

Secretome profiling reveals temperature-dependent growth of *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitous opportunistic fungus. In this study, systematic analyses were carried out to study the temperature adaptability of *A. fumigatus*. A total of 241 glycoside hydrolases and 69 proteases in the secretome revealed the strong capability of *A. fumigatus* to degrade plant biomass and protein substrates. In total, 129 pathogenesis-related proteins detected in the secretome were strongly correlated with glycoside hydrolases and proteases. The variety and abundance of proteins remained at temperatures of 34°C–45°C. The percentage of endo-1,4-xylanase increased when the temperature was lowered to 20°C, while the percentage of cellobiohydrolase increased as temperature was increased, suggesting that the strain obtains carbon mainly by degrading xylan and cellulose, and the main types of proteases in the secretome were aminopeptidases and carboxypeptidases. Only half of the proteins were retained and their abundance declined to 9.7% at 55°C. The activities of the remaining β -glycosidases and proteases were merely 35% and 24%, respectively, when the secretome was treated at 60°C for 2 h. Therefore, temperatures >60°C restrict the growth of *A. fumigatus*.

***Aspergillus fumigatus*, functional secretome, temperature adaptability, opportunistic pathogen, saprophytic fungus**

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INTRODUCTION

Aspergillus fumigatus is a well-adapted saprophytic fungus that can survive and propagate under a wide range of environmental conditions, and, accordingly, is abundant in soil and decaying organic matter (Abadet al., 2010). The strong adaptability of *A. fumigatus* correlates with the diversity of functional genomic genes and the flexibility of the metabolic system (Nierman et al., 2005; Cramer, 2016). Due to the adaptability to high temperature and other industrial conditions, *A. fumigatus* has been used as high performance cell factory for enzyme production (Saqib et al., 2012; Wang et al., 2012; Jena et al., 2016); however, as an opportunistic human fun-

gal pathogen, it causes life-threatening invasive aspergillosis, which results in high mortality among immunodeficient patients (30%–90%). Therefore, the threat it poses to human health should not be overlooked (Latgé, 1999; Nierman et al., 2005; Ghazaei, 2017).

In recent years, the genomes of ten *A. fumigatus* strains have been sequenced, and large-scale genome comparisons have shown that no gene sets are shared exclusively by *A. fumigatus* or any other human pathogen, but its genomic characteristics are consistent with most saprophytic fungi (Denning et al., 2002; Nierman et al., 2005; Tekaiia and Latgé, 2005) compared with most typical saprophytic fungi, such as *Trichoderma reesei*, *Neurospora crassa*, and *A. niger*, *A. fumigatus* has a relatively higher number of glycoside hydrolase and protease superfamily genes. In addition, more

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fungi-specific transcription factors have been identified in their genome, indicating that *A. fumigatus* has the ability to degrade plant cell wall polysaccharides and proteins to acquire carbon and nitrogen sources (Tekaiia and Latgé, 2005) and has more complex and redundant gene expression regulatory mechanisms. In addition, a large expansion in the InterPro category of the major facilitator super family in *A. fumigatus* reflects their strong ability to obtain soluble substances. The number of identified oligosaccharide transporters in *A. fumigatus* is about twice that of *T. reesei* and *N. crassa*, which is consistent with the capacity for degrading plant cell wall (Miao et al., 2015). In addition, the *A. fumigatus* genome contains 22 secondary metabolism gene clusters and 16 different secondary metabolites have been identified, including gliotoxin, a broad range antimicrobial (Frisvad and Samson, 1990; Sugui et al., 2007; Gibbons et al., 2012). The *A. fumigatus* genome is also rich in specific enzymes, such as catalases, superoxide dismutases, and glutathione transferases (Burns et al., 2005; Abadet et al., 2010). All of these features equip *A. fumigatus* to survive and propagate under various conditions.

Temperature has a significant effect on the growth of microorganisms, as small temperature changes can distinguish optimal and lethal growth conditions (Leuenberger et al., 2017). Microbial diversity studies have revealed differences in the distribution of *A. fumigatus* under different temperature environments. A large quantity of *A. fumigatus* or its conidia is detectable under a normal temperature, such as the air over a sludge compost pile or garden and greenhouse soil (Warcup, 1951; Millner et al., 1977; Kozakiewicz and Smith, 1994; Paulussen et al., 2017). However, the distribution is extremely different at high temperature; for example, *A. fumigatus* was undetectable in corn straw self-heating compost (Zhang et al., 2015), and only trace amounts were detected on the surface of the compost during the early fermentation period in a 90-m³ fermenter (Zhang et al., 2016). These differences highlight the drastic impact of environmental temperatures that may be encountered by *A. fumigatus* in nature.

Genomic information determines the structure and topology of *A. fumigatus* metabolic and regulatory networks. These networks are dynamically modulated by various environmental perturbations, and the status of the entire network determines the state of fungal growth (Bensimon et al., 2012). In recent years, proteomic techniques have been applied to identify and quantify changes in network wiring in response to stimuli or under different physiological conditions, and these techniques have been used to detect abundance changes at network nodes. In addition, the ability to correlate network wiring to the phenotype is constantly advancing (Bensimon et al., 2012; Culibrk et al., 2016). Therefore, proteomic techniques have gradually become effective tools to build the dynamic network linkage between the genotype and

phenotype.

Fungi are exodigesters and produce a panel of degradation enzymes necessary for the production of nutrients from the environment and for nonsaprophytes to penetrate host tissues (Girard et al., 2013). As a result, the functional secretome explains the dynamic network linkage between the genotype and phenotype and reveals the adaptation of *A. fumigatus* under fluctuating environmental elements, such as temperature. *A. fumigatus* has been widely studied due to its crucial significance in industrial and clinical applications. For example, many thermotolerant lignocellulolytic enzymes have been purified from *A. fumigatus* and their properties have been studied (Katsimpouras et al., 2016; Robledo et al., 2016). Thermally induced pathogenic proteins (Hsp70/Hsp90) that occur during infection have been molecularly characterized (O'Meara and Cowen, 2014; Martinez-Rossi et al., 2016). However, there is no complete and systematic study about the adaptations of *A. fumigatus* at the proteomics level, and the determinants of thermal sensitivity of the proteomes remain largely uncharacterized (Leuenberger et al., 2017). Studies of these issues have great importance for applications and effective prevention of *A. fumigatus*. Therefore, in this study, *A. fumigatus* was cultured by solid-fermentation under different temperatures from 20°C to 60°C, and growth preferences were systematically analyzed via proteomics from physiological and biochemical perspectives.

RESULTS

Influence of temperature on the physiological characteristics and activities of *A. fumigatus*

A strain of *A. fumigatus* was isolated from a self-heating biomass compost pile and named *A. fumigatus* AF14. Solid fermentations were carried out from temperatures of 20°C–60°C, and the growth state of the strain was monitored by the changes in extracellular reducing sugar and protein concentrations. Extracellular reducing sugars accumulated during the first 2 days of culture, but began to decline on day 3 (Figure 1A), mainly because the polysaccharide substrates had been degraded into simple soluble sugars by glycoside hydrolases and absorbed by the strain. Therefore, changes in reducing sugar content could be an indicator of the growth and metabolic level of this fungus. As shown in Figure 1A, peak reducing sugar content was obtained on day 2 at 37°C–40°C; subsequently, fungal growth entered the exponential phase. The time needed to reach peak sugar content was delayed 2–3 days if the culture temperature was raised or lowered; therefore, temperature significantly affected fungal growth.

The strain acquired reducing sugars from the environment as a carbon source to provide energy for growth, and protein secretion levels were negatively correlated with reducing sugar content (Figure 1A and B). Protein secretion lev-

els were also significantly affected by temperature. Extracellular proteins began to be secreted on day 2 at 37°C and reached maximum levels on day 4, but were barely detectable at 60°C (Figure 1B). It should be noted that the extracellular proteins included large numbers of biomass degrading enzymes (Figure 1C). Endoglucanase activities were detected on day 1 at 37°C–45°C, which was earlier than xylanase activities (Figure 1D), and maximum endoglucanase and xylanase activities were both reached on day 4. Similar results were found in a study of *A. fumigatus* L9 (Adav et al., 2013), suggesting that temperature strongly influences the secretion of extracellular functional proteins.

The variety and concentration changes in extracellular functional components can be visualized by a functional zymogram (Xing et al., 2013; Gong et al., 2015). As shown in Figure 2A, the maximum number of high concentration

endoglucanase bands (seven isozyme bands) was detected in the temperature range of 30°C–40°C. Changes in the types of isozymes mainly occurred below 30°C or above 45°C; for example, EGL5 and EGL6 were undetectable at these temperatures. Similar results were observed for xylanases (Figure 2B): changes occurred based on concentration rather than the type of isozymes when the fungus was cultured at 30°C–37°C. Xyn2 and Xyn4 were significantly affected by low temperature, whereas Xyn5 was barely detected at higher temperatures. Few endoglucanase and xylanase bands were present in the functional zymogram at 55°C, suggesting a dramatic decline in activated protein levels. These data are consistent with other results (Figure 1C and D), both of which demonstrate the reduced ability of this fungus to secrete activated proteins. Therefore, growth of *A. fumigatus* was inhibited when temperature reached 55°C.

