any community of coprophilous fungi has been identified by molecular means (Herrera et al., 2011). The ITS sequences now form the only Popset (population data study set) from coprophilous fungi in the NCBI database (Section 3.4.5) and serve as a good starting point for studying the evolutionary relatedness of coprophilous fungi in the future. Furthermore, two fungal isolates from koala faeces had significantly different ITS sequences to those of any other fungi in the NCBI database, suggesting the discovery of two strains of a new species of *Mucoraceae* (Section 3.4.7).

2) **Discovery that fungi from koala faeces secrete a diverse range of enzymes.** The agar plate assays (Publication 1; Section 3.4.8.3) revealed a diversity of enzymes secreted by the fungal isolates, providing new insight into how coprophilous fungi might gain nutrition and subsist on herbivore faeces. The hypothesis that fungi from koala faeces would secrete enzymes that break down plant biomass was realised as approximately 70 % of the fungal isolates secreted enzymes involved in cellulose, hemicellulose and lignin degradation (Table

1, Publication 1). In addition, documented evidence was generated for the nutritional theory of coprophilous fungal succession (Section 3.4.8.3); the first fungal colonisers of the faeces only secreted proteases, breaking down the easily degraded protein in the faeces, and the later colonisers secreted enzymes to break down the more recalcitrant plant cell wall polymers.

3) **Characterisation of novel enzymes secreted by fungi from koala faeces.** Liquid enzyme assays and zymogram gels (Publication 2; Section 4.2) were used to establish the activity profiles of the novel enzymes and reveal attributes that could be of interest for industrial applications. Two fungal isolates from koala faeces, *Gelasinospora cratophora* A10 and *Trichoderma atroviride* A2, were particularly good secretors of protein, including heat tolerant hemicellulases, endoglucanases and β-glucosidases, making the isolates possible candidates for strain development by classical mutagenesis and screening or genetic engineering (Section 6.2.1). Xylanase(s), mannanase(s), endoglucanase(s) and β-glucosidase(s) with optimal activities at neutral to alkaline pH were secreted by *Doratomyces stemonitis* C8, a feature that would suit paper, textile or detergent manufacture.

4) **Secretome analysis of a coprophilous fungus**. The secretome of *D. stemonitis* C8 from koala faeces was analysed by mass spectrometry (Publication 4; Section 5.3), revealing a broad range of enzymes involved in the degradation of cellulose, hemicellulose, pectin, lignin and protein. The abundance of a GH10 endo-1,4-β-xylanase and a high number of enzymes for the degradation of pectin were particularly defining features of the *D. stemonitis* C8 secretome that distinguish it from the secretomes of some industrially exploited fungi such as *Trichoderma reesei*. To date, the current study represents the only published proteomic analysis of the secretome of a coprophilous fungus, expanding fungal secretome research beyond previously explored saprophytic (Vinzant et al., 2001; Oda et al., 2006; Sato et al., 2007; Tsang et al., 2009; Vanden Wymelenberg et al., 2010), pathogenic (Mueller et al., 2008; Paper et al., 2007; Shah et al., 2009) and symbiotic fungal species (Nagendran et al., 2009). Furthermore, identifications were obtained for novel enzymes that could be of interest

for industrial applications. In particular, these include a GH10 endo-1,4-β-xylanase, GH5 endo-1,4-β-mannanase, GH5 endoglucanase, GH74 endoglucanase and GH3 β-glucosidase with relatively high pH optima (Publication 2).

5) **Development of an effective proteomic analysis workflow protocol for identifying proteins from an organism without a sequenced genome.** As the genome of *D. stemonitis* has not been sequenced, the analysis of the *D. stemonitis* C8 secretome (Publication 4) required the development of techniques supplementary to those typically used for protein identification involving organisms with a sequenced genome. Protein gel electrophoresis and mass spectrometry formed the basis of the analysis, and cross-species identification (Section 1.9.3.3) was found to be an effective method of establishing the identities and predicted functions of *D. stemonitis* C8 proteins based on significant similarities to proteins from other fungal species in the NCBI database. The importance of using *de novo* sequencing (Section 1.9.3.3) was clearly demonstrated as protein identifications that had been overlooked by automated Mascot searches were revealed (Section 5.4.2). Furthermore, the enzyme assays and zymogram gels (Publication 2) provided good support for the identifications made. In addition, a new and rapid technique to identify enzymes displaying activity in zymogram gels using mass spectrometry was presented (Publication 3; Publication 4). The combination of methods used to identify proteins in the secretome of *D. stemonitis* C8 provides a new and effective "toolbox" for identifying proteins (particularly enzymes) from any organism without a sequenced genome in the future.

6.2 Future Prospects

This work provides a potential stimulus for many future projects. As a direct result of the work, enzymes have been identified that could have potential for industrial applications. The procedures involved in the production of novel enzymes for possible use in industry are outlined in Section 6.2.1. On a broader level, the work provides an impetus to explore further into the world of coprophilous fungi to continue to advance knowledge about how the fungi subsist on faeces and to potentially identify more novel enzymes of interest to industry, as discussed in Section 6.2.2.

6.2.1 The development of novel enzymes for industrial applications

The identification of enzymes of interest from the fungal isolates from koala faeces (Publication 2; Publication 4) is a very early step towards potentially utilising the enzymes in an industrial setting. Of prime importance, a specific need for an enzyme with the attributes characterised would be required, for example to improve the efficiency, cost or environmental impact of an existing manufacturing process. The investment costs involved in developing a novel enzyme for the application of interest would then need to be weighed against the expected long term profits to determine if the venture was commercially justifiable (Aehle, 2007).

After a suitable enzyme is found, decisions need to be made about how the enzyme could be produced in an economically viable manner. Two broad options could be considered: strain improvement to enhance enzyme production in the native host (Section 6.2.1.1), or isolation of the gene encoding the enzyme(s) of interest and recombinant expression in a high secreting industrially established production host (Section 6.2.1.2). The option chosen depends on the suitability of the native host for commercial utilisation, for example whether the native host could be given a "GRAS" (Generally Recognised As Safe) status by regulatory authorities, and whether growth habits and culturing requirements of the species suit industrial fermentation conditions (Nevalainen et al., 2003).

6.2.1.1 Strain improvement to enhance enzyme production in the native host

Fungal strain improvement programs can be undertaken using traditional mutagens such as NTG (N'-methyl-N'-nitro-N'-nitrosoguanidine) or UV (ultra-violet) radiation, followed by mass screening to select high-performing mutants. The secreted enzyme yields of *Trichoderma*, *Aspergillus* and *Penicillium* species have been markedly increased using random mutagenesis and screening programs, in combination with improving cultivation techniques (reviewed in Nevalainen, 2001). For example, the protein secretion of *T. reesei* has been improved from mg/L in the wild-type QM6a (Mandels et al., 1971) to over 40 g/L in mutant *T. reesei* strains (Durand et al., 1988), and further to 100 g/L by improving fermentation conditions (Cherry and Fidantsef et al., 2010).

The strains *Trichoderma atroviride* A2 and *Gelasinospora cratophora* A10 isolated from koala faeces in this work could be possible candidates for strain improvement programs because the isolates grew well in cheap liquid medium, their natural protein secretion was quite substantial and they exhibited numerous enzyme activities that could be of interest to industry (moderately heat tolerant endoglucanase, xylanase, mannanase, and β-glucosidase activities, for example; Publication 2). Furthermore, *T. atroviride* A2 and *G. cratophora* A10 are closely related to fungi that have already have a GRAS status (*Trichoderma reesei* and *Neurospora crassa* respectively; www.fda.gov), increasing the likelihood that these strains could also be accepted for commercial use (Nevalainen et al., 2003).

6.2.1.2 Gene isolation and recombinant expression in an established production host

In many cases the industrial production of a novel enzyme of interest in a natural fungal host is not practicable and molecular techniques must be employed to isolate the gene encoding the enzyme for recombinant expression in an established microbial host. This forms the most likely scenario for the potential industrial production of the enzymes from the fungal isolates from koala faeces in this work. Amplification of the gene of interest could be achieved using degenerate primers based on peptide sequences identified from the enzymes by mass

spectrometry; for example, peptide sequences identified by mass spectrometry were used to design degenerate primers for the isolation of a laccase gene from the edible fungus *Pleurotus sapidus* (Linke et al., 2005). Likewise, the sequences of peptides identified from enzymes in the *D. stemonitis* C8 secretome (Supplementary Tables S1 - S4, Publication 4, Appendix 1) provide a good starting point for the construction of degenerative primers for gene isolation. Degenerative primers can also be based on conserved regions of known genes encoding similar enzymes, as used for the isolation of a cellobiohydrolase gene from the thermophilic ascomycetous *Myriococcum thermophilum* (Zámocký et al., 2008). Genes encoding enzymes responsible for activities displayed in the agar plate (Publication 1) and enzyme assays (Publication 2) by the fungal isolates from koala faeces could be isolated in this way, without any prior amino acid sequence information.

Once isolated, the gene (cDNA) encoding the enzyme of interest could be expressed in *E. coli* for primary characterisation of the enzyme product, followed by expression of the gene under a strong inducible promoter in a high secreting filamentous fungal host, such as a *Trichoderma* or *Aspergillus* species (de Faria et al., 2002; Kiiskinen et al., 2004; Rodríguez et al., 2008). *Pichia pastoris* is also used as an expression host of heterologous fungal enzymes (Berrin et al., 2000; Kawai et al., 2003; Spadiut et al., 2010). Further characterisation of the enzyme would then need to be undertaken in laboratory shake flasks and pilot-scale fermenters before the industrial value of the enzyme could become better established.

Genetic engineering could be used to increase the copy number of genes encoding enzymes of interest, in a similar manner that glucoamylase and feruloyl esterase production have been increased in *Aspergillus niger* (Verdoes et al., 1993; Levasseur et al., 2004) and endoglucanase production enhanced in *Trichoderma reesei* (Miettinen-Oinonen and Suominen, 2002). The deletion of genes encoding proteases may reduce the degradation of a recombinant enzyme, a task that has been achieved in *Aspergillus* species (Yoon et al., 2010).

6.2.2 A world of coprophilous fungi awaits attention

Fungal isolates that may warrant further investigation have been identified from koala faeces throughout this work. Isolates that displayed enzyme activities in the agar plate assays (Publication 1) but that were not studied further could be grown in a liquid medium for further enzyme characterisation using various enzyme assays and zymogram gels. Enzyme activity profiles may reveal new enzymes with industrial potential. Mass spectrometric analysis could be used to identify the proteins in the secretomes of the fungal isolates, in a similar way that identifications were obtained from the secretome of *D. stemonitis* C8 (Publication 4). Furthermore, a variety of different liquid media could be used to determine the influence of different substrates on protein production and secretion. For example, liquid medium containing *Eucalyptus* leaf material or koala faeces may induce the secretion of a different array of enzymes than those secreted in the general hydrolase-inducing medium used in this work (Publication 2; Publication 4). Other characteristics of the fungi from koala faeces could also be of interest, such as the secretion of antifungal substances (Section 3.4.2; Section 3.4.9), which may have potential for pharmaceutical or biocontrol applications. Ultimately, genome sequencing would reveal a great deal more about the coprophilous fungi from koala faeces, a possibility that has become more feasible with the development of cheaper and faster sequencing technologies such as 454 pyrosequencing (Margulies et al., 2005).

Beyond the immediate sources of further research directly generated from this work, the work in itself forecasts the potential value of similar studies on coprophilous fungi from the faeces of other animals in the future. Techniques used for the isolation and identification of fungi in this study provide good means to identify a range of coprophilous fungi from the faeces of other animals to generate comparative data on species variability in different coprophilous communities. Comparisons could be made between the enzymes and other proteins produced by coprophilous fungi from the faeces of different species of animal, using enzyme assays, zymography and mass spectrometry to reveal the diversity in the protein arrays. Moreover,

coprophilous fungi appear to hold high potential as sources of enzymes of industrial interest. Coprophilous fungi from the faeces of Australian herbivores such as wombats, kangaroos and possums may be particularly good sources of enzymes for efficient plant biomass degradation due to the tough, recalcitrant nature of the foliage of the native plants of Australia.

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Appendix 1: Supplementary material for Publication 4 (Section 5.3)

The following pages contain a printed form of the supplementary material that is available on-line for Publication 4 (Section 5.3).

Figure S1 is an image of the 2D gel produced from the electrophoresis of protein in the *D. stemonitis* C8 supernatant. The image is the same as Fig. 1 in Publication 4 (Section 5.3) except that the spots that were excised and subjected to mass spectrometric analysis are numbered from 1 - 119. The protein spots are referred to by their numbers in the supplementary peptide tables (Tables S1 - S2).

Tables S1 - S4 contain details of the peptide sequences of proteins from the *D. stemonitis* C8 supernatant that match to proteins in the NCBI database:

Table S1: MALDI TOF/TOF MS/MS data from 2D gel

Table S2: Q-TOF LC MS/MS data from 2D gel

Table S3: Q-TOF LC MS/MS data from 1D gel

Table S4: Q-TOF LC MS/MS data from 2D mannanase zymogram

Abbreviations used in the tables:

Spt no – spot number, referring to protein spots on the 2D gel (Fig. S1) that were excised and subjected to mass spectrometric analysis

Prt scr – protein score, as determined by the Mascot search engine.

pI – isoelectric point

PMF pep – the total number of peptide matches obtained by Peptide Mass Fingerprinting (PMF). The number of unique peptide matches is indicated in brackets.

MS/MS pep – the total number of peptide matches generated by tandem mass spectrometry. The number of unique peptides is indicated in brackets.

Miss – the number of amino acids that differ between the analysed peptide and its closest match in the NCBI database.

Ions scr – ions score of the MS/MS peptide, as determined by the Mascot search engine.

Expect – the probability that the peptide match would occur by chance, as determined by the Mascot search engine for PMF and MS/MS peptide matches, or by the NCBI database for *de novo* peptides.

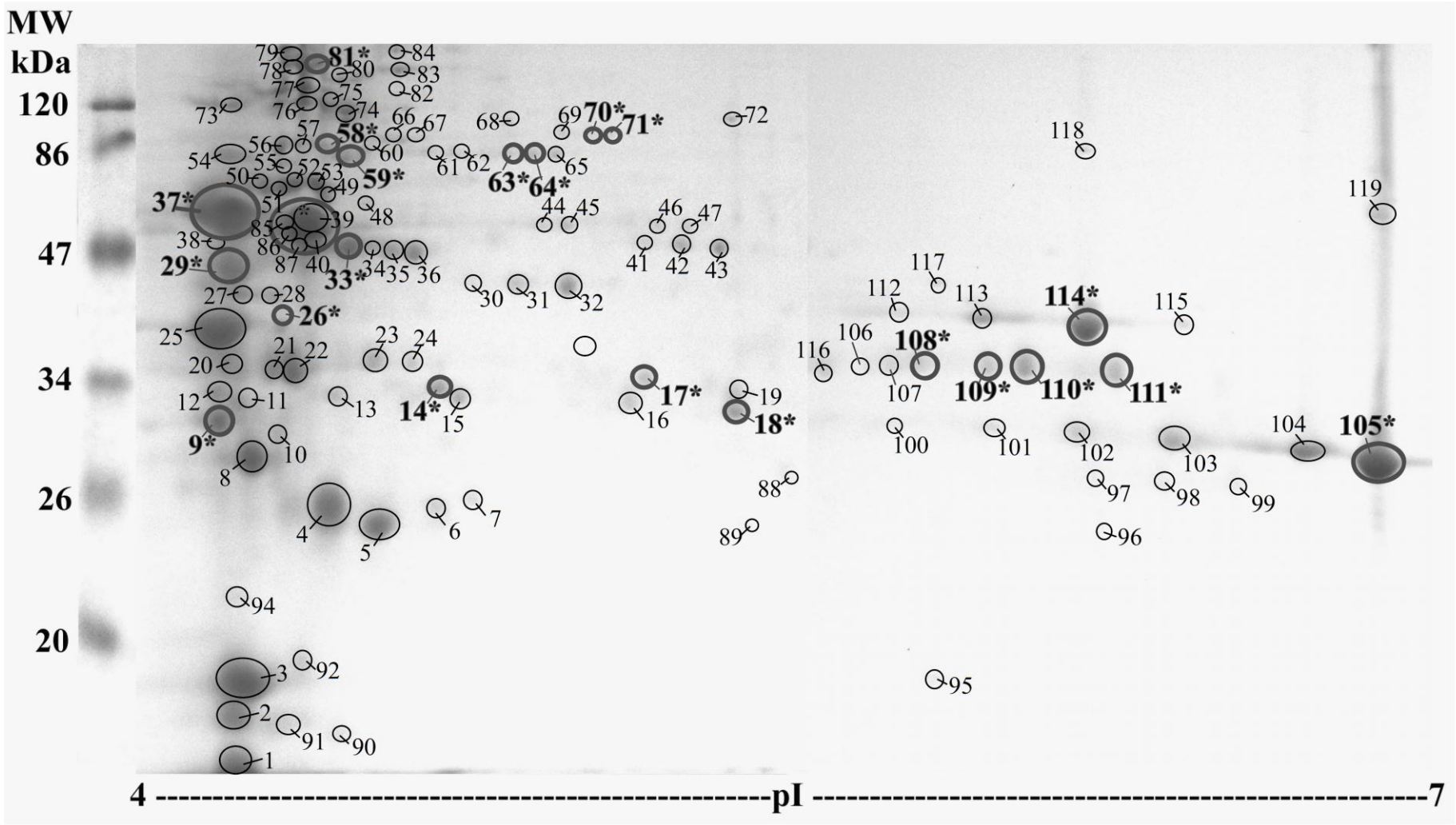

Figure S1. The 120 spots excised for MALDI TOF/TOF MS/MS from the 2D gel of *Doratomyces stemonitis* C8 supernatant (Table S1).
Spots 4, 5, 8, 25, 29, 37, 104, 105, 114 and the area encompassing spots 39, 40 and 85-7 were reanalysed from a replicate gel by Q-TOF LC MS/MS (Table S2).
Spots with an asterick and indicated in bold were identified above significance level and the protein names are included in Figure 1 and Table 1 of the main article.
For spots 14 and 58, significant identifications were only obtained from Q-TOF LC MS/MS of the 1D SDS-PAGE gel (Table S3). Spots 93 and 120 - Blanks.

Table S1: Protein identifications by MALDI TOF/TOF MS/MS of protein spots from the 2D gel of *Doratomyces stemonitis* C8 supernatant (Fig. 1, Publication 4)

The following table contains protein identifications made using MALDI-TOF/TOF MS/MS analysis of protein spots from the 2D gel of *D. stemonitis* C8 supernatant (Fig. 1, Publication 4). The 120 protein spots excised from the 2D gel for MALDI TOF/TOF MS/MS analysis are numbered in Supplementary Fig. S1. Proteins identified above significance level by MALDI TOF/TOF MS/MS (Table S1) or Q-TOF LC MS/MS (Table S2) are shown in Table 1 and Fig. 1 of Publication 4, and are indicated in bold with asterick in Tables S1 and S2 (red text) and in Supplementary Fig. S1.

Peaklists were generated by Data Explorer (Applied Biosystems) and searched against the NCBInr database (www.ncbi.nlm.nih.gov) using the Mascot search engine (Matrix Sciences) as described in Materials and Methods. Peptide sequences shown in the table were generated by Mascot unless preceded by the words *"de novo"*. *De novo* sequencing was carried out on good quality unassigned spectra using Mascot Distiller (Matrix Sciences). Included in the table are proteins that could not be identified above significance level ($p < 0.05$, protein score > 71) or for which only one matching *de novo* peptide was found (Expect < 0.001 or Expect > 0.001). In many cases these proteins or proteins of the same function were subsequently identified by Q-TOF LC MS/MS of the 1D SDS-PAGE gel (Table 1; Supplementary Table S3).

No significant matches to proteins in the NCBI database were obtained for protein spots with numbers not listed in the table below.

** Pectin esterase and α-L-rhamnosidase did not score significant peptide matches from MALDI TOF/TOF MS/MS data but identification was subsequently confirmed by Q-TOF LC MS/MS of protein bands from the 1D SDS-PAGE gel (Table S2).

Table S1: Protein identifications by MALDI TOF/TOF MS/MS of protein spots from the 2D gel of *Doratomyces stemonitis* C8 supernatant (Fig. 1, Publication 4; Supp. Fig. S1)

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	PMF pep	MS/MS pep	Miss	Peptide sequence	Ions scr	Expect
9 *	**subtilisin-like protease**	**subtilisin protease; proteinase K-like (S8)**	***Neotyphodium lolii***	**gi|170674493**	**-**	**40**	**5.2**	**-**	**2(2)**	**4**	***de novo* GYGTHVAGTIGGATYGVAK**	**-**	**4.00E-04**
										5	***de novo* VLDAQGSGSNSGVIAGFDCWR**	**-**	**2.00E-05**
10	hypothetical protein CHGG_03409	pectate lyase	*Chaetomium globosum* CBS 148.51	gi|116208232	60	29	4.9	1(1)	1(1)	0	K.VFQFNGR.G	55	6.90E-01
12	1,4-beta-D-glucan cellobiohydrolase B	cellobiohydrolase I (GH7)	*Aspergillus niger*	gi|145230535	-	56	4.1	-	1(1)	4	*de novo* HGNHDFYGPGLTVDTSK	-	1.00E-03
14 **	**hypothetical protein**	**pectin esterase**	***Podospora anserina* S mat+**	**gi|171682268**	**46**	**34**	**9.1**	**2(1)**	**1(1)**	**0**	**R.IWNAGDER.T**	**41**	**5.20E-02**
										0	**R.IWNAGDER.T**	**PMF only**	**-**
	pectin esterase precursor	pectin esterase	*Neurospora crassa* OR74A	gi|164428261	-	35	9.2	1(1)	1(1)	1	*de novo* KSQADGLSNDQTATLR	-	2.00E-05
	hypothetical protein SNOG_06018	pectin esterase	*Phaeosphaeria nodorum* SN15	gi|169605971	-	198	8.4	-	1(1)	3	*de novo* TEGVTFAEYNNSGK	-	6.70E-02
17 *	**pectate lyase B**	**pectate lyase**	***Verticillium albo-atrum***	**gi|261353894**	**72**	**34**	**5.1**	**4(2)**	**2(2)**	**1**	**R.RTGNVIIR.N**	**14**	**1.70E+01**
										1	**R.RTGNVIIR.N**	**PMF only**	**-**
										0	**R.TGNVIIR.N**	**52**	**7.20E-03**
										0	**R.TGNVIIR.N**	**PMF only**	**-**
18 *	**Abf2**	**α-L-arabinofurano-sidase (GH62)**	***Hypocrea jecorina* (QM6a)**	**gi|31747156**	**-**	**35**	**6.4**	**-**	**2(2)**	**3**	***de novo* NASGATAFTNDISHGDLIR**	**-**	**3.00E-04**
										5	***de novo* QREKQYLFIVEAIGADGR**	**-**	**3.00E-04**
21	endo-1,4-beta-mannosidase	endo-1,4-beta-mannosidase (GH5)	*Aspergillus clavatus* NRRL 1	gi|121709475	-	39	5.4	-	1(1)	1	*de novo* GSDAIFAWELANEPR	-	2.00E-05
	hypothetical protein CHGG_10708	endo-1,4-beta-mannosidase (GH5)	*Chaetomium globosum* CBS 148.51	gi|116205649	-	46	5.0	-	1(1)	3	*de novo* GSDAIFAWELANEPR	-	1.00E-04
22	endo-1,4-beta-mannosidase	endo-1,4-beta-mannosidase (GH5)	*Aspergillus clavatus* NRRL 1	gi|121709475	-	39	5.4	-	1(1)	1	*de novo* GSDAIFAWELANEPR	-	2.00E-05
	hypothetical protein CHGG_10708	endo-1,4-beta-mannosidase (GH5)	*Chaetomium globosum* CBS 148.51	gi|116205649	-	46	5.0	-	1(1)	3	*de novo* GSDAIFAWELANEPR	-	1.00E-04
25	hypothetical protein SNOG_01690	endoglucanase (GH6)	*Phaeosphaeria nodorum* SN15	gi|169597801	-	41	5.2	-	1(1)	6	*de novo* AGNAVADLGTFVWVDTR	-	1.60E-01
	conserved hypothetical protein	endoglucanase (GH6)	*Chaetomium globosum* CBS 148.51	gi|116208232	-	42	4.7	-	1(1)	8	*de novo* AGNAVADLGTFVWVDTR	-	1.70E+00