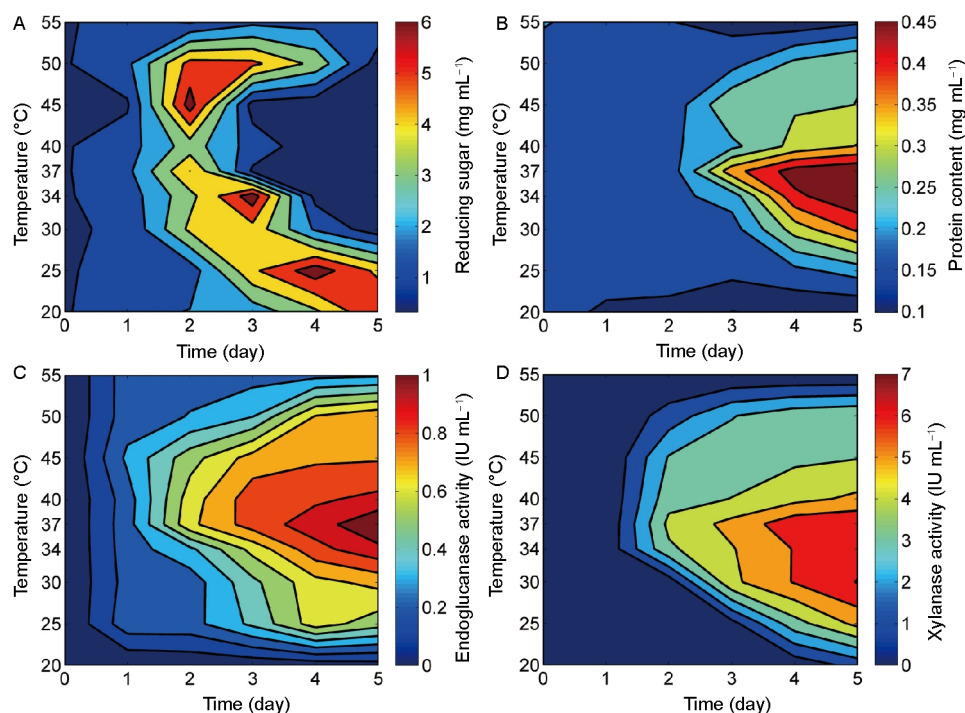


Figure 1 Dynamic changes in the concentrations of extracellular reducing sugars (A) and proteins (B) as well as activities of endoglucanases (C) and xylanases (D) during 5 days of solid-state fermentation.

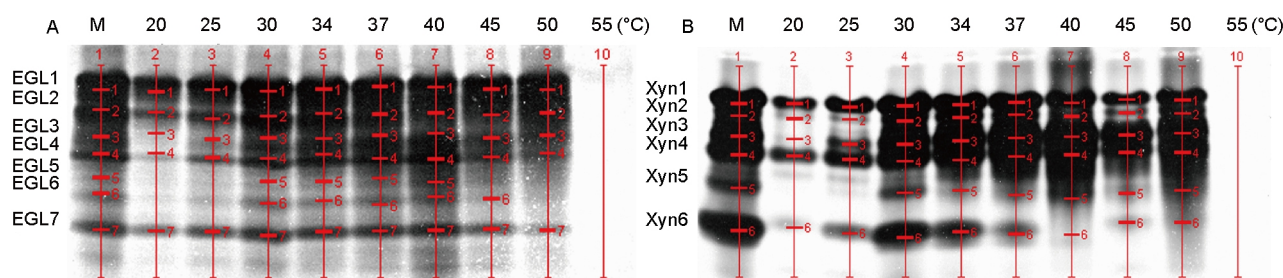


Figure 2 Day 4 functional zymogram of endoglucanases (A) and xylanases (B) secreted by *Aspergillus fumigatus* AF14 cultured at different temperatures. M, the crude exoenzyme secreted by *A. fumigatus* under the optimal culture condition was used as an enzyme marker.

Thermostability of the major glycoside hydrolases and proteases

The quantities (variety and concentration) and activities of extracellular enzymes were both significantly affected by environmental temperature. The thermostability of extracellular glycoside hydrolases and proteases secreted by *A. fumigatus* AF14 are shown in Figure 3 and Figure S1 in Supporting Information. Crude enzymes from the strain cultured at 37°C were incubated for 2 h at temperatures of 30°C–80°C, and endoglucanase activities were extremely stable at <60°C, suggesting high thermostability of the endoglucanases. Combined with previous results (Figure 2A), these results suggest that the few endoglucanase activities in the functional zymogram were due to the reduced ability of *A. fumigatus* to secrete activated proteins, rather than the influence of high temperature. In addition, the activity of each of the endoglucanase isozymes decreased by more than 50% when the temperature reached 70°C (Figure 3A). Similar results were observed for xylanase activities (Figure 3B), except Xyn5 and Xyn6 were inactivated faster than the others when the temperature was increased. Extracellular β -glucosidases and proteases had relatively lower thermostability compared with that of Xyn5 and Xyn6 (Figure 3C and D). These results show that the activities of β -glucosidases and proteases decreased progressively with increasing temperature, and that only one protease isozyme was detected with 24% activity when temperature was raised to 60°C (Figure 3D). The lower thermostability of the extracellular β -glucosidases compared with endoglucanases would hinder cello-oligosaccharide hydrolysis and carbon absorption. Proteases secreted by *A. fumigatus* AF14 were more sensitive to high temperature, which further affected fungal

growth at the elevated temperatures.

Secretome insights into the composition of the glycoside hydrolases

The highest activities and abundances of glycoside hydrolases were obtained on day 4, according to the enzyme activity and zymogram assay results (Figures 1 and 2). Thus, the secretome composition of *A. fumigatus* AF14 on day 4 was further analyzed by LTQ-Orbitrap, and 484±30 proteins were identified. The correlation between the peptide spectrum match (PSM) values of the identified proteins and the corresponding activities was >80% (Figure 4A), suggesting that changes in functional protein contents can be roughly measured by the PSM values (Liu et al., 2004; Saykhedkar et al., 2012).

Further classification according to their biological functions indicated significantly higher abundance of glucoside hydrolases, followed by proteases and pathogenic-related proteins (Figure 4A). The correlation between the three proteins at different temperatures was 80% (Figure 4B). These proteins remained relatively high in variety and abundance at temperatures of 34°C–45°C, and the correlation between secreted proteins at 34°C and 37°C was 93.4% (Figure 4A and C), suggesting that the variety and abundance of proteins was stable in this temperature range. However, increasing or decreasing the temperature changed the secretion of proteins both in variety and abundance. For example, extracellular protein content increased as temperature was increased and reached the maximum at 37°C. Protein abundance declined to 38% at 50°C compared to that at 37°C. Merely half of the proteins were retained and their abundance declined to 9.7% when the temperature was increased to 55°C (Figures 4A

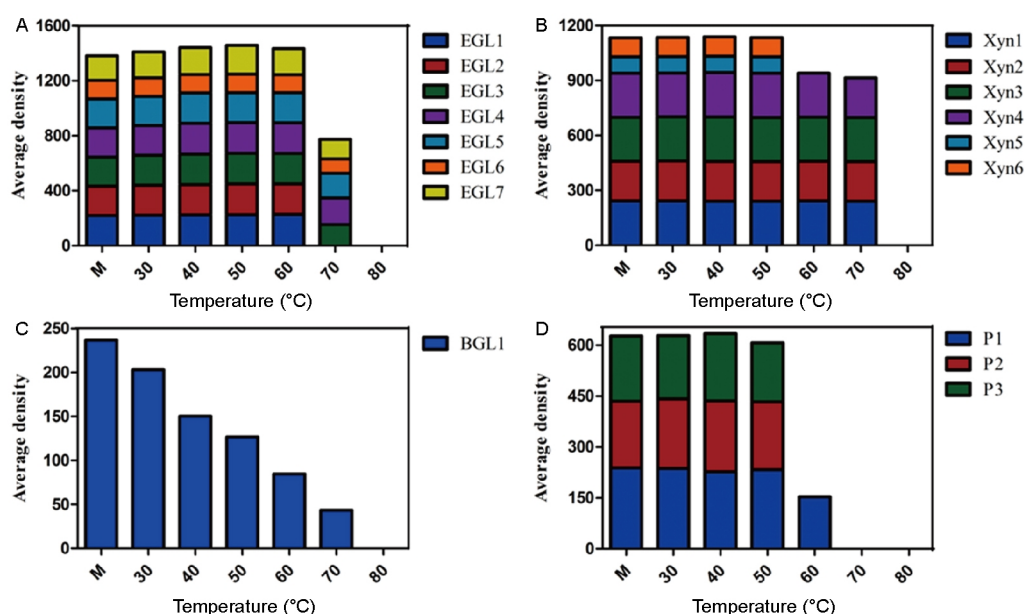


Figure 3 Thermostability of the major glycoside hydrolases and proteases secreted by *Aspergillus fumigatus* AF14 at 37°C. The average density of each isozyme band was used to describe residual activity. A, Endoglucanase activities. B, Xylanase activities. C, β -glucosidase activities. D, Protease activities.

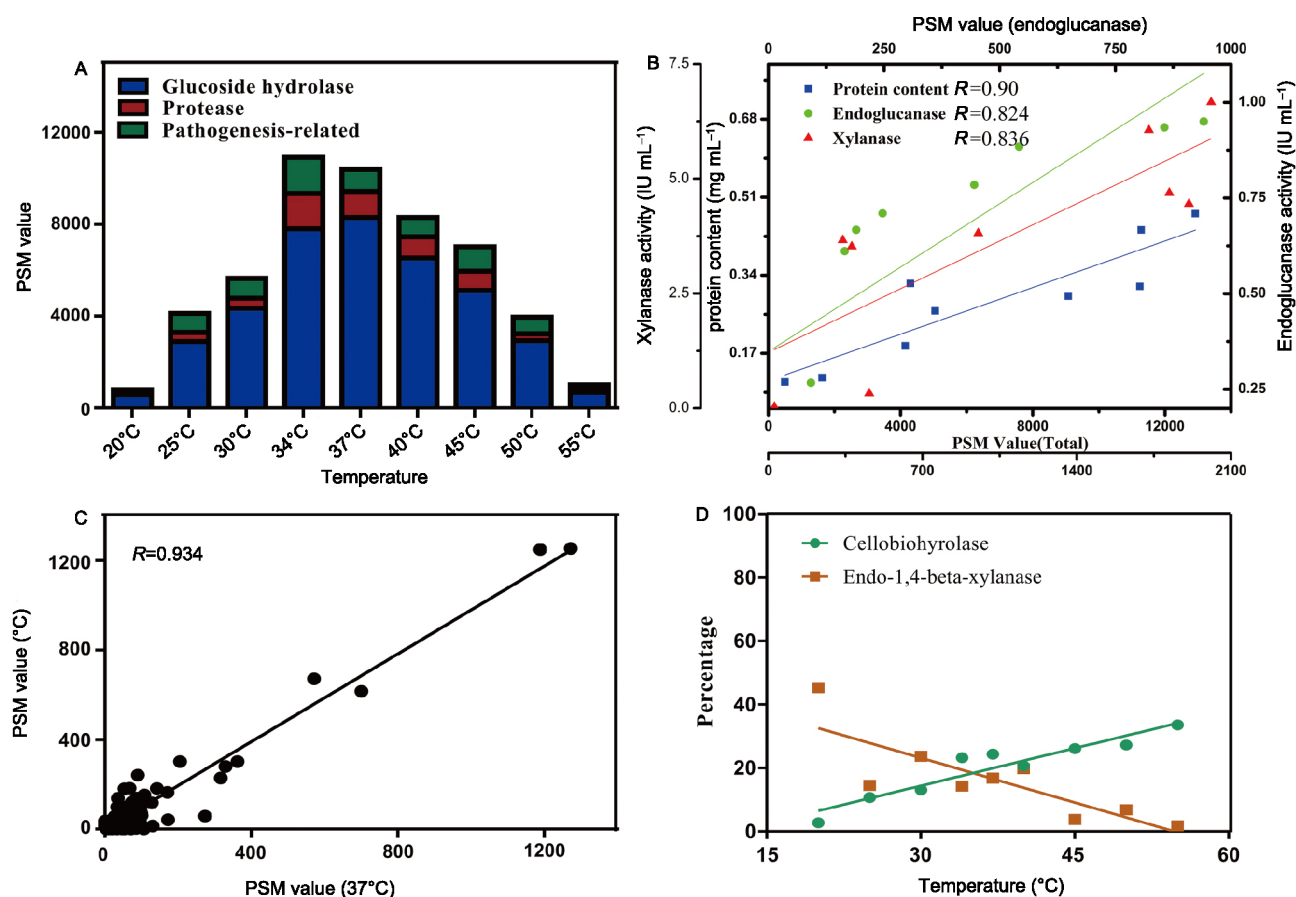


Figure 4 Correlation analysis of proteins secreted by *Aspergillus fumigatus* AF14 at different temperatures. A, Protein abundance under different temperatures. B, Correlation between PSM values and corresponding enzyme activities. C, Correlation between secretion at 34°C and 37°C. D, The percentage of cellobiohydrolases and endo-1,4- β -xylanases at different temperatures.

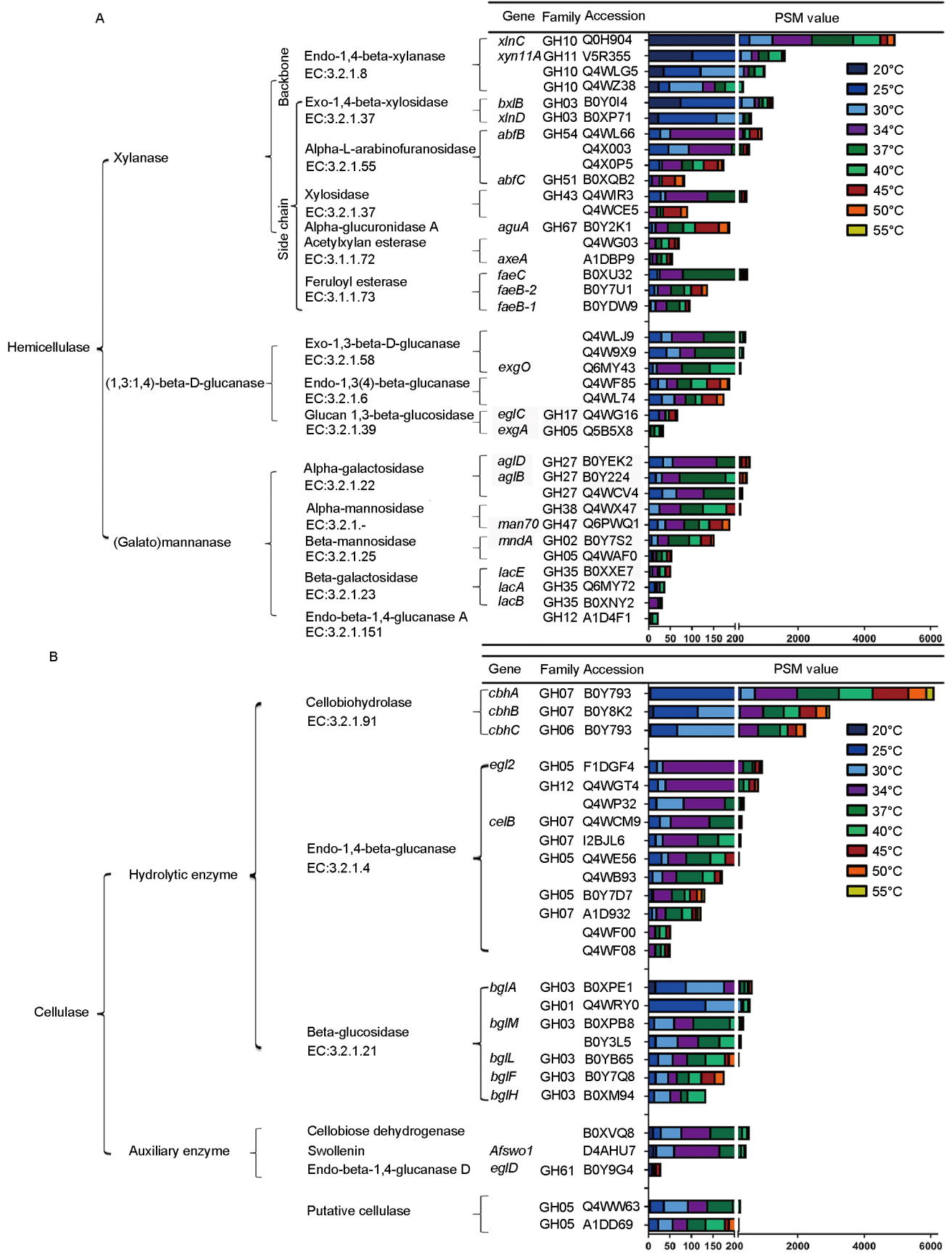
and 5–7), revealing a significant effect of temperature on the composition of the *A. fumigatus* secretome.

At least 241 proteins were classified as glycoside hydrolases in the *A. fumigatus* AF14 secretome. All cellulases and xylanases in the genome were detected by comparison with the genomic data, and other types of glycoside hydrolases were also partially detected, accounting for 56.4% of all glycoside hydrolase genes (Figure S2 in Supporting Information). Among all detected proteins, at least 12 endoglucanases and four xylan-backbone hydrolases were identified (Figure 5), and these numbers were higher than those detected in the zymogram, suggesting that the proteomic technique has higher resolution than the functional zymogram for identifying proteins. Although the LTQ-Orbitrap accurately identified the proteins, it failed to characterize the activities of the functional proteins. For instance, the abundance of proteins detected by LTQ-Orbitrap at 55°C was relatively higher than that at 20°C; however, isozyme bands were hardly detected at 55°C in contrast to the zymogram results (Figure 2). Therefore, the combination of the LTQ-Orbitrap and functional zymogram results comprehensively reflected the changes in activities and composition of the functional proteins.