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	PMF pep	MS/MS pep	Miss	Peptide sequence	Ions scr	Expect
26 *	**carbohydrate esterase family 15**	**4-O-methyl-glucuro-noyl esterase (CE15)**	***Schizophyllum commune* H4-8**	**gi|300099970**	**77**	**42**	**4.4**	**1(1)**	**1(1)**	**0**	**K.GALMAGALEPR.V**	**77**	**3.50E-05**
29	endoglucanase I	endoglucanase (GH6)	*Robillarda* sp. Y-20	gi|6855474	22	42	4.7	1(1)	1(1)	0	K.GAYAILDPHNYMR.Y + Oxidation(M)	-	-
33 *	**glycosyl hydrolase family 43 protein**	**β-xylosidase (GH43)**	***Aspergillus fumigatus* Af293**	**gi|70982855**	**72**	**49**	**4.5**	**5(4)**	**1(1)**	**0**	**R.VVERPK.V**	**PMF only**	**-**
										0	**0 K.DDDGSAYLLTEDRPNGLR.I**	**53**	**1.80E-03**
										0	**K.NGVYFMFGSQLTATNDNK.Y**	**PMF only**	**-**
										0	**K.YENGDSSQR.Y**	**PMF only**	**-**
										0	**K.YENGDSSQR.Y**	**PMF only**	**-**
37	1,4- beta-D glucan cellobiohydrolase B precursor	cellobiohydrolase I (GH7)	*Aspergillus niger*	gi|145230535	-	56	4.1	-	1(1)	4	*de novo* HGNHDFYGPGLTVDTSK	-	1.00E-03
	hypothetical protein MGG_10712	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39971383	-	49	5	-	1(1)	6	*de novo* HGNHDFYGPGLTVDTSK	-	1.60E-01
39	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	-	50	5.3	-	1(1)	0	*de novo* HEYGTNIGSR	-	6.80E-02
51	cellulose 1,4-beta-cellobiosidase	cellobiohydrolase I (GH7)	*Chaetomium thermophilum*	gi|156712282	-	56	5.2	-	1(1)	4	*de novo* QGDETFYGPGLTVDTNQK	-	6.00E-04
52	cellulose 1,4-beta-cellobiosidase	cellobiohydrolase I (GH7)	*Chaetomium thermophilum*	gi|156712282	-	56	5.2	-	1(1)	6	*de novo* QGDETFYGPGLTVDWER	-	1.60E-01
53	hypothetical protein MGG_06834	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39977899	-	48	6.6	-	1(1)	4	*de novo* WLDSTYPETSTSQAPR	-	6.70E-02
54	Cel74a	endoglucanase (GH74)	*Hypocrea jecorina* (QM6a)	gi|31747160	-	87	5.4	-	1(1)	8	*de novo* AVYSADADTVLLSSQSSGVLR	-	6.60E-02
58 **	**hypothetical protein**	**bacterial α-L-rhamnosidase (GH78)**	***Podospora anserina* S mat+**	**gi|171694950**	**68**	**79**	**5.1**	**4 (3)**	**1(1)**	**0**	**R.TFVVAGGR.F**	**PMF only**	**-**
										0	**R.TFVVAGGR.F**	**58**	**1.70E-03**
										0	**K.QQGNETEAEK.W**	**PMF only**	**-**
										0	**K.GTMFEK.Y + Oxidation (M)**	**PMF only**	**-**
59 *	**cellulose-binding beta-glucosidase**	**β-glucosidase (GH3)**	***Coprinopsis cinerea okayama***	**gi|299755823**	**136**	**86**	**8.6**	**4 (2)**	**2(2)**	**0**	**R.LPYTIGK.R**	**PMF only**	**-**
										0	**R.LPYTIGK.R**	**32**	**2.70E-07**
										1	**R.HFDKNNIEPR.Y**	**PMF only**	**-**
										1	**R.HFDKNNIEPR.Y**	**94**	**5.80E-01**

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	PMF pep	MS/MS pep	Miss	Peptide sequence	Ions scr	Expect
59	hypothetical protein MGG_09353	β-glucosidase (GH3)	*Magnaporthe grisea* 70-15	gi|39971383	-	49	5	-	1(1)	1	*de novo* HYLLNEQEVNR	-	2.80E-02
60	cellulose-binding beta-glucosidase	β-glucosidase (GH3)	*Coprinopsis cinerea okayama*	gi|299755823	57	86	8.6	1(1)	1(1)	1	R.HFDKNNIEPR.Y	-	1.60E-03
63	hypothetical protein	GMC oxidoreductase	*Podospora anserina* S mat+	gi|171683764	50	72	5.3	4(3)	1(1)	1	R.GGKVLLTSTDPR.D	PMF only	-
										1	R.GLRVVDASAFPR.V	PMF only	-
										0	R.VVDASAFPR.V	PMF only	-
										0	R.VVDASAFPR.V	43	8.00E-02
64 *	**hypothetical protein**	**GMC oxidoreductase**	***Podospora anserina* S mat+**	**gi|171683764**	**79**	**72**	**5.3**	**2(1)**	**1(1)**	**0**	**R.VVDASAFPR.V**	**PMF only**	**-**
										0	**R.VVDASAFPR.V**	**74**	**5.60E-05**
70 *	**catalase**	**catalase**	***Aspergillus clavatus***	**gi|121712100**	**81**	**80**	**5.7**	**4(2)**	**2(2)**	**1**	**K.EGKETPVFVR.F**	**PMF only**	**-**
										1	**K.EGKETPVFVR.F**	**29**	**1.30E+00**
										0	**R.GVDFTEDPLLQGR.I**	**PMF only**	**-**
										0	**R.GVDFTEDPLLQGR.I**	**33**	**4.80E-01**
	catalase B	catalase	*Paracoccidioides brasiliensis* Pb03	gi|225683111	49	82	6.7	2(1)	1(1)	0	R.GIDFSEDPLLQGR.V	49	1.10E-02
										0	R.GVDFTEDPLLQGR.I	PMF only	-
71	catalase	catalase	*Aspergillus clavatus*	gi|121712100	33	80	5.7	3(2)	1(1)	1	K.EGKETPVFVR.F	PMF only	-
										0	R.GVDFTEDPLLQGR.I	PMF only	-
										0	R.GVDFTEDPLLQGR.I	33	5.20E-01
	catalase B	catalase	*Paracoccidioides brasiliensis* Pb03	gi|225683111	49	82	6.7	2(1)	1(1)	0	R.GIDFSEDPLLQGR.V	49	1.10E-02
										0	R.GVDFTEDPLLQGR.I	PMF only	-
75	alpha-glucuronidase precursor	α-glucuronidase (GH67)	*Aspergillus terreus* NIH2624	gi|115385461	43	93	5.8	3(2)	1(1)	1	K.GIDQQRFR.E	PMF only	-
										0	R.VSEIVTGR.R	PMF only	-
										0	R.VSEIVTGR.R	43	3.30E+01
76	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	57	50	5.3	1(1)	1(1)	0	K.HEYGTNIGSR.F	51	2.30E-06

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	PMF pep	MS/MS pep	Miss	Peptide sequence	Ions scr	Expect
77	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	-	50	5.3	-	-	0	*de novo* HEYGTNIGSR	-	6.80E-02
80	beta-1,4-glucosidase	β-glucosidase (GH3)	*Botryotinia fuckeliana*	gi|154302511	57	105	5.1	2(1)	1(1)	0	K.HFVGNEQEHFR.Q	57	1.20E-03
										0	K.HFVGNEQEHFR.Q	PMF only	-
81 *	**cellulose-binding beta-glucosidase**	**β-glucosidase (GH3)**	***Coprinopsis cinerea okayama***	**gi|299755823**	**136**	**86**	**8.6**	**2(1)**	**1(1)**	**1**	**R.HFDKNNIEPR.Y**	**PMF only**	**-**
										1	**R.HFDKNNIEPR.Y**	**74**	**3.10E-05**
	beta-1,4-glucosidase	β-glucosidase (GH3)	*Botryotinia fuckeliana* B05.10	gi|154302511	57	105	5.1	1(1)	2(2)	0	K.HFVGNEQEHFR.Q	57	1.20E-03
										0	*de novo* EGLYIDYR	-	1.40E+00
85	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	57	50	5.3	2(2)	1(1)	1	K.TVDTNRK.F	PMF only	-
										0	K.HEYGTNIGSR.F	46	1.70E-02
86	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	66	50	5.3	2(1)	1(1)	1	R.LSQFFVQDGRK.I	PMF only	-
										0	K.HEYGTNIGSR.F	54	2.50E-03
87	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	66	50	5.3	2(1)	1(1)	1	R.LSQFFVQDGRK.I	PMF only	-
										0	K.HEYGTNIGSR.F	52	4.10E-03
106	hypothetical protein CHGG_00304	endo-1,4-β-xylanase (GH10)	*Chaetomium globosum* CBS 148.51	gi|116179352	34	41	7.1	2(1)	1(1)	0	R.VLGEDFVGIAFR.A	PMF only	-
										0	R.VLGEDFVGIAFR.A	28	1.40E+00
107	hypothetical protein CHGG_00304	endo-1,4-β-xylanase (GH10)	*Chaetomium globosum* CBS 148.51	gi|116179352	49	41	7.1	2(1)	1(1)	0	R.VLGEDFVGIAFR.A	PMF only	-
										0	R.VLGEDFVGIAFR.A	42	5.30E-02
108 *	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**103**	**41**	**7.1**	**2(1)**	**2(2)**	**0**	**R.VLGEDFVGIAFR.A**	**PMF only**	**-**
										0	**R.VLGEDFVGIAFR.A**	**95**	**2.80E-07**
										4	***de novo* SSDNPLLFDSNYEPK**	**-**	**4.00E-03**
109 *	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**99**	**41**	**7.1**	**2(1)**	**1(1)**	**0**	**R.VLGEDFVGIAFR.A**	**PMF only**	**-**
										0	**R.VLGEDFVGIAFR.A**	**92**	**4.90E-07**
110 *	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**141**	**41**	**7.1**	**2(2)**	**4(4)**	**0**	**R.DSVFSR.V**	**22**	**4.90E+00**
										0	**R.VLGEDFVGIAFR.A**	**104**	**3.40E-08**

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	PMF pep	MS/MS pep	Miss	Peptide sequence	Ions scr	Expect
110 (cont)										**3**	***de novo* SSDNPLLFDSNYQPK**	**-**	**5.00E-04**
										4	***de novo* LYIDDYNLDIANCMK**	**-**	**1.00E-03**
111 *	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**135**	**41**	**7.1**	**2(2)**	**3(3)**	**0**	**R.DSVFSR.V**	**17**	**1.30E+01**
										0	**R.VLGEDFVGIAFR.A**	**104**	**3.30E-08**
										1	***de novo* LYIDDYNLDIANYAK**	**-**	**1.00E-06**
114	extracellular metalloproteinase	metallopeptidase (M36)	*Artroderma benhamiae*	gi|74612199	58	70	6.8	1(1)	1(1)	0	R.DSSFEAGTVIHEYTHGLSNR.L	58	8.00E-04

Table S2: Protein identifications by Q-TOF LC MS/MS of protein spots from the 2D gel of *Doratomyces stemonitis* C8 supernatant (Fig. 1, Publication 4)

The following table contains protein identifications obtained from the ten largest protein spots on the 2D gel (Supplementary Fig. S1), which were re analysed by Q-TOF LC MS/MS as described in Materials and Methods.

Proteins identified above significance level are shown in Table 1 and Fig. 1 of Publication 4 and are indicated in bold with asterick in the table below (red text) and in Supplementary Fig. S1.

Peaklists were generated by Mascot Distiller (Matrix Sciences) and searched against the NCBInr database (www.ncbi.nlm.nih.gov) using the Mascot search engine (Matrix Sciences). Peptide sequences shown in the table were generated by Mascot unless preceded by the words *"de novo".De novo* sequencing was carried out on good quality unassigned spectra using Mascot Distiller.

Included in the table are proteins that could not be identified above significance level ($p < 0.05$, protein score > 43) or for which only one matching *de novo* peptide was found (Expect < 0.001 or Expect > 0.001).

Note: A large area of protein encompassing the original spots 39, 40, 85, 86 and 87 analysed by MALDI TOF/TOF MS/MS (Supplementary Fig. 1; Table S1) was analysed as one spot by Q-TOF LC MS/MS.