All xylanases (four backbone and 12 side-chain hydrolases)

and β -(1,3:1,4)-glucanases were highly abundant (Figure 5), corresponding with their high enzymatic activities (Figure 2). Three of the identified endo-xylanases (Q0H904, Q4WLG5, and Q4WZ38) were derived from the GH10 family. Endo-xylanases degrade the xylan backbone into smaller units that are more easily absorbed by *A. fumigatus* AF14 (Gong et al., 2016). A large number of xylanases and β -(1,3:1,4)-glucanases have been identified at high abundance in *A. fumigatus* L9 and *A. fumigatus* Z5 proteomic studies (Adav et al., 2013; Liu et al., 2013), suggesting that *A. fumigatus* is an efficient degrader of xylan and β -(1,3:1,4)-glucan, which are rich in monocots. Only the side-chain hydrolases of (galacto)mannase but not the backbone hydrolases were detected due to the absence of an inducer in the substrates, suggesting that glycoside hydrolases secreted by *A. fumigatus* tend to degrade monocots more than polysaccharides when cultivated with substrates from monocots.

All pectin and xyloglucan backbone hydrolases, except rhamnogalacturonyle, β -1,6-galactanase, and β -1,4-galactanase were detected at low abundance (Figure 5 and Figure S2 in Supporting Information), whereas the side-chain hydrolases, including glucuronanlyase, β -glucuronidase, and α -fucosidase were undetectable. The genomic data suggested



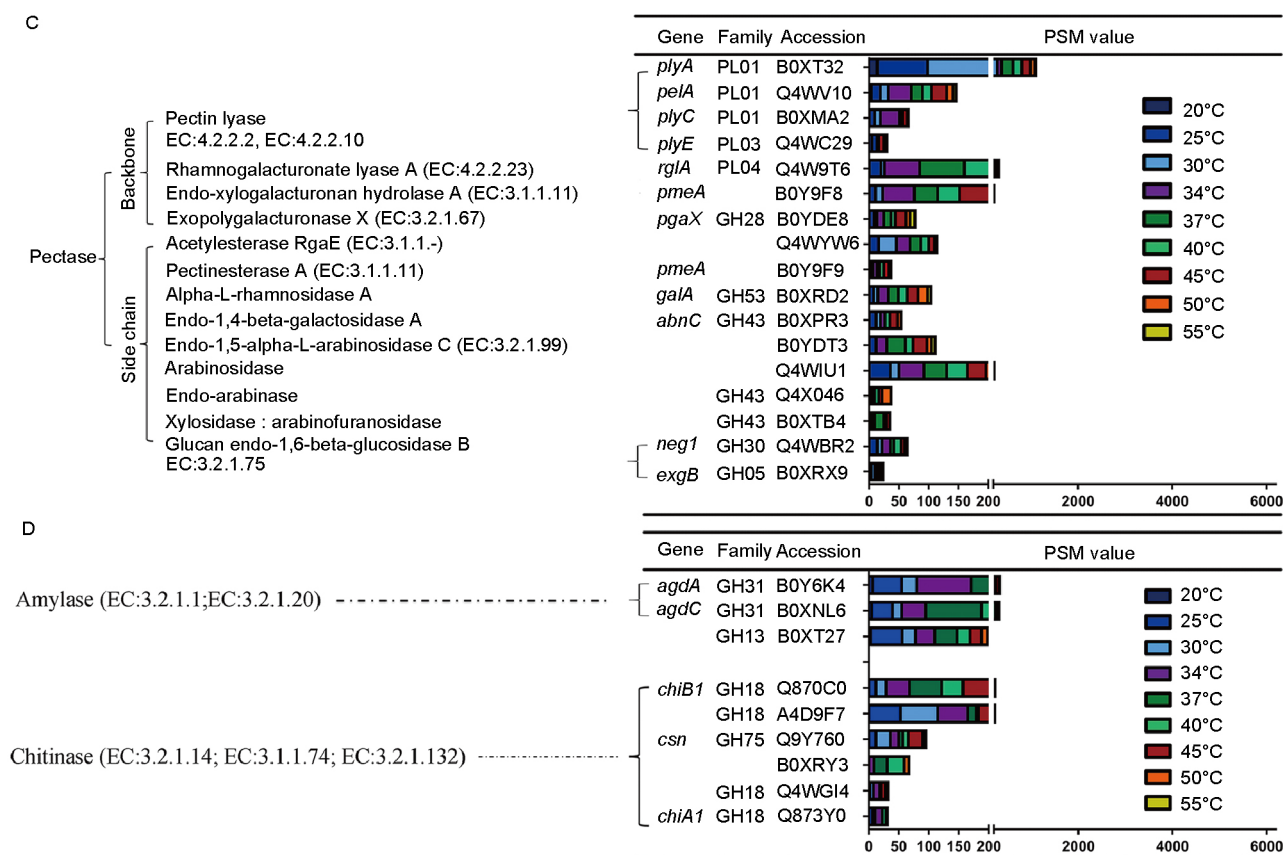


Figure 5 The main glucoside hydrolases secreted by *Aspergillus fumigatus* AF14 during solid-fermentation under different temperatures identified by LTQ-Orbitrap.

that *A. fumigatus* has no ability to completely degrade these two polysaccharides (Figure S2 in Supporting Information). The fungus reduced secretion of the corresponding proteins when the substrate did not contain these two polysaccharides, reflecting adaptation of *A. fumigatus* to substrate composition.

As reported previously (Wesenberg et al., 2003), the laccase (Q4WRN7) for lignin degradation was identified in the secretome (Figure 5). No proteins homologous to lignin peroxidase or manganese peroxidase, which are secreted by other lignin-degrading fungi such as *Phanerochaete chrysosporium* (Wesenberg et al., 2003; Tekaiia and Latgé, 2005), was found, suggesting that *A. fumigatus* plays a major role in the decay of leaves rather than hardwood. In addition, three amylases and six chitinases were detected, at relatively high abundances. Combined with the above results, the enzyme system secreted by *A. fumigatus* when cultured with complex substrates helped to obtain carbon sources from various components. Moreover, 25 polysaccharide-degrading enzymes containing carbohydrate-binding domains were detected (Figure S2 in Supporting Information), suggesting the strong capability of *A. fumigatus* to degrade insoluble substrates.

The variety and concentrations of extracellular gluco-

side hydrolases changed according to temperature (Figure 5). *A. fumigatus* secreted four endo-1,4- β -xylanases and two exo-1,4- β -xylosidases (B0Y014 and B0XP71) at 20°C, and the two endo-1,4- β -xylanases (Q0H904 and V5R355) were at relatively high abundance. The percentage of endo-1,4- β -xylanase in the secretome decreased gradually when the temperature was increased (Figure 4D), suggesting that endo-1,4- β -xylanases play a major role producing carbon at low temperatures. Both the variety and abundance of glycoside hydrolases increased when the temperature was increased. The proteins were more abundant at temperatures of 34°C–45°C. For example, only one endoglucanase and two β -glucanases were detected at 20°C; however, 12 endoglucanases and seven β -glucosidases were identified at temperatures of 34°C–45°C, indicating that the synergistic function of isoenzymes greatly improved the efficiency of the *A. fumigatus* AF14 to obtain carbon sources. Three 1,4- β -D-glucan cellobiohydrolases were detected under all temperatures, and their abundance at this temperature range was 77–116 times higher than those cultured at 20°C. These results suggest that *A. fumigatus* can degrade crystalline and amorphous cellulose, reflecting the strong ability to utilize different carbon sources. However, when the temperature was raised to 55°C, the variety and abundance of the glyco-