Table S2: Protein identifications by Q-TOF LC MS/MS of protein spots from the 2D gel of *Doratomyces stemonitis* C8 supernatant (Fig. 1, Publication 4; Supp. Fig. S1)

Spt No.	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Miss	Peptide matches	Ions scr	Expect
4	hypothetical protein	endoglucanase (GH45)	uncultured soil fungus	gi|299766954	-	12	4.9	7	*de novo* TSPCVEATNTGGER (Scan 10828)	-	1.54E+02
	hypothetical protein	endoglucanase (GH45)	*Podospora anserina* DSM 980	gi|171687659	-	26	7.4	7	*de novo* TSPCVEATNTGGER (Scan 10828)	-	1.54E+02
	hypothetical protein	endoglucanase (GH45)	*Leptosphaeria maculans*	gi|312217600	-	35	4.8	7	*de novo* TSPCVEATNTGGER (Scan 10828)	-	1.54E+02
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	0	*de novo* SECDGFPEK (Scan 10832)	-	3.90E-01
	hypothetical protein	endoglucanase (GH45)	*Leptosphaeria maculans*	gi|312217600	-	35	4.8	1	*de novo* SECDGFPEK (Scan 10832)	-	4.10E+00
	hypothetical protein	endoglucanase (GH45)	*Magnaporthe grisea* 70-15	gi|39951371	-	23	6.6	1	*de novo* SECDGFPEK (Scan 10832)	-	4.10E+00
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	2	*de novo* SHCSGFPEK (Scan 10838)	-	6.07E+02
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	3	*de novo* SESPGFPEK (Scan 10844)	-	1.97E+03
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	3	*de novo* SDPDGFPEK (Scan 10858)	-	4.53E+02
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	2	*de novo* PECAGFPEK (Scan 10867)	-	5.80E+01
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	3	*de novo* PECAGFPEK (Scan 10879)	-	4.53E+02
	hypothetical protein	endoglucanase (GH45)	*Podospora anserina* DSM 980	gi|171687659	-	26	7.4	4	*de novo* MEVEATNTGER (Scan 10963)	-	2.79E+02
	hypothetical protein	endoglucanase (GH45)	*Leptosphaeria maculans*	gi|312217600	-	35	4.8	1	*de novo* LTFTGGPVSGK (Scan 10996)	-	7.90E-01
	hypothetical protein	endoglucanase (GH45)	*Podospora anserina* DSM 980	gi|171687659	-	26	7.4	1	*de novo* LTFTGGPVSGK (Scan 10996)	-	7.90E-01
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	2	*de novo* LTFTGGPVSGK (Scan 10996)	-	4.60E+00
	hypothetical protein	endoglucanase (GH45)	*Leptosphaeria maculans*	gi|312217600	-	35	4.8	1	*de novo* ELTFTGGPVSGK (Scan 11064)	-	7.50E-02
	hypothetical protein	endoglucanase (GH45)	*Podospora anserina* DSM 980	gi|171687659	-	26	7.4	1	*de novo* ELTFTGGPVSGK (Scan 11064)	-	7.50E-02
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	2	*de novo* ELTFTGGPVSGK (Scan 11064)	-	4.40E-01
	glycosyl hydrolase family 45	endoglucanase (GH45)	*Glomerella graminicola* M1.001	gi|310789959	-	25	4.9	1	*de novo* TAYGWAAVR (Scan 11116, 11132, 11142)	-	4.20E+00

Spt No.	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Miss	Peptide matches	Ions scr	Expect
4 (cont)	hypothetical protein	endoglucanase (GH45)	*Podospora anserina* DSM 980	gi|171687659	-	26	7.4	2	*de novo* TAYGWAAVR (Scan 11116)	-	4.40E+01
5	unnamed protein product	endo-1,4-beta-xylanase (GH11)	*Podospora anserina* S mat+	gi|171694490	-	25	8.5	2	*de novo* QTYNIYESTR (Scan 10898)	-	1.50E+01
	hypothetical protein CHGG_05235	endo-1,4-beta-xylanase (GH11)	*Chaetomium globosum* CBS 148.51	gi|116197274	-	25	8.7	2	*de novo* QTYNIYESTR (Scan 10898)	-	1.50E+01
	endo-1,4-beta-xylanase B precursor	endo-1,4-beta-xylanase (GH11)	*Phanerochaete chrysosporium*	gi|167599626	-	30	5.7	4	*de novo* QTYNIYESTR (Scan 10898)	-	8.80E+01
	endo-1,4-beta-xylanase B precursor	endo-1,4-beta-xylanase (GH11)	*Phanerochaete chrysosporium*	gi|167599626	-	30	5.7	2	*de novo* ATYDIYESTR (Scan 10907)	-	2.60E+00
	GH11 endo-beta-1,4-xylanase	endo-1,4-beta-xylanase (GH11)	*Podospora anserina* S mat+	gi|312124718	-	28	8.5	1	*de novo* ATYDIYESTR (Scan 10907)	-	6.30E+00
	hypothetical protein CHGG_05235	endo-1,4-beta-xylanase (GH11)	*Chaetomium globosum* CBS 148.51	gi|116197274	-	25	8.7	3	*de novo* ATYDIYESTR (Scan 10907)	-	8.80E+01
	unnamed protein product	endo-1,4-beta-xylanase (GH11)	*Podospora anserina* S mat+	gi|171694490	-	25	8.5	2	*de novo* QTYNIYESTR (Scan 10923)	-	1.50E+01
	hypothetical protein CHGG_05235	endo-1,4-beta-xylanase (GH11)	*Chaetomium globosum* CBS 148.51	gi|116197274	-	25	8.7	2	*de novo* QTYNIYESTR (Scan 10923)	-	1.50E+01
	endo-1,4-beta-xylanase B precursor	endo-1,4-beta-xylanase (GH11)	*Phanerochaete chrysosporium*	gi|167599626	-	30	5.7	4	*de novo* QTYNIYESTR (Scan 10923)	-	8.80E+01
	unnamed protein product	endo-1,4-beta-xylanase (GH11)	*Podospora macrospora*	gi|289621622	-	24	9.0	2	*de novo* QTYDIYESTR (Scan 10927)	-	8.40E+00
	endo-1,4-beta-xylanase B precursor	endo-1,4-beta-xylanase (GH11)	*Phanerochaete chrysosporium*	gi|167599626	-	30	5.7	2	*de novo* QTYDIYESTR (Scan 10927)	-	1.50E+01
	GH11 endo-beta-1,4-xylanase	endo-1,4-beta-xylanase (GH11)	*Podospora anserina* S mat+	gi|312124718	-	28	8.5	1	*de novo* QTYDIYESTR (Scan 10927)	-	6.30E+00
	hypothetical protein CHGG_05235	endo-1,4-beta-xylanase (GH11)	*Chaetomium globosum* CBS 148.51	gi|116197274	-	25	8.7	3	*de novo* QTYDIYESTR (Scan 10927)	-	8.80E+01
	cellobiohydrolase I	cellobiohydrolase I (GH7)	uncultured fungus	gi|291197124	-	180	4.7	0	*de novo* ALSLKFVTK (Scan 11190)	-	3.10E+00
	exoglucanase 1	cellobiohydrolase I (GH7)	*Cochliobolus carbonum*	gi|3913802	-	48	5.7	0	*de novo* ALSLKFVTK (Scan 11190)	-	3.10E+00
	unnamed protein product	cellobiohydrolase I (GH7)	*Sordaria macrospora*	gi|289613465	-	49	5.2	1	*de novo* TSGDALSLKFVTK (Scan 11211)	-	7.00E-03
	cellobiohydrolase	cellobiohydrolase I (GH7)	*Melanocarpus Albomyces*	gi|203282246	-	48	4.5	1	*de novo* TSGDALSLKFVTK (Scan 11211)	-	7.00E-03
	unnamed protein product	cellobiohydrolase I (GH7)	*Sordaria macrospora*	gi|289613465	-	49	5.2	1	*de novo* TSGDALSLKFVTK (Scan 11231)	-	1.30E-02
	cellobiohydrolase	cellobiohydrolase I (GH7)	*Melanocarpus Albomyces*	gi|203282246	-	48	4.5	1	*de novo* TSGDALSLKFVTK (Scan 11231)	-	1.30E-02
	endo-1,4-beta-xylanase	endo-1,4-beta-xylanase (GH11)	*Penicillium canescens*	gi|227343519	-	24	6.4	5	*de novo* SPLVEYYDDGAYK (Scan 11278)	-	8.30E+00

Spt No.	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Miss	Peptide matches	Ions scr	Expect
5 (cont)	endo-1,4-beta-xylanase B precursor	endo-1,4-beta-xylanase (GH11)	*Phanerochaete chrysosporium*	gi|167599626	-	30	5.7	7	*de novo* SPLVEYYDDGAYK (Scan 11278)	-	4.90E+01
	hypothetical protein	cellobiohydrolase I (GH7)	*Chaetomium globosum* CBS 148.51	gi|116200349	-	49	4.6	2	*de novo* TSGDALSLKFNTK (Scan 11356)	-	4.40E-01
	unnamed protein product	endo-1,4-beta-xylanase (GH11)	*Podospora macrospora*	gi|289621622	-	24	9.0	2	*de novo* TSGDALSLKFNTK (Scan 11356)	-	5.90E-01
	cellobiohydrolase	cellobiohydrolase I (GH7)	*Melanocarpus Albomyces*	gi|203282246	-	48	4.5	2	*de novo* TSGDALSLKFNTK (Scan 11356)	-	5.90E-01
8	hypothetical protein	Cip1	*Gibberella zeae* PH-1	gi|46124177	-	26	6.1	0	*de novo* IPSGDVYVR (Scan 10802)	-	7.00E-01
	Cip1	Cip1	*Hypocrea jecorina*	gi|31747158	-	33	4.9	2	*de novo* IPSGDVYVR (Scan 10802)	-	2.40E+01
	hypothetical protein	Cip1	*Podospora anserina* S mat+	gi|171682848	-	25	6.2	2	*de novo* IPSGDVYVR (Scan 10802)	-	2.40E+01
	hypothetical protein	Cip1	*Gibberella zeae* PH-1	gi|46124177	-	26	6.1	0	*de novo* PSGDVYVR (Scan 10830, 10854, 10875, 10895, 10935)	-	6.50E+00
	Cip1	Cip1	*Hypocrea jecorina*	gi|31747158	-	33	4.9	1	*de novo* PSGDVYVR (Scan 10830, 10854, 10875, 10895, 10935)	-	3.80E+01
	hypothetical protein	Cip1	*Podospora anserina* S mat+	gi|171682848	-	25	6.2	1	*de novo* PSGDVYVR (Scan 10830, 10854, 10875, 10895, 10935)	-	3.80E+01
	hypothetical protein	Cip1	*Gibberella zeae* PH-1	gi|46124177	-	26	6.1	0	*de novo* AIPSGDVYVR (Scan 10906, 10942, 10958, 10970-4, 10990, 11006, 11042, 11078, 11098, 11118, 11154, 11215, 11230, 11247, 11830, 12199)	-	1.30E-01
	Cip1	Cip1	*Hypocrea jecorina*	gi|31747158	-	33	4.9	2	*de novo* AIPSGDVYVR (Scan 10906, 10942, 10958, 10970-4, 10990, 11006, 11042, 11078, 11098, 11118, 11154, 11215, 11230, 11247, 11830, 12199)	-	2.60E+01
	hypothetical protein	Cip1	*Podospora anserina* S mat+	gi|171682848	-	25	6.2	2	*de novo* AIPSGDVYVR (Scan 10906, 10942, 10958, 10970-4, 10990, 11006, 11042, 11078, 11098, 11118, 11154, 11215, 11230, 11247, 11830, 12199)	-	2.60E+01
	Carboxypeptidase S1	Serine carboxypeptidase B	*Arthroderma benhamiae* CBS 112371	gi|302507494	-	72	5.3	2	*de novo* YHILSTSTCTK (Scan 11184-8)	-	2.50E+00
25	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Podospora anserina* S mat+	gi|171677885	-	43	5.2	7	*de novo* RALAPNPVNHLGVVIYDLPGR (Scan 11959)	-	5.40E-02

Spt No.	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Miss	Peptide matches	Ions scr	Expect
25 (cont)	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	4.8	11	*de novo* RALAPNPVNHLGVVIYDLPGR (Scan 11959)	-	1.70E-01
	glycoside hydrolase family 6 protein	cellobiohydrolase II (CBHII, GH6)	*Schizophyllum commune* H4-8	gi|302677600	-	41	4.4	1	*de novo* PGAEELAAVYK (Scan 12071)	-	1.40E+00
	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Podospora anserina* S mat+	gi|171677885	-	43	5.2	2	*de novo* PGAEELAAVYK (Scan 12071)	-	2.00E+01
	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	4.8	2	*de novo* PGAEELAAVYK (Scan 12071)	-	2.00E+01
	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Podospora anserina* S mat+	gi|171677885	-	43	5.2	3	*de novo* PGAEEPKEYK (Scan 12083)	-	5.00E+02
	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	4.8	3	*de novo* PGAEEPKEYK (Scan 12083)	-	5.00E+02
	hypothetical protein	cellobiohydrolase II (CBHII, GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	4.8	5	*de novo* VMYLDSGLDEYK (Scan 12430)	-	2.06E+02
29 *	**Endoglucanase 1**	**GH5 Endoglucanase**	***Robillarda* sp.Y-20**	**gi|6855474**	**101**	**41**	**4.3**	**0**	**R.VAFLLER.M**	**19.49**	**1.80E+01**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**27.55**	**1.00E+00**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**50.16**	**6.10E-03**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**46.94**	**1.30E-02**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**52**	**4.20E-03**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**57.84**	**1.10E-03**
								0	**K.GAYAILDPHNYMR.Y + Oxidation (M)**	**57.75**	**1.30E-03**
37 *	**unnamed protein product**	**PA_ScAPY_like: aminopeptidase Y**	***Podospora anserina* S mat+**	**gi|171677143**	**73**	**54**	**5.8**	**0**	**R.VNYNVIAETK.G**	**73.19**	**4.90E-05**
	hypothetical protein SNOG_08778	**aminopeptidase Y; PA_ScAPY_like**	***Phaeosphaeria nodorum SN15***	**gi|169611336**	**59**	**54**	**4.9**	**0**	**K.LQDIADANGGNR.A**	**59.41**	**5.70E-04**
	exoglucanase	**cellobiohydrolase I (CBHI, GH7)**	***Humicola grisea* var. *thermoidea***	**gi|4204214**	**57**	**50**	**5.3**	**0**	**K.HEYGTNIGSR.F**	**57.25**	**8.40E-04**
	Cel74a	**endoglucanase (GH74)**	***Hypocrea jecorina***	**gi|31747160**	**52**	**87**	**5.4**	**0**	**R.TDIGGLYR.L**	**51.78**	**8.10E-03**

Spt No.	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Miss	Peptide matches	Ions scr	Expect
39, 40, 85-7 *	hypothetical protein MGG_10712	cellobiohydrolase I (CBHI, GH7)	*Magnaporthe grisea* 70-15	gi|39971383	211	49	5	0	R.FNEVGGWSK.L	62.21	2.50E-04
								0	R.FNEVGGWSK.L	61.1	3.50E-04
								0	R.FNEVGGWSK.L	55.57	1.30E-03
								0	R.FNEVGGWSK.L	59.69	5.10E-04
								0	R.FNEVGGWSK.L	57.33	9.20E-04
								0	R.FNEVGGWSK.L	59.42	5.70E-04
								0	R.FNEVGGWSK.L	62.26	3.50E-04
								0	R.FNEVGGWSK.L	63.82	2.50E-04
								0	R.FNEVGGWSK.L	41.1	8.00E-02
	exoglucanase	cellobiohydrolase I (CBHI, GH7)	*Humicola grisea* var. *thermoidea*	gi|4204214	63	50	5.3	0	K.HEYGTNIGSR.F	31.47	2.40E-01
								0	K.HEYGTNIGSR.F	50.36	4.60E-03
								0	K.HEYGTNIGSR.F	39.9	5.10E-02
105 *	hypothetical protein CHGG_00304	endo-1,4-beta-D-xylanase (GH10)	*Chaetomium globosum* CBS 148.51	gi|116179352	76	41	7.1	0	R.VLGEDFVGIAFR.A	76.37	2.60E-05
114 *	metalloprotease MEP5	metallopeptidase (M36)	*Microsporum canis* CBS 113480	gi|238845207	79	69	7.3	0	R.NAIIDADK.N	49.36	1.90E-02
								0	R.NAIIDADK.N	56.35	3.80E-03
								0	K.NDGPTPVFENGVPK.D	12.19	4.40E+01
								0	K.NDGPTPVFENGVPK.D	7.42	1.40E+02
								0	R.DSSFEAGTVIHEYTHGLSNR.L	56.37	3.40E-03
								0	R.DSSFEAGTVIHEYTHGLSNR.L	10.73	7.40E+01

Table S3: Protein identifications by Q-TOF LC MS/MS of protein bands from the 1D SDS-PAGE gel of *Doratomyces stemonitis* C8 supernatant

The following table contains protein identifications obtained from the 1D SDS-PAGE gel Proteins identified above significance level are shown in Table 1 of Publication 4 and are indicated in red in the Table S3.

Peaklists were generated by Mascot Distiller (Matrix Sciences) and searched against the NCBInr database (www.ncbi.nlm.nih.gov) using the Mascot search engine (Matrix Sciences) as described in Materials and Methods. Peptide sequences shown in the table were generated by Mascot unless preceded by the words *"de novo"*. *De novo* sequencing was carried out on good quality unassigned spectra using Mascot Distiller.

Included in the table are proteins that could not be identified above significance level ($p < 0.05$, protein score > 43) or for which only one matching *de novo* peptide was found (Expect < 0.001 or Expect > 0.001).

Table S3: Protein identifications by Q-TOF LC MS/MS of protein bands from the 1D SDS-PAGE gel of *D. stemonitis* C8 supernatant

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
1 75-150 kDa	catalase B	catalase	*Paracoccidioides brasiliensis* Pb03	gi|225683111	95	82	0	R.GIDFSEDPLLQGR.V	95.22	3.30E-07
	unnamed protein product	GMC oxidoreductase	*Podospora anserina* S mat+	gi|171691498	67	154	0	R.SNYDLLVK.H	67.01	2.70E-04
	hypothetical protein BC1G_09832	β-glucosidase (GH3)	*Botryotinia fuckeliana* B05.10	gi|154302511	66	105	0	K.HFVGNEQEHFR.Q	65.62	3.10E-04
	Cel74a	endoglucanase (GH74)	*Hypocrea jecorina*	gi|31747160	64	87	0	R.TDIGGLYR.L	63.57	6.70E-04
							0	R.LAVDPANSNIIYFGAR.S	27.76	1.60E+00
	unnamed protein product	bacterial alpha-L-rhamnosidase (GH78)	*Podospora anserina* S mat+	gi|171694950	62	79	0	R.TFVVAGGR.F	37.81	2.70E-01
							0	K.VIDIWPDLTR.R	48.4	1.60E-02
							0	K.VIDIWPDLTR.R	57.33	2.10E-03
							2	*de novo* LATGAESGWDYTSR (Scan 10243)	-	9.00E-04
	hypothetical protein MGG_10038	β-glucosidase (GH3)	*Magnaporthe grisea* 70-15	gi|39966399	49	68	0	R.LPFDLPR.S	49.13	1.70E-02
	H antigen precursor	β-glucosidase (GH3)	*Ajellomyces capsulatus*	gi|671684	46	93	0	R.TPFTWGR.T	46.12	3.90E-02
							0	R.TPFTWGR.T	23.79	6.70E+00
	exo-beta-1,3-glucanase	polygalacturonase	*Aspergillus nidulans* FGSC A4	gi|259488941	-	84	4	*de novo* DGAVGDGSTDDSDAIQK (Scan 9856)	-	2.00E-03
							4	*de novo* WVGAVQGDGSTDDSDAIQK (Scan 9862)	-	2.00E-03
							4	*de novo* QPGAVGDGSTDDSDAIQK (Scan 9867)	-	2.00E-03
							4	*de novo* QPGAVGDGSTDDSDAIQK (Scan 9887, 9894)	-	2.00E-03
							6	*de novo* NGAVQGDGSTDDSDAIQK (Scan 9854)	-	3.70E-02
							7	*de novo* PGDQVGDGSTDDSDAIQK (Scan 9863)	-	6.60E-02
							5	*de novo* MHVGDGSTDDSDAIQK (Scan 9863)	-	6.60E-02
2 58-75 kDa	hypothetical protein FG03626.1	GMC oxidoreductase	*Gibberella zeae* PH-1	gi|46115568	274	64	0	K.GVGDSGPVQAVVNR.V	132.25	6.40E-11
							0	K.GVGDSGPVQAVVNR.V	132.28	6.40E-11

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
2 (cont.) 58-75 kDa							0	**K.VLGGSSALNLMTYDR.A + Ox (M)**	**89.32**	**1.20E-06**
							7	*de novo* DYTEYIVVGGGTAGPALTAR (Scan 10227)	-	1.10E-02
	Choline dehydrogenase	GMC oxidoreductase	*Verticillium albo-atrum* VaMs.102	gi|302408731	-	66	7	*de novo* DYTEYIVVGGGTAGPALTAR (Scan 10227)	-	2.00E-03
	hypothetical protein SNOG_08778	**aminopeptidase Y; PA_ScAPY_like**	***Phaeosphaeria nodorum* SN15**	**gi|169611336**	**89**	**54**	**0**	**K.LQDIADANGGNR.A**	**89.11**	**1.50E-06**
	hypothetical protein BC1G_09832	**β-glucosidase (GH3)**	***Botryotinia fuckeliana* B05.10**	**gi|154302511**	**72**	**105**	**0**	**K.HFVGNEQEHFR.Q**	**71.62**	**7.70E-05**
	unnamed protein product	**GMC oxidoreductase**	***Podospora anserina* S mat+**	**gi|171691498**	**70**	**154**	**0**	**R.SNYDLLVK.H**	**70.2**	**1.30E-04**
	hypothetical protein	polygalacturonase	*Tuber melanosporum* Mel28	gi|296425456	-	88	4	*de novo* VGNPGDVGVVEISELTFETK (Scan 10466)	-	2.00E-05
							5	*de novo* NGAVGDGSTDDSDAIQK (Scan 9715, 9722)	-	1.70E+00
							6	*de novo* RNHDTGNVEISQNTFETK (Scan10478)	-	1.70E+00
	exo-beta-1,3-glucanase	polygalacturonase	*Aspergillus nidulans* FGSC A4	gi|259488941	-	84	3	*de novo* NGAVGDGSTDDSDAIQK (Scan 9715, 9722)	-	2.00E-03
							7	*de novo* VGNPGDVGVVEISELTFETK (Scan 10466)	-	2.90E-01
	hypothetical protein FG09445.1	polygalacturonase	*Gibberella zeae* PH-1	gi|46135859	-	84	0	*de novo* LGNQQFTAR (Scan 9771)	-	8.60E-01
	1,4-beta-D-glucan-cellobiohydrolyase	cellobiohydrolase I (GH7)	*Aspergillus nidulans* FGSC A4	gi|67516425	-	56	2	*de novo* QGDETFYGPGLTVDTNK (Scan 10131)	-	3.00E-05
							5	*de novo* PGEETFYGPGLTVDTNK (Scan 10199)	-	9.00E-04
							8	*de novo* EGDETFYGPGLTVDGTGQK (Scan 10079)	-	2.00E-02
							8	*de novo* LPGAVLVFSLWDDGYANFR (Scan 10467)	-	6.60E-02
	hypothetical protein	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39977899	-	48	9	*de novo* WLDSTYPETSTSQAPR (Scan 10059, 10075)	-	6.70E-02
	exoglucanase	cellobiohydrolase I (GH7)	*Verticillium albo-atrum* VaMs.102	gi|302412114	-	29	1	*de novo* VSGSLVLDSNWR (Scan 10211)	-	2.10E-02
3	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**108**	**41**	**0**	**R.VLGEDFVGIAFR.A**	**108.41**	**1.60E-08**
50-58 kDa	**hypothetical protein MGG_10712**	**cellobiohydrolase I (GH7)**	***Magnaporthe grisea* 70-15**	**gi|39971383**	**95**	**49**	**0**	**R.FNEVGGWSK.L**	**51.4**	**8.80E-03**
							0	**R.FNEVGGWSK.L**	**59.19**	**1.50E-03**
							0	**R.FNEVGGWSK.L**	**64.02**	**4.80E-04**