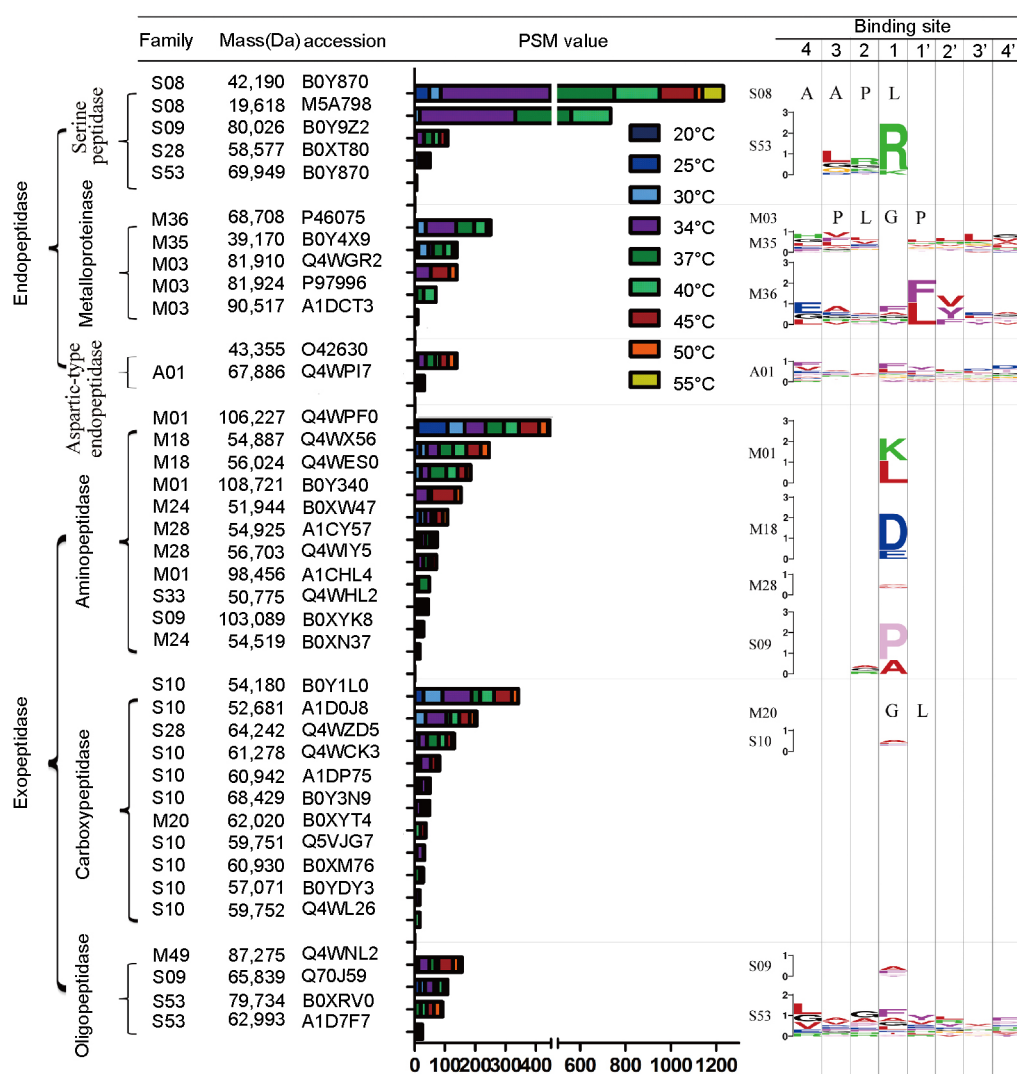


Figure 6 The main proteases secreted by *Aspergillus fumigatus* AF14 during solid-fermentation under different temperatures identified by LTQ-Orbitrap.

sidehydrolases decreased sharply. For example, five endoglucanases became undetectable and the abundance of detectable proteins declined by 91.4%. Xylan backbone-degrading enzymes decreased in diversity by 50% and in abundance by 84%. In general, mean abundance of glycoside hydrolases decreased approximately 90% at 55°C compared with the optimal temperature conditions, which reflected a significant reduction in glycoside hydrolase secretion and blockage of acquisition of fungal carbon sources to a great extent. In addition, it is interesting to note that the percentage of three identified cellobiohydrolases (B0Y793, B0Y8K2, and B0XWL3) in the entire secretome increased as temperature was increased (Figure 4D); therefore, it can be speculated that cellobiohydrolases played a major role obtaining a carbon source when *A. fumigatus* AF14 was cultured at high temperature.

Secretome insights into the composition of proteases

Saprophytic *A. fumigatus* also produced large amounts of

proteases. At least 69 proteases were detected by proteomics (Figure 6), including all of the carboxypeptidases and oligopeptidases, and most aminopeptidases, metalloproteinases, and peptidases, accounting for 74.2% of all proteases in the genome (Table S1 in Supporting Information). The identified proteases were classified into 45 exopeptidases and 24 endopeptidases, and the abundance of the exopeptidases was about 4.2 times greater than that of the endopeptidases. More types of proteases were detected in the *A. fumigatus* secretome compared with the proteases secreted by *A. niger* under the same culture conditions, and protein abundance was three to five times higher than that of *A. niger* (Table S2 in Supporting Information).

Analysis of the proteolytic substrates using the Merops database (<http://merops.sanger.ac.uk/>) suggested that the identified proteases bind a variety of amino acids, and they had a bias toward charged amino acids (R, D, E, K, and H), hydroxyamino acids (S, Y), and those with non-bulky side chains (A), especially in the -1 and +1 binding sites. These

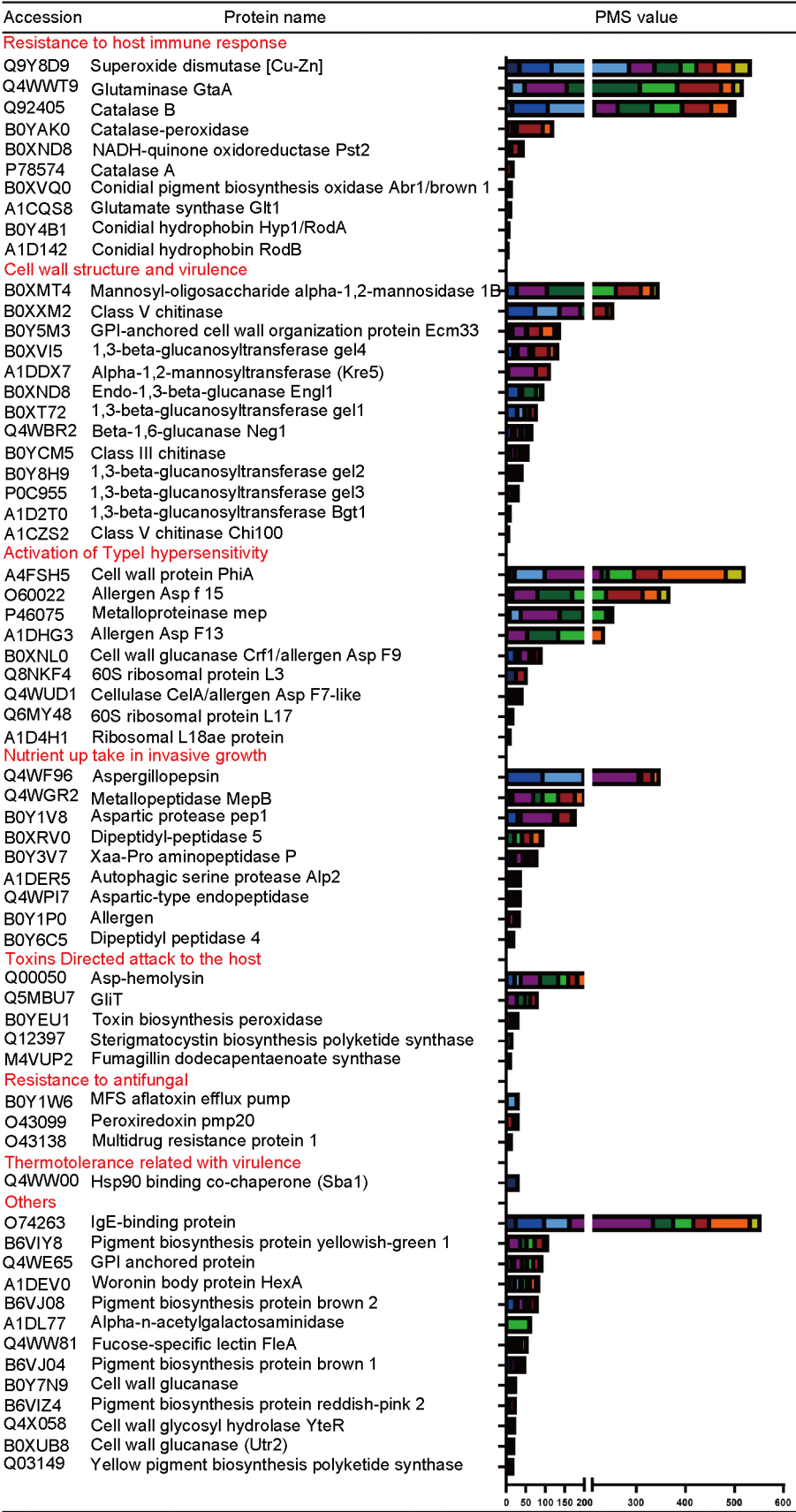


Figure 7 Pathogenesis-related proteins produced by *Aspergillus fumigatus* AF14 under different temperatures identified by LTQ-Orbitrap.

amino acids frequently appear on the surface of natural proteins (Wolfenden et al., 1981; Rose et al., 1985; Creighton, 1993). Hence, we speculated that various natural substrate proteins could be degraded by the proteases secreted by *A. fumigatus* without denaturation and deconstruction. Only two to three amino acids need to be bound by the proteases during hydrolysis (Tables S2 and S3 in Supporting Information), which reflects the flexible sequence-specific requirements for proteolytic substrates (Fuchs et al., 2013). Taken together, these results indicate that diverse natural substrates can be degraded into oligopeptides or amino acids by the proteases for efficient growth in the natural environment.