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
3 (cont.) 50-58 kDa							12	*de novo* VAATDGCTTVDGEVVIDANSVR (Scan 10262)	-	6.60E-02
							4	*de novo* WLDSTYPETSTSQAPR (Scan 10087)	-	6.70E-02
							2	*de novo* VDGEVVIDANWR (Scan 10247)	-	2.10E-02
							3	*de novo* TSGDALSLKFVTK (Scan 10274)	-	3.90E-01
							8	*de novo* SMGGESADGCDFNPYR (Scan 10131)	-	7.00E-01
							8	*de novo* HNHGDFYGPGLTVDTSK (Scan 9971)	-	3.00E+00
	unnamed protein product	cellobiohydrolase I (GH7)	*Sordaria macrospora*	gi|289620161	-	49	6	*de novo* HNHGDFYGPGLTVDTSK (Scan 9971)	-	6.00E-03
	hypothetical protein SS1G_04945	cellobiohydrolase I (GH7)	*Sclerotinia sclerotiorum*	gi|156055188	-	60	6	*de novo* SMGGESADGCDFNPYR (Scan 10131)	-	1.50E-02
	isoamyl alcohol oxidase	**isoamyl alcohol oxidase**	***Gibberella zeae***	**gi|82779927**	**87**	**62**	**0**	**K.DENPDLFWALR.G**	**87.12**	**2.30E-06**
	hypothetical protein SNOG_08778	**aminopeptidase Y; PA_ScAPY_like**	***Phaeosphaeria nodorum* SN15**	**gi|169611336**	**85**	**54**	**0**	**K.LQDIADANGGNR.A**	**84.64**	**4.20E-06**
							15	*de novo* VPVTSSTSLQELVTLDDLFAGSQK (Scan 10406)	-	1.30E+01
	hypothetical protein	aminopeptidase Y; PA_ScAPY_like	*Aspergillus nidulans* FGSC A4	gi|67902916	-	54	12	*de novo* VPVTSSTSLQELVTLDDLFAGSQK (Scan 10406)	-	1.30E+01
	glycosyl hydrolase family 43 protein	**β-xylosidase (GH43)**	***Aspergillus fumigatus* Af293**	**gi|70982855**	**74**	**50**	**0**	**K.DDDGSAYLLTEDRPNGLR.I**	**73.6**	**3.80E-05**
	hypothetical protein BC1G_09832	**β-glucosidase (GH3)**	***Botryotinia fuckeliana* B05.10**	**gi|154302511**	**69**	**105**	**0**	**K.HFVGNEQEHFR.Q**	**69.16**	**1.40E-04**
	exo-beta-1,3-glucanase	β-glucosidase (GH3)	*Aspergillus nidulans* FGSC A4	gi|259488941	-	84	4	*de novo* DGAVGDGSTDDSDAIQK (Scan 9695)	-	2.00E-03
	hypothetical protein CHGG_10708	**endo-1,4-β-mannanase (GH5)**	***Chaetomium globosum* CBS 148.51**	**gi|116205649**	**67**	**46**	**0**	**K.TVEWTDQWIR.D**	**66.95**	**2.40E-04**
							4	*de novo* NHDDGFTIFLDDEEAKTLVYK (Scan 10546)	-	2.00E-09
							8	*de novo* PFDTPKTIFLDDEEAQTLVYK (Scan 10536)	-	4.00E-04
							7	*de novo* YEHQQTIFLDDEEAQTLVYK (Scan 10591)	-	4.00E-04
							8	*de novo* YVPTPKTIFLDDEEAQTLVYK (Scan 10603)	-	4.00E-04

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
3 (cont.) 50-58 kDa							8	*de novo* MASHRCTIFLDDEEAQTLVYK (Scan 10635)	-	4.00E-04
							8	*de novo* TMPPLFTIFLDDEEAQTLVYK (Scan 10567)	-	3.00E-05
	hypothetical protein MPER_02553	**GMC oxidoreductase**	***Moniliophthora perniciosa* FA553**	**gi|238607896**	**60**	**7**	**0**	**R.AGAAILER.F**	**60.46**	**1.30E-03**
	hypothetical protein CIMG_03818	**hypothetical protein**	***Coccidioides immitis* RS**	**gi|119187541**	**55**	**48**	**1**	**R.KTGQLLSLR.A**	**55.07**	**4.40E-03**
	exoglucanase	**cellobiohydrolase I (GH7)**	***Humicola grisea var. thermoidea***	**gi|4204214**	**54**	**50**	**0**	**K.HEYGTNIGSR.F**	**18.18**	**2.00E+01**
							0	**K.HEYGTNIGSR.F**	**54.28**	**4.90E-03**
							2	*de novo* TSGDALSLKFVTK (Scan 10274, 10362, 10378, 10423)	-	3.70E-02
							1	*de novo* VDGEVVIDANWR (Scan 10247)	-	6.00E-03
							5	*de novo* FAGLYTHGCDYNPYR (Scan 10224)	-	5.00E-02
							11	*de novo* VAATDGCTTVDGEVVIDANSVR (Scan 10262)	-	8.80E-02
	unnamed protein product	**hypothetical protein**	***Candida glabrata***	**gi|50288161**	**49**	**164**	**0**	**K.DIIGFLNR.N**	**48.89**	**2.00E-02**
	hypothetical protein FG09018.1	**hypothetical protein**	***Gibberella zeae* PH-1**	**gi|46132978**	**48**	**35**	**0**	**R.AVEEVMR.D**	**48.41**	**2.20E-02**
	Pc21g09510	**FAD/FMN-containing dehydrogenase**	***Penicillium chrysogenum* Wisconsin 54-1255**	**gi|211589700**	**47**	**64**	**1**	**K.CQNKDLFWAIR.G**	**47.08**	**2.20E-02**
	hypothetical protein NCU02192	**hypothetical protein**	***Neurospora crassa* OR74A**	**gi|164426947**	**46**	**23**	**0**	**R.LDGTCAALLPK.I**	**45.68**	**3.50E-02**
	rhamnogalacturonan lyase	**rhamnogalacturonan lyase (PL4)**	***Pyrenophora tritici-repentis* Pt-1C-BFP**	**gi|189193225**	**42**	**54**	**0**	**R.SLTVGTYR.G**	**42.29**	**7.00E-02**
							1	*de novo* VLPGTYTFTVYK (Scan 9950)	-	9.00E-03
	unnamed protein product	cellobiohydrolase I (GH7)	*Podospora anserina* S mat+	gi|171682802	-	51	6	*de novo* FTVVTQFHTNAAGDLTDIKR (Scan 10050)	-	4.00E-05
							8	*de novo* FTVVTQFHTNAAGDLTVTR (Scan 10130)	-	6.60E-02
	hypothetical protein	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39973029	-	48	10	*de novo* VAATDGCTTVDGEVVIDANSVR (Scan 10262)	-	8.00E-04
							1	*de novo* VDGEVVIDANWR (Scan 10247)	-	2.00E-03
	cellulase CEL2	cellobiohydrolase I (GH7)	*Leptosphaeria maculans*	gi|7804885	-	49	2	*de novo* PAVGDQQTETHPK (Scan 9658)	-	9.00E-03
							3	*de novo* PAVGDQETETHPK (Scan 9671)	-	6.70E-02

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
3 (cont.)										
	alpha-mannosidase	α-mannosidase (GH47)	*Ajellomyces capsulatus* H143	gi|240274614	-	58	4	*de novo* WKYDFQESFLFAEVFK (Scan 10527)	-	9.00E-04
4 38-50 kDa	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**327**	**41**	**0**	**K.WDAIEPSR.G**	**30.05**	**1.50E+00**
							0	**R.VLGEDFVGIAFR.A**	**73.31**	**5.30E-05**
							0	**R.VLGEDFVGIAFR.A**	**90.17**	**1.10E-06**
							0	**R.VLGEDFVGIAFR.A**	**85.26**	**3.40E-06**
							0	**R.VLGEDFVGIAFR.A**	**46.13**	**2.80E-02**
							0	**R.VLGEDFVGIAFR.A**	**82.98**	**5.70E-06**
							0	**R.VLGEDFVGIAFR.A**	**81.51**	**8.00E-06**
							0	**R.VLGEDFVGIAFR.A**	**70.58**	**9.90E-05**
							0	**K.LYINDYNLDIANYAK.V**	**90.32**	**8.60E-07**
							0	**R.NTLTQVIQNHVTTMVTR.Y + Ox (M)**	**32.98**	**4.40E-01**
							9	*de novo* KDMGDEATDEIFAEDGSLR (Scan 10663)	-	6.60E-02
	hypothetical protein	endo-1,4-β-xylanase (GH10)	*Magnaporthe grisea* 70-15	gi|39973147	-	40	10	*de novo* WVAWTALAVLESYAATGVEVAYTELDVR (Scan 11394)	-	7.00E-06
	endoxylanase D	endo-1,4-β-xylanase (GH10)	*Gibberella zeae* PH-1	gi|46139579	-	41	5	*de novo* QFTFSNADAVVSFAQADGK (Scan 10451)	-	1.60E-01
	unnamed protein product	endo-1,4-β-xylanase (GH10)	*Sordaria macrospora*	gi|289617806	-	42	1	*de novo* LYYNDYNLEAPSPK (Scan 10139, 10172)	-	1.00E-05
	metalloprotease MEP5	**metallopeptidase (M36)**	***Microsporum canis***	**gi|238845207**	**100**	**70**	**0**	**R.NAIIDADK.N**	**67.36**	**3.10E-04**
							0	**R.DSSFEAGTVIHEYTHGLSNR.L**	**61.06**	**6.20E-04**
							0	**R.DSSFEAGTVIHEYTHGLSNR.L**	**45.7**	**2.10E-02**
							8	*de novo* YHDVLYVLGFTEAAGNFEVNNDGQNK (Scan 10656)	-	2.00E-05
							9	*de novo* TINPSSYVDASTTQLFYTANR (Scan 10404)	-	9.00E-04
							7	*de novo* TNAAIAQSNPSGGSAYLNNPR (Scan 9880)	-	3.00E-03

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
4 (cont.) 38-50 kDa							8	*de novo* PFSFDSGVVIHEYTHGWKR (Scan 10263)	-	6.00E-03
							9	*de novo* WVAIAQSNPSGGSAYLNNPR (Scan 9886)	-	5.00E-02
							9	*de novo* SPSYVTQGDASTTQLFYTANR (Scan 10360)	-	6.60E-02
							11	*de novo* SPSYATNVDASTTZLFYTANR (Scan 10374)	-	6.60E-02
							12	*de novo* PSSAKPGPVDASTTQLFTANR (Scan 10384)	-	8.80E-02
	extracellular elastinolytic metalloproteinase	metallopeptidase (M36)	*Verticillium albo-atrum* VaMs.102	gi|261357727	-	59	6	*de novo* TNAAIAQSNPSGGSAYLNN PR (Scan 9880)	-	2.00E-06
								de novo WVAIAQSNPSGGSAYLNNPR (Scan 9886)	-	4.00E-05
	hypothetical protein SNOG_10695	metallopeptidase (M36)	*Phaeosphaeria nodorum* SN15	gi|169615080	-	67	6	*de novo* TINPSSYVDASTTQLFYTANR (Scan 10404)	-	3.00E-06
							7	*de novo* SPSYATNVDASTTZLFYTANR (Scan 10374)	-	9.00E-04
							10	*de novo* PSSAKPGPVDASTTQLFTANR (Scan 10384)	-	3.00E-03
	hypothetical protein MPER_02553	**GMC oxidoreductase**	***Moniliophthora perniciosa* FA553**	**gi|238607896**	**58**	**7**	**0**	**R.AGAAILER.F**	**58.1**	**2.20E-03**
	hypothetical protein MGG_02371	GMC oxidoreductase	*Magnaporthe oryzae* 70-15	gi|145606362	-	67	4	*de novo* NPPSGAELLGIYYPR (Scan 10494)	-	4.00E-03
	hypothetical protein MGG_10712	**cellobiohydrolase I (GH7)**	***Magnaporthe grisea* 70-15**	**gi|39971383**	**50**	**49**	**0**	**R.FNEVGGWSK.L**	**49.6**	**1.30E-02**
							15	*de novo* TDTADWDFYFAGSNAYYFPFGGKADAEK (Scan 10723)	-	2.00E-06
							8	*de novo* PGYGADFYGPGLTVDTSK (Scan 9991)	-	3.00E-03
							3	*de novo* TSGDALSLKFVTK (Scan 10290)	-	3.90E-01
	unnamed protein product	cellobiohydrolase I (GH7)	*Sordaria macrospora*	gi|289620161	-	49	7	*de novo* PGYGADFYGPGLTVDTSK (Scan 9991)	-	6.00E-03
							1	*de novo* TSGDALSLKFVTK (Scan 10290)	-	6.00E-03
	rhamnogalacturonan lyase	**rhamnogalacturonan lyase (PL4)**	***Pyrenophora tritici-repentis* Pt-1C-BFP**	**gi|189193225**	**49**	**54**	**0**	**R.SLTVGTYR.G**	**48.98**	**1.50E-02**
	exoglucanase	**cellobiohydrolase I (GH7)**	***Humicola grisea* var. *thermoidea***	**gi|4204214**	**48**	**50**	**0**	**K.HEYGTNIGSR.F**	**48.42**	**1.90E-02**
							3	*de novo* TSGDALSLEFVTK (Scan 10322)	-	2.00E-05