Similar to the glycoside hydrolases, protease secretion was also regulated by temperature. As shown in Figure 6, most of the proteases had higher abundances at 34°C–45°C, except the three carboxypeptidases (B0Y1L0, A1D0J8, and Q4WCK3), and the diversity and abundance of proteases were both affected by culture temperature, which was different from the glycoside hydrolases where significant changes occurred in protein abundance rather than variety. The maximum secretion of proteases occurred at 34°C, whereas that of glycoside hydrolases was at 37°C, suggesting that proteases are more sensitive to high temperature, consistent with the thermostability assay (Figure 3). Only a few proteases at trace amounts were detected when the temperature was lowered to 20°C or raised to 55°C, which included aminopeptidases and carboxypeptidases, and their degradation products were smaller peptide fragments or amino acids, which were more easily absorbed by the strain. This is an adaptation to obtain nitrogen when cultured under unfavorable temperature conditions. These results suggest that the quantities of proteases secreted by *A. fumigatus* did not hardly counteract the rapid loss of activity when the fungus was cultured at 55°C. Therefore, the ability of *A. fumigatus* to obtain nitrogen could be greatly weakened by elevating the temperature.

Secretome insights into the composition of pathogenesis-related proteins

A. fumigatus is an opportunistic human pathogen. According to a previous study on the classification of pathogenic proteins (Abadet al., 2010), large quantities of proteins (27%) in the secretome have been identified as pathogenesis-related, which contribute to the swift adaptation and rapid growth of the fungus in the host environment (Figure 7). Some of the identified proteins were highly pathogenic, including 23 proteins that participate in activation of type I hypersensitivity and nutrient uptake during invasive growth, of which Asp-hemolysin (Q00050) and GliT (Q5MBU7) are typically immunosuppressive and cytotoxic, respectively. Proteins, such as aspergillopepsin (Q4WF96), Pep1 (B0Y1V8), and MepB (Q4WGR2), hydrolyze tissue proteins, such as collagen, elastin, casein, and fibrinogen (Figure 7). All of these

functional proteins contribute to fungal growth by releasing nutrients from destroyed host cells; however, this directly damages the host (Latgé, 1999; Abad et al., 2010).

The GPI-anchored protein (Q4WE65), efflux pumps (043099, 043138, and B0Y1W6), proteins that provide resistance to the host immune response (Q4WWT9, Q92405, Q9Y8D9, P78574, Q4WKD8, B0XVQ0, A1D142, and B0Y4B1), and autophagic-related proteins (A1DER5) were also identified (Figure 7). These proteins were present at relatively high quantities in the proteome and play an important role in swift adaptation to the host environment (Latgé, 1999; Abad et al., 2010).

Secretion of pathogenesis-related proteins was also regulated by temperature, and these proteins had relatively high abundances at temperatures of 34°C–45°C, which was similar with the results of glycoside hydrolases and proteases (Figure 7), suggesting that secretion of pathogenesis-related proteins is closely related to strain growth and metabolism. If *A. fumigatus* were used as a lignocellulolytic strain in a large-scale industrial application, it would secrete a high abundance of glycoside hydrolases and a large amount of pathogenesis-related proteins, which would pose an increased threat to certain populations. Although the abundance and varieties of pathogenesis-related proteins was reduced at 55°C, these proteins are detrimental to humans, suggesting the significance of controlling *A. fumigatus* growth and distribution.

DISCUSSION

A. fumigatus is an opportunistic human pathogen and is ubiquitous in nature due to its saprophytic life cycle. The potential hazard caused by this fungus is recognized as a worldwide problem (Sheikh-Ali et al., 2014). Numerous *A. fumigatus* strains have been sequenced. In this study, the physiological and biochemical characteristics of *A. fumigatus* AF14 were comprehensively analyzed at the proteomic level, providing a linkage between genotype and phenotype (growth state). More glycoside hydrolases were identified in the *A. fumigatus* genome compared with those of *T. reesei*, *A. niger*, and *N. crassa* (Adav et al., 2013; Liu et al., 2013), which makes it easier for the fungus to obtain carbon sources and energy by degrading natural biomass (Miao et al., 2015). Analysis of the composition and dynamic changes of the glycoside hydrolases in the secretome revealed that *A. fumigatus* regulated protein secretion according to culture conditions. For example, *A. fumigatus* preferred to degrade monocots when cultured on substrates derived from monocot plants. Increasing the secretion ratio of endo-1,4- β -xylanase in the secretome made it possible for xylan to be degraded into smaller xylo-oligosaccharides that can be used directly by the strain under low temperature. The proportion of cellulases in the *A. fumigatus* secretome increased at high temperature through hydrolysis of non-side-chain crystallization or

amorphous cellulose to obtain a carbon source; thus, ensuring growth under unfavorable temperature conditions. These results reflect the adaptability of *A. fumigatus* in acquiring carbon sources.

Compared with other *Aspergillus* species, such as *A. oryzae*, *A. niger*, *A. flavus*, *A. terreus*, and *A. nidulans*, more proteases were detected in the *A. fumigatus* secretome. Further analysis revealed their biases toward shorter substrate binding sequences and amino acids containing charges and hydroxy and non-bulky side chains, especially in the -1 and +1 binding sites. Therefore, natural substrate proteins are easily degraded by these proteases without denaturation and deconstruction, which reflected the strong capability of *A. fumigatus* to obtain nitrogen in a natural environment. The main types of proteases under lower or higher temperature were aminopeptidases and carboxypeptidases, which can degrade substrates into small peptides or even amino acids that can be used directly by the strain. These results show the adaptability of *A. fumigatus* for acquiring nitrogen sources.

A. fumigatus was more thermotolerant in comparison with most other air-borne fungi, such as *Penicilliumchrysogenum*, *Cladosporiumherbarum*, *Botrytis cinerea*, *Penicilliumexpansum*, *Penicilliumbrevicaulis*, and *A. flavus*. Thermolabile strains may not survive when the temperature is increased (Singh et al., 2003), whereas *A. fumigatus* persisted and secreted proteins to support rapid growth under a wide range of pH and moisture conditions, even when the temperature was raised to 50°C (Figure S3 in Supporting Information). These characteristics make it easy for *A. fumigatus* to dominate a saprophytic environment under high temperature conditions, and it would be almost impossible to efficiently reduce the amount of *A. fumigatus* in a saprophytic environment just by changing the substrate, pH, or moisture conditions (Figures S3 and S4 in Supporting Information).

At least 129 kinds of virulence proteins were identified in the secretome, accounting for 27% of all proteins detected, and secretion of these proteins was simultaneous with that of the glycoside hydrolases and proteases. These proteins contribute to fungal colonization, swift adaptation to the host environment, and prevent of damage due to host self-protection mechanisms. *A. fumigatus* is quite efficient at natural protein degradation, which enables it to hydrolyze host tissue proteins and promote infection processes (Shenet et al., 2007). Once an infection is established in an immunodeficient population, it would do great harm to the host (McDonagh et al., 2008). If *A. fumigatus* was used as a lignocellulolytic strain for large-scale industrial applications without removing its infectious characteristics, it would pose an increased threat to some populations. However, knockout of pathogenesis-related proteins is a difficult task and would limit growth of the strains because of a decline in environmental adaptability. Therefore, appropriate methods are required to reduce the potential hazards from pathogenesis-related proteins.

A. fumigatus can easily be isolated from garden and greenhouse soil (Adav et al., 2013; Köhler et al., 2015). The proportion of *A. fumigatus* was 30.98% of the fungal community on day 5 if fermentation temperature of a laboratory fermentor composted (1 m in height and diameter) with corn straw as the substrate remained within 37°C–50°C (Figure S5 in Supporting Information). This observation indicates that *A. fumigatus* is thermotolerant, and this is closely related to its dominant growth in self-heating composts. In contrast, no *A. fumigatus* was detected when fermentation temperature was 60°C in the middle layer of natural corn straw compost (Zhang et al., 2015). By blending and mixing the contents of the fermentor, the other layers of the compost would also reach 60°C, which would inhibit the growth of *A. fumigatus* on a large scale. No *A. fumigatus* was detected in the middle layer of compost in a 90-m³ fermentor with corn straw and cow dung as substrates when the fermentation temperature was 75°C (Zhang et al., 2016). These results are similar to the growth characteristics of *A. fumigatus* when cultured under solid-state fermentation. In conclusion, *A. fumigatus* is a heat-resistant fungus rather than a thermophilic one, and continuous 60°C conditions in compost would lead to a dramatic reduction in growth of *A. fumigatus*, and further inhibit its distribution. Reducing exposure to the hemicellulose components, such as xylan, may reduce the threat in infection-susceptible areas with relative lower temperatures, such as wards with immunodeficient patients.

In addition, the functional secretome approach used here can be widely applied to physiological and biochemical studies of various microorganisms. Inactivated proteins were not distinguished by the LTQ-Orbitrap. Therefore, combining the results of LTQ-Orbitrap and a functional zymogram comprehensively reflected the changes in activities and composition of the functional proteins.

MATERIALS AND METHODS

Composting performance and sampling

The compost experiment was carried out in a cylindrical fermentor (diameter: 1 m; height: 1 m) in Shandong Province, China. The substrate ratio, initial water content, and the inoculated microorganisms were the same as used in our previous study (Zhang et al., 2016). The weight and height of the compost were ~0.9 tons and 0.9 m, respectively. The composting process lasted for 5 days. Samples were collected every 24 h at a height of 0.45 m from three different parallel sites at a depth of ~10 cm, and they were mixed to obtain the final sample with a weight of ca. 500 g that was used for further study. The temperature and moisture of the samples were also measured during the composting process.

Pyrosequencing and taxonomic classification

Total DNA from the biomass compost samples was extracted

using the PowerSoil® DNA Isolation Kit (MO-BIO Laboratories, USA), according to the manufacturer's instructions. DNA concentration was determined at 260 nm using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., USA). The barcoded fusion primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') were used to amplify the 16S rRNA genes, and 1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and 2043R (5'-GCTGCGTTCTTCATC-GATGC-3') primers were used to amplify the internal transcribed spacer rRNA from the extracted DNA. The taxonomic classification and statistical analysis of the pyrosequencing data were performed as described by Zhang et al. (Zhang et al., 2015).

Microorganism cultivation and secretome extraction

The *A. fumigatus* strain used in this study was isolated from a compost sample and was maintained in archeosporial status in 20% glycerol solution at -80°C until further experiments. The solid-state fermentation experimental design included three independent replicates and a control for each test condition. A 100 µL aliquot of a spore suspension with a concentration of 1×10^7 conidia mL⁻¹ was inoculated into the three flasks containing 0.4 g (NH₄)₂SO₄ and 10 mL water, as well as 10 g of mixed corn straw/wheat bran (CSWB) medium at different ratios (corn straw: wheat bran=1:0, 3:1, 1:1, 1:3, 0:1); the control was not inoculated. Each flask containing medium was sterilized at 115°C for 30 min before use. The control group was placed at 37°C for 5 days and sterilization was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Figure S6 in Supporting Information). Inoculated flasks with a CSWB ratio of 1:1 were cultured in an incubator at 20°C, 25°C, 30°C, 34°C, 37°C, 40°C, 45°C, 50°C, 55°C, and 60°C; other flasks were cultured at 50°C. The sampling interval was 24 h, when 100 mL of distilled water was added to each flask and shaken for 1 h at 200 r min⁻¹ and 4°C. Subsequently, the medium was filtered through eight layers of distilled gauze and centrifuged for 20 min at 10,000×g to obtain the supernatant, which was stored at 4°C prior to further study.

Extracellular reducing sugar and glycoside hydrolase activity assays

The concentration of extracellular reducing sugars and quantitative measurement of glycoside hydrolase activities were determined by the dinitrosalicylic acid (DNS) method. Gradient volumes of 1 mg mL⁻¹ glucose were used to prepare the standard curve, and 1% (w/v) sodium carboxymethylcellulose (CMC) and 1% (w/v) xylan dissolved in citric acid/disodium hydrogen phosphate buffer at pH 5.0 were employed to determine the activities of endoglucanases and xylanases, respectively. The reaction mixture containing 600 µL substrate solution and 400 µL fermentation broth was allowed to

react at 50°C for 30 min, along with a control consisting of an inactivated fermentation instead of the enzyme sample. An 800 µL aliquot of DNS was added to end the reaction, and the mixtures were boiled for 10 min. Each mixture was brought up to 10 mL with water, and the optical density value was determined at 550 nm. Finally, enzyme activity was defined as international units of activity through the standard curve. Coomassie Brilliant Blue was employed to determine protein content (Bradford, 1976).

Native polyacrylamide gel electrophoresis analysis

Native polyacrylamide gel electrophoresis was used to visualize the variety and concentrations of the major glycoside hydrolases and proteases. The zymogram analysis of endoglucanases, xylanases, and β-glucosidases was performed as described by Zhang et al. (Zhang et al., 2012). After electrophoresis, the gel was incubated in 2% (w/v) CMC solution or 1% (w/v) xylan solution at 50°C for 30 min to detect endoglucanase and xylanase activities, respectively. Then, the gel was stained with a 0.5% (w/v) Congo Red solution for 20 min and destained with 1 mol L⁻¹ NaCl solution. The gel images were visualized using a BenQ scanner 7550R (BenQ Corp., Taipei, China). To determine β-glucosidase activities, 0.1% (w/v) aesculin and 0.5% (w/v) FeCl₃ solution were used as the substrate. After electrophoresis, the gels were allowed to react with the substrate at 50°C for 50 min and visualized directly using a scanner. Protease zymograms were obtained as described by Lantz and Caborowski (Lantz and Caborowski, 1994).

Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis and database searches

The day 4 crude enzyme solutions were concentrated by ultrafiltration (3-kD cut-off membrane; Sigma-Aldrich, USA) for the proteomic study. The concentrated enzymes were precipitated with trichloroacetic acid, and the protein powder obtained was dissolved in double-distilled water. After determining the protein concentration, 10 µL of the protein solution was added to 50 µL of degeneration buffer (0.5 mol L⁻¹ TrisHCl, 2.75 mmol L⁻¹ EDTA, 6 mol L⁻¹ guanidine HCl (all from Sigma-Aldrich)). The proteins were treated with 30 µL of 1 mol L⁻¹ dithiothreitol (Sigma-Aldrich) for reduction and with 50 µL of 1 mol L⁻¹ iodoacetamide for alkylation. Then, 500 µL of high performance liquid chromatography water and 360 µL of 25 mmol L⁻¹ NH₄HCO₃ were respectively used to wash the samples on a MicroconYM-10 membrane (3-kD cut-off; Sigma-Aldrich) at 1,000×g. The samples were digested with 1:25 (w/w) trypsin at 37°C overnight. After desalination by passage through a C18 Ziptip (Sigma-Aldrich), the peptide samples were dissolved in 10 µL of 50% (v/v) acetonitrile in water with a final concentration of 0.1% (v/v) trifluoroacetic acid. The peptides were prepared, and LC-MS/MS with a

Prominence nano LC system (Shimadzu, Japan) coupled with an LTQ-OrbitrapVelos Pro ETD mass spectrometer (Thermo Fisher Scientific) was used to extract and analyze the peptides. Dynamic exclusion was used within 60 s to avoid repetitively selecting peptides. Three technical replicates were performed for each sample. The LC-MS/MS analysis was conducted as described by Gong et al. (Gong et al., 2015). Alignment of all of the MS/MS spectra was performed using Proteome Discoverer software 1.4 (Thermo Fisher Scientific) with the SEQUEST algorithm against the *A. fumigatus* reference database (<http://www.uniprot.org>). Enzyme limits were set at full tryptic cleavage at both ends for the MS/MS spectra database search and 0.8-kD mass tolerance was set for the fragment ions during the SEQUEST searches. Oxidation was set as a dynamic modification and carbamido methylation was set as a static modification. A label-free method was employed to determine the relative abundances of proteins and characterized by PSMs. PSM values can be used for relative quantification after optimization of the LTQ-Orbitrap and related algorithms based on good confidence in the data at the relative quantitative level (Zhou et al., 2010).

Data screening

Automatic decoy database searches were used to filter the search results using a false discovery rate of 0.05. Protein identifications were accepted if a probability < 0.05 was achieved and at least two peptides were identified ($P < 0.05$). Three biological repeats were used for all samples, and proteins were kept if identified twice.

Pearson's correlation coefficient (R value) was calculated by the formula:

$$R = \sum_{i=1}^n \frac{(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (i = 1, 2, \dots, n),$$

where n is sample size; x_i or y_i is the i -th element in sample, and $\bar{x}$ or $\bar{y}$ is the average value of the samples.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Figure S1 Thermostability of major glycoside hydrolases and proteases secreted by *A. fumigatus* AF14 at 37°C.

Figure S2 Comparison of genome and proteome between *A. fumigatus* Af14 and *A. niger* ATCC1015.

Figure S3 Concentration of reducing sugar and extracellular proteins as well as the activities of endoglucanases and xylanases when *A. fumigatus* AF14 was cultivated under different pH (A and C) and moisture (B and D). Protein bands detected by SDS-PAGE were also presented (C and D).

Figure S4 Dynamic changes of concentration of extracellular reducing sugars and proteins as well as the glycoside hydrolase activities of *A. fumigatus* AF14 during the 5 days solid-state fermentation under different ratios (CS/WB:1:0, 3:1, 1:1, 1:3, 0:1, respectively) of corn straw (CS) and wheat bran (WB) as substrates.

Figure S5 Fermentation cylinder (A) and microbial diversity in the bottom layer (0.15 m) of the fermentor during 5 days composting (B); dynamic changes of *A. fumigatus* relative abundance and compost temperature as well as water content of samples were also illustrated (C).

Figure S6 The SDS-PAGE of sample from control group. In experiment, the samples were concentrated 10 times.