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
4 (cont.) 38-50 kDa							2	*de novo* TSGDALSLKFVTK (Scan 10290)	-	3.70E-02
							9	*de novo* QPLVLVFSIWDDGYANFR (Scan 10554)	-	5.20E-01
	hypothetical protein CHGG_10708	**endo-1,4-β-mannanase(GH5)**	***Chaetomium globosum* CBS 148.51**	**gi|116205649**	**48**	**46**	**0**	**K.TVEWTDQWIR.D**	**48.21**	**1.80E-02**
							4	*de novo* NHDDGFTIFLDDEEAQTLVYK (Scan 10592, 10593, 10594)	-	2.00E-09
							10	*de novo* TDTADWDFYFAGSNAYYFPFGGKADAEK (Scan 10723)	-	7.00E-06
							9	*de novo* QPLVLVFSIWDDGYANFR (Scan 10644)	-	4.00E-04
	hypothetical protein MGG_11999	**endo-1,4-β-mannanase (GH5)**	***Magnaporthe grisea* 70-15**	**gi|145606609**	**48**	**45**	**1**	**R.AMVTRYADSPAIMAWEIANEPR.C**	**47.71**	**1.20E-02**
							14	*de novo* TDTADWDFYFAGSNAYYFPFGGKADAEK (Scan 10723)	-	7.00E-06
	hypothetical protein MGG_06524	**histidine phosphatase**	***Magnaporthe grisea* 70-15**	**gi|145608592**	**47**	**51**	**0**	**R.IGGDSELSPR.G**	**47.38**	**2.50E-02**
	endoglucanase I	**endoglucanase (GH5)**	***Robillarda* sp.Y-20**	**gi|6855474**	**44**	**41**		**K.GAYAILDPHNYMR.Y**	**44.4**	**3.80E-02**
								K.GAYAILDPHNYMR.Y	**32.62**	**5.70E-01**
							3	*de novo* AVDGSPGLYETLWLK (Scan 10659)	-	8.00E-04
							7	*de novo* DYAFIDESTVDLFVDEKH (Scan 10578, 10595)	-	8.90E-02
							5	*de novo* AVDGSPGLYETVESPK (Scan 10647)	-	2.10E-02
							6	*de novo* AVDGSPGLYETIFALK (Scan 10423, 10482)	-	1.60E-01
							5	*de novo* AVDGSPGLYETIMRR (Scan 10542)	-	1.60E-01
							5	*de novo* AVDGSPGLYETLDRR (Scan 10559)	-	1.60E-01
							3	*de novo* ATPGTGLPGVFGK (Scan 10231)	-	4.10E+00
	glycosyl hydrolase family 43 protein	β-xylosidase (GH43)	*Aspergillus fumigatus* Af293	gi|70982855	-	50	12	*de novo* VEGLFKDDDGSAYLLPLGYAVLVAR (Scan 10459, 10460)	-	2.00E-04

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
4 (cont.)	conserved hypothetical protein	cellobiohydrolase II (GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	11	*de novo* RALADCCGVGILGVVIYDLPGR (Scan 10891)	-	1.50E-02
							4	*de novo* RAKVADLGTFVWVDTR (Scan 10736)	-	3.70E-02
	alpha-mannosidase	α-mannosidase (GH47)	*Ajellomyces capsulatus* H143	gi|240274614	-	58	5	*de novo* WYTAAFQESFLFAEVFK (Scan 10580)	-	1.50E-02
5 30-38 kDa	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**484**	**41**	**0**	**K.WDAIEPSR.G**	**27.57**	**2.60E+00**
							0	**K.WDAIEPSR.G**	**33.17**	**7.30E-01**
							0	**R.VLGEDFVGIAFR.A**	**73.26**	**5.30E-05**
							0	**R.VLGEDFVGIAFR.A**	**73.31**	**5.30E-05**
							0	**R.VLGEDFVGIAFR.A**	**57.6**	**2.00E-03**
							0	**R.VLGEDFVGIAFR.A**	**64.89**	**3.70E-04**
							0	**R.VLGEDFVGIAFR.A**	**86.53**	**2.50E-06**
							0	**R.VLGEDFVGIAFR.A**	**71.03**	**8.90E-05**
							0	**R.VLGEDFVGIAFR.A**	**22.99**	**5.70E+00**
							0	**R.VLGEDFVGIAFR.A**	**68.02**	**1.80E-04**
							0	**R.VLGEDFVGIAFR.A**	**64.23**	**4.30E-04**
							0	**R.VLGEDFVGIAFR.A**	**89.02**	**1.40E-06**
							0	**R.VLGEDFVGIAFR.A**	**82.82**	**5.90E-06**
							0	**R.VLGEDFVGIAFR.A**	**68.44**	**1.60E-04**
							0	**R.VLGEDFVGIAFR.A**	**77.16**	**2.20E-05**
							0	**R.VLGEDFVGIAFR.A**	**78.7**	**1.50E-05**
							0	**K.LYINDYNLDIANYAK.V**	**87.15**	**1.80E-06**
							0	**R.NTLTQVIQNHVTTMVTR.Y + Ox (M)**	**37.13**	**1.70E-01**
							0	**R.NTLTQVIQNHVTTMVTR.Y + Ox (M)**	**20.74**	**7.30E+00**
							8	*de novo* RTYVIVVNEIFAEDGMSR	-	8.90E-02
							3	*de novo* NTFGQVTNENSFK (Scan 9822)	-	1.00E+01

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
5 (cont.)							9	*de novo* QFTFSNADAVVSFAQAD (Scan 10458, 10490)	-	1.80E+01
30-38 kDa	endoxylanase D	endo-1,4-β-xylanase (GH10)	*Gibberella zeae* PH-1	gi|46139579	-	41	5	*de novo* QFTFSNADAVVSFAQADGK (Scan 10458, 10490)	-	2.00E-04
								de novo NTFGQVTNENSFK (Scan 9822)	-	3.90E-01
	Abf2 putative arabinofuranosidase	**α -L-arabinofuranosidase (GH62)**	***Hypocrea jecorina* (QM6a)**	**gi|31747156**	**97**	**35**	**0**	**K.ANSGATWTNDISHGDLIR.S**	**97.44**	**1.60E-07**
								de novo KQYLFIVEAIGADGR (Scan 10519)		4.00E-04
	unnamed protein product	**cellobiohydrolase I (GH7)**	***Podospora anserina***	**gi|171676762**	**67**	**56**	**0**	**R.YGGVCDPDGCDFNSYR.M**	**66.67**	**2.00E-04**
							5	*de novo* TVVTQFHTNAAGPLAYIK (Scan 10207)	-	2.90E-01
	cellulose 1,4-beta-cellobiosidase	cellobiohydrolase I (GH7)	*Chaetomium thermophilum*	gi|156712282	-	56	4	*de novo* TVVTQFHTNAAGPLAYIK (Scan 10207)	-	2.80E-02
							7	*de novo* DPLVLVFSIWDDGYANFR (Scan 10627)	-	3.70E-02
	exoglucanase	**cellobiohydrolase I (GH7)**	***Humicola grisea* var. *thermoidea***	**gi|4204214**	**57**	**50**	**0**	**K.HEYGTNIGSR.F**	**57.03**	**2.60E-03**
							3	*de novo* YVTEHQYGTNIGSR (Scan 9818)		1.50E-02
	rhamnogalacturonan lyase	**rhamnogalacturonan lyase (PL4)**	***Pyrenophora tritici-repentis* Pt-1C-BFP**	**gi|189193225**	**56**	**54**	**0**	**K.ASGGPFFR.S**	**55.98**	**3.20E-03**
							2	*de novo* VLPGTYTFTVYK (Scan 10012)	-	7.10E-01
	hypothetical protein MGG_10712	**cellobiohydrolase I (GH7)**	***Magnaporthe grisea* 70-15**	**gi|39971383**	**56**	**49**	**0**	**R.FNEVGGWSK.L**	**55.77**	**3.20E-03**
							6	*de novo* DHFYGPGLTVDTSK (Scan 10039, 10138)	-	3.20E+01
							9	*de novo* WLDSTYPETSTSQAPR (Scan 10144)	-	9.90E+00
	1,4-beta-D-glucan cellobiohydrolase B precursor	cellobiohydrolase I (GH7)	*Aspergillus niger*	gi|145230535	-	56	2	*de novo* FYGPGLTVDTYAP (Scan 10138)	-	2.80E-02
	hypothetical protein MGG_06834	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39977899	-	48	3	*de novo* WLDSTYPETSTSQAPR (Scan 10144)	-	6.70E-02
	hypothetical protein MGG_04922	**α-L-arabinofuranosidase (GH43)**	***Magnaporthe grisea* 70-15**	**gi|39940636**	**49**	**40**	**0**	**R.YIISQPR.E**	**49.18**	**2.00E-02**
							2	*de novo* LPDDKWAIDGALFR (Scan 10570)		3.00E-04
	YALI0F18392p	**hypothetical protein**	***Yarrowia lipolytica***	**gi|50556336**	**45**	**80**	**1**	**R.QEYEGALRR.N**	**44.56**	**4.70E-02**

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
5 (cont.) 30-38 kDa	**Glyceraldehyde-3-phosphate dehydrogenase**	**Glyceraldehyde-3-phosphate dehydrogenase**	***Paracoccidioides brasiliensis* Pb01**	**gi|30580398**	**44**	**36**	**0**	**K.VGINGFGR.I**	**27.4**	**2.90E+00**
							1	**K.AAVKAASEGELK.G**	**44.02**	**5.10E-02**
							1	**K.AAVKAASEGELK.G**	**41.84**	**8.30E-02**
	hypothetical protein CHGG_10708	endo-1,4-β-mannanase (GH5)	*Chaetomium globosum* CBS 148.51	gi|116205649	-	46	4	*de novo* NHDDGFTIFLDDEEAQTLVYK (Scan10674)	-	2.00E-09
								de novo AIFAWELANEPR (Scan 10691)	-	3.00E-03
	Pc21g14050	endo-1,4-β-mannanase (GH5)	*Penicillium chrysogenum* WiScanonsin 54-1255	gi|255955337	-	46	2	*de novo* STAIFAWELANEPR (Scan 10543)	-	8.00E-04
							4	*de novo* WGSIFAWELADEPR (Scan 10592-94)	-	9.00E-04
							3	*de novo* TAWYTNAAAQTVYR (Scan 10163, 10196)	-	3.00E-03
							5	*de novo* YFAGSDSYWIFSLR (Scan 10523)	-	6.70E-02
	mannan endo-1,4-beta-mannosidase	endo-1,4-β-mannanase (GH5)	*Verticillium albo-atrum* VaMs.102	gi|261355398	-	50	1	*de novo* STAIFAWELANEPR (Scan 10543)	-	1.00E-04
							3	*de novo* TWYTDAAAQAEYR (Scan 10107)	-	9.00E-03
							3	*de novo* TWYTNAAAEAEYR (Scan 10212)	-	1.20E-02
	glycosyl hydrolase family 43 protein	β-xylosidase (GH43)	*Aspergillus fumigatus* Af293	gi|70982855	-	50	0	*de novo* GLFKDDDGSAYLLTEDR (Scan 10504-12)	-	3.00E-08
	conserved hypothetical protein	cellobiohydrolase II (GH6)	*Chaetomium globosum* CBS 148.51	gi|116201509	-	42	6	*de novo* KNVETFGAALATAGFPNHAIIDCSR (Scan 10614)	-	2.00E-05
	hypothetical protein NCU06949	subtilisin protease;proteinase K-like (S8)	*Neurospora crassa* OR74A	gi|85090020	-	46	5	*de novo* AGTNVLDGYEWAVDDIIAK (Scan 10578)	-	2.00E-04
							2	*de novo* MDGYEWAVNDIIAK (Scan 10812)	-	1.00E-04
							3	*de novo* LDGYEWAVNDIIAK	-	3.00E-03
	serine protease	subtilisin protease;proteinase K-like (S8)	*Ophiocordyceps sinensis*	gi|161897705	-	40	2	*de novo* SAAVNQAAAALVK (Scan 10067)	-	2.90E-01
6 17-30 kDa	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**359**	**41**	**0**	**K.WDAIEPSR.G**	**30.76**	**1.30E+00**
							0	**R.VLGEDFVGIAFR.A**	**73.25**	**5.30E-05**
							0	**R.VLGEDFVGIAFR.A**	**86.33**	**2.60E-06**

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
6 (cont.) 17-30 kDa							0	R.VLGEDFVGIAFR.A	62.79	5.90E-04
							0	R.VLGEDFVGIAFR.A	75.28	3.30E-05
							0	R.VLGEDFVGIAFR.A	89.8	1.20E-06
							0	R.VLGEDFVGIAFR.A	72.02	7.10E-05
							0	R.VLGEDFVGIAFR.A	65.34	3.30E-04
							0	R.VLGEDFVGIAFR.A	85.2	3.40E-06
							0	K.LYINDYNLDIANYAK.V	92.32	5.50E-07
							0	R.NTLTQVIQNHVTTMVTR.Y	32.61	4.80E-01
							0	R.NTLTQVIQNHVTTMVTR.Y	32.81	4.60E-01
								de novo NTFGQVTNENSFK (Scan 9750)	-	1.00E+01
	endoxylanase D	endo-1,4-β-xylanase (GH10)	*Gibberella zeae* PH-1	gi|46139579	-	41	5	*de novo* FTFSNADAVVSFAQADGK (Scan 10414, 10446)	-	6.00E-05
							2	*de novo* NTFGQVTNENSFK (Scan 9750)	-	3.90E-01
	hypothetical protein	endo-1,4-β-xylanase (GH10)	*Phaeosphaeria nodorum* SN15	gi|169625326	-	35	2	*de novo* NTFGQVTNENSFK (Scan 9750)	-	2.10E-02
	subtilisin-like protease	**subtilisin protease;proteinase K-like (S8)**	***Neotyphodium lolii***	**gi|170674493**	**99**	**40**	**0**	**K.GSVANMSLGGSK.S**	**99.33**	**1.40E-07**
	Abf2 putative arabinofuranosidase	**α -L-arabinofuranosidase (GH62)**	***Hypocrea jecorina* (QM6a)**	**gi|31747156**	**87**	**35**	**0**	**K.ANSGATWTNDISHGDLIR.S**	**87.47**	**1.60E-06**
							13	*de novo* DCKAGLATNAFIVEAIGADGR (Scan 10472)		1.60E+01
	unnamed protein product	**GMC oxidoreductase**	***Podospora anserina* S mat+**	**gi|171683764**	**64**	**72**	**0**	**R.VVDASAFPR.V**	**26.53**	**3.40E+00**
							0	**R.VVDASAFPR.V**	**63.87**	**6.30E-04**
	hypothetical protein MGG_10712	**cellobiohydrolase I (GH7)**	***Magnaporthe grisea* 70-15**	**gi|39971383**	**64**	**49**	**0**	**R.FNEVGGWSK.L**	**51.05**	**9.50E-03**
							0	**R.FNEVGGWSK.L**	**53.08**	**6.00E-03**
	metalloprotease MEP5	**metallopeptidase (M36)**	***Microsporum canis***	**gi|238845207**	**59**	**70**	**0**	**R.DSSFEAGTVIHEYTHGLSNR.L**	**58.88**	**1.00E-03**
	hypothetical protein BC1G_09832	**β-glucosidase (GH3)**	***Botryotinia fuckeliana* B05.10**	**gi|154302511**	**51**	**106**	**0**	**K.EGLYIDYR.H**	**51.33**	**9.10E-03**
	unnamed protein product	**cellobiohydrolase I (GH7)**	***Podospora anserina***	**gi|171676762**	**50**	**56**	**0**	**R.YGGVCDPDGCDFNSYR.M**	**49.67**	**1.00E-02**
	predicted protein	**hypothetical protein**	***Laccaria bicolor* S238N-H82**	**gi|170112093**	**48**	**53**	**1**	**K.TLKTLGMLPR.R**	**47.52**	**2.40E-02**

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
6 (cont.) 17-30 kDa	subtilisin-like proteinase Mp1	subtilisin protease;proteinase K-like (S8)	*Magnaporthe poae*	gi|17369936	-	42	3	*de novo* SAPNAITVGATDVDNQR (Scan 9844)	-	1.00E-04
							5	*de novo* YSPASAPNAITVGAPAADR (Scan 9991)	-	1.00E-04
							4	*de novo* VLDGYEWAVKTILAK (Scan 10554)	-	7.00E-01
	subtiliase	subtilisin protease;proteinase K-like (S8)	*Ophiostoma pliferum*	gi|15808805	-	28	5	*de novo* TAGZGTYAYVVDTGVYVGHK (Scan 10444)	-	4.00E-06
							3	*de novo* FLAVAAGNESADAK (Scan 10755)	-	9.00E-02
							3	*de novo* FLAVAAGNESGEAK (Scan 10248)	-	1.60E-01
	subtilase	subtilisin protease;proteinase K-like (S8)	*Ophiostoma floccosum*	gi|15808791	-	29	2	*de novo* FLAVAAGNESGEAK (Scan 10248)	-	2.10E-02
	serine protease	subtilisin protease;proteinase K-like (S8)	*Ophiocordyceps sinensis*	gi|161897705	-	40	2	*de novo* SAAVNQAAAALVK (Scan 10140, 10159, 10176)	-	2.90E-01
	pectate lyase	pectate lyase	*Chaetomium globosum* CBS 148.51	gi|116208232	-	29	3	*de novo* GTVSVSGFYTEDYGK (Scan 10187)	-	3.70E-02
7 13-17 kDa	**hypothetical protein CHGG_00304**	**endo-1,4-β-xylanase (GH10)**	***Chaetomium globosum* CBS 148.51**	**gi|116179352**	**296**	**41**	**0**	**R.VLGEDFVGIAFR.A**	**65.33**	**3.30E-04**
							0	**R.VLGEDFVGIAFR.A**	**80.4**	**1.00E-05**
							0	**R.VLGEDFVGIAFR.A**	**76.87**	**2.30E-05**
							0	**R.VLGEDFVGIAFR.A**	**57.01**	**2.20E-03**
							0	**R.VLGEDFVGIAFR.A**	**85.86**	**2.90E-06**
							0	**R.VLGEDFVGIAFR.A**	**71.86**	**7.40E-05**
							0	**K.LYINDYNLDIANYAK.V**	**112.78**	**4.90E-09**
	hypothetical protein FG05352.1	**Major Facilitator Superfamily**	***Gibberella zeae* PH-1**	**gi|46121949**	**72**	**62**	**0**	**K.GPTLEEIAR.I**	**72.42**	**7.20E-05**
	hypothetical protein MGG_10712	**cellobiohydrolase I (GH7)**	***Magnaporthe grisea* 70-15**	**gi|39971383**	**54**	**49**	**0**	**R.FNEVGGWSK.L**	**53.81**	**5.10E-03**
	Endo-1,4-beta-xylanase F1	**endo-1,4-β-xylanase (GH10)**	***Aspergillus oryzae***	**gi|74582795**	**51**	**35**	**0**	**R.DSVFSQVLGEDFVR.I**	**50.54**	**8.70E-03**
	unnamed protein product	**pectin esterase**	***Podospora anserina* S mat+**	**gi|171682268**	**48**	**34**	**0**	**R.IWNAGDER.T**	**47.99**	**2.40E-02**
	acetate kinase	**acetate kinase**	***Pyrenophora tritici-repentis* Pt-1C-BFP**	**gi|189206552**	**47**	**45**	**0**	**R.QAVVQGVK.C**	**47.45**	**2.30E-02**

Band (MW)	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	Miss	Peptide matches	Peptide score	Expect
8 0-13 kDa	hypothetical protein CHGG_00304	endo-1,4-β-xylanase (GH10)	*Chaetomium globosum* CBS 148.51	gi|116179352	154	41	0	R.VLGEDFVGIAFR.A	74.42	4.10E-05
							0	R.VLGEDFVGIAFR.A	35.63	3.10E-01
							0	K.LYINDYNLDIANYAK.V	120.24	8.80E-10
							0	R.NTLTQVIQNHVTTMVTR.Y	40.75	7.30E-02
	hypothetical protein SNOG_08778	aminopeptidase Y; PA_ScAPY_like	*Phaeosphaeria nodorum SN15*	gi|169611336	92	54	0	K.LQDIADANGGNR.A	91.71	8.20E-07
	exoglucanase	cellobiohydrolase I (GH7)	*Humicola grisea var. thermoidea*	gi|4204214	60	50	0	K.HEYGTNIGSR.F	59.83	1.40E-03
	unnamed protein product	GMC oxidoreductase	*Podospora anserina* S mat+	gi|171683764	55	72	0	R.VVDASAFPR.V	54.55	5.40E-03
	hypothetical protein MGG_10712	cellobiohydrolase I (GH7)	*Magnaporthe grisea* 70-15	gi|39971383	53	49	0	R.FNEVGGWSK.L	53.29	5.70E-03
	rhamnogalacturonan lyase	rhamnogalacturonan lyase (PL4)	*Pyrenophora tritici-repentis* Pt-1C-BFP	gi|189193225	48	54	0	R.SLTVGTYR.G	47.97	1.90E-02
	unnamed protein product	pectin esterase	*Podospora anserina* S mat+	gi|171682268	47	34	0	R.IWNAGDER.T	46.9	3.10E-02

Table S4: Protein identifications by Q-TOF LC MS/MS of spots from 2D mannanase zymogram produced from *Doratomyces stemonitis* C8 supernatant (Fig. 3, Publication 4)

The following table contains protein identifications obtained from 2D mannanase zymogram (Fig. 3, Publication 4). All protein identifications shown in the table below were made above significance level ($p < 0.05$, protein score > 43).

Peaklists were generated by Mascot Distiller (Matrix Sciences) and searched against the NCBInr database (www.ncbi.nlm.nih.gov) using the Mascot search engine (Matrix Sciences) as described in Materials and Methods.

No significant peptide matches to database proteins were found from the spectra produced from spot 3 (Fig. 3, Publication 4) when analysed using the Mascot search engine or by *de novo* sequencing (Mascot Distiller).

Table S4: Protein identifications by Q-TOF LC MS/MS of spots from 2D mannanase zymogram produced from *D. stemonitis* C8 supernatant (Fig. 3, Publication 4)

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Matches (unique peptides)	Miss	Peptide sequence	Peptide score	Expect
1	hypothetical protein MGG_11999	endo-1,4-beta-mannosidase (GH5)	*Magnaporthe grisea* 70-15	gi|145606609	394	45	5.1	29 (1)	0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	44.72	4.10E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.9	2.00E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.55	2.20E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	53.91	5.00E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.86	2.00E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	56.59	2.80E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	50.66	1.10E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	51.45	9.10E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	51.79	8.50E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.55	2.30E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	54.89	4.20E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.97	2.00E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	62.89	6.60E-04
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	53.98	5.20E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.57	2.30E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	52.32	7.60E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	54.48	4.60E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	49.9	1.30E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	51.68	8.90E-03

Spt no	Protein name	Description	Species	Accession No.	Prt scr	Mass (kDa)	pI	Matches (unique peptides)	Miss	Peptide sequence	Peptide score	Expect
1 (cont.)									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	48.39	1.90E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	53.52	5.80E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	55.23	4.00E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	50.72	1.10E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.44	2.40E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	48.95	1.70E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	53.91	5.50E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	54.51	4.80E-03
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.58	2.40E-02
									0	R.YADSPAIMAWEIANEPR.C + Oxidation (M)	47.04	2.80E-02
2	unnamed protein product	endo-1,4-beta-mannosidase (GH5)	*Podospora anserina* S mat+	gi|171677227	187	47	8.2	11 (1)	0	K.DSPTIFGWELANEPR.C	55.41	3.10E-03
									0	K.DSPTIFGWELANEPR.C	45.37	3.10E-02
									0	K.DSPTIFGWELANEPR.C	48.5	1.50E-02
									0	K.DSPTIFGWELANEPR.C	43.05	5.30E-02
									0	K.DSPTIFGWELANEPR.C	52.57	6.00E-03
									0	K.DSPTIFGWELANEPR.C	56.57	2.40E-03
									0	K.DSPTIFGWELANEPR.C	52.13	6.70E-03
									0	K.DSPTIFGWELANEPR.C	37.69	1.90E-01
									0	K.DSPTIFGWELANEPR.C	57.91	1.80E-03
									0	K.DSPTIFGWELANEPR.C	63.71	4.70E-04
									0	K.DSPTIFGWELANEPR.C	40.63	9.50E-02
3	-	-	-	-	-	-	-	-	-	-	-	-