Table S1 Number of enzymes related to protein degradation in *A. fumigatus* genome and secretome

Table S2 Binding sites and cleavage sites of proteases secreted by *A. fumigatus* and *A. niger* under solid fermentation identified by LTQ-orbitrap

Table S3 Binding sites and cleavage sites of proteases identified in *A. fumigatus* genome

The supporting information is available online at <http://life.scichina.com> and <https://link.springer.com>. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

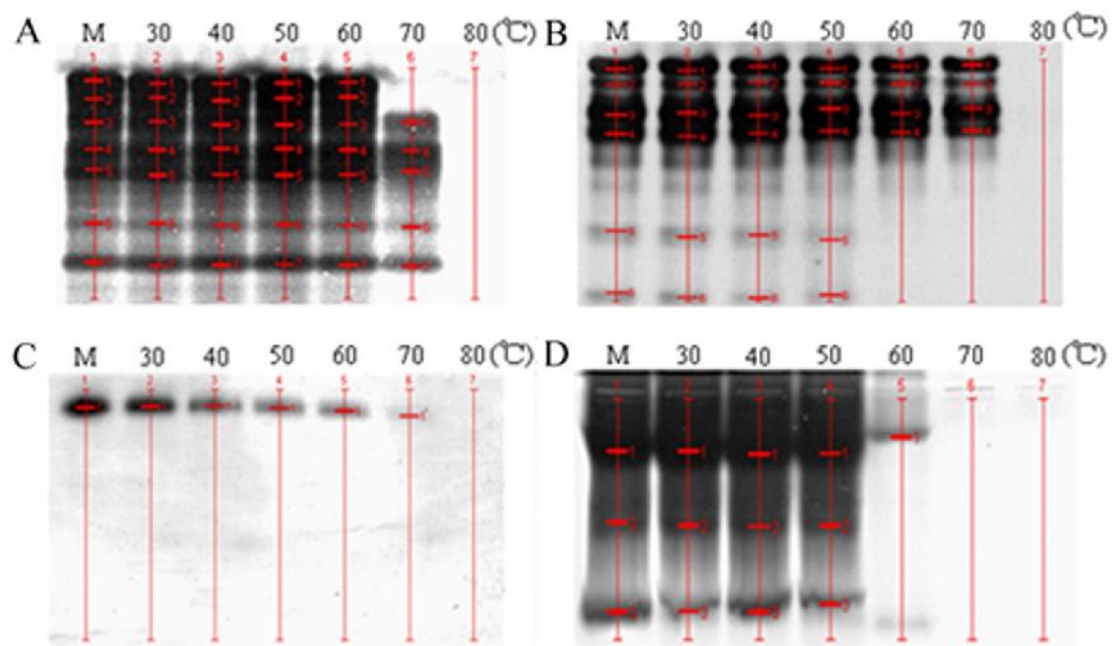
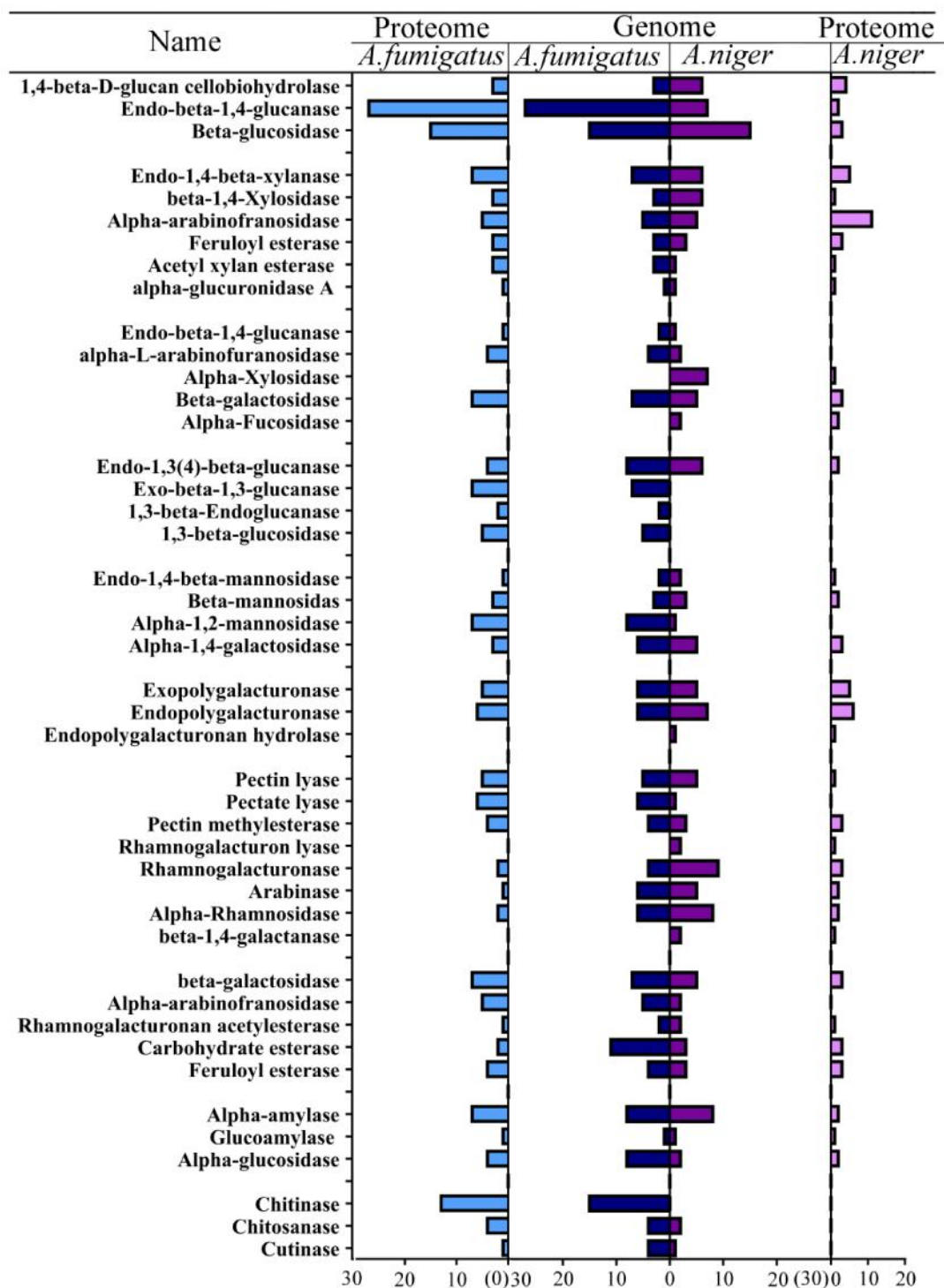


Figure S1 Thermostability of major glycoside hydrolases and proteases secreted by *A. fumigatus* AF14 at 37°C. A, endoglucanase activities; B, xylanase activities; C, beta-glucosidase activities; D, protease activities.



FigureS2 Comparison of genome and proteome between *A.fumigatus* Af14 and *A.niger* ATCC1015.

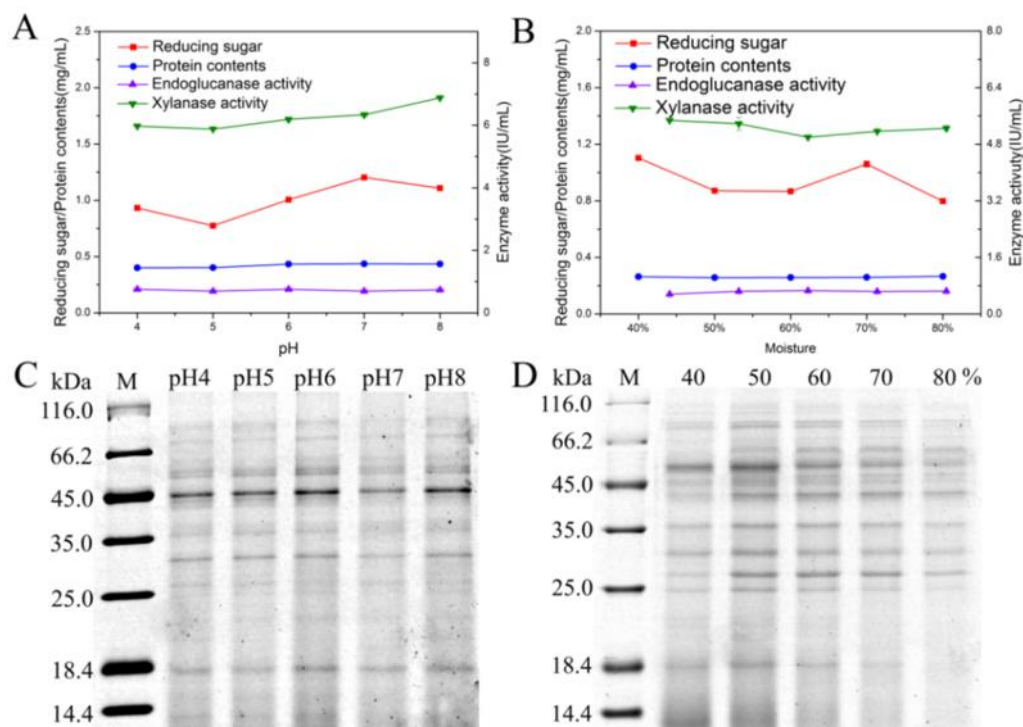


Figure S3 Concentration of reducing sugar and extracellular proteins as well as the activities of endoglucanases and xylanases when *A. fumigatus* AF14 was cultivated under different pH (A, C) and moisture (B, D). Protein bands detected by SDS-PAGE were also presented (C, D).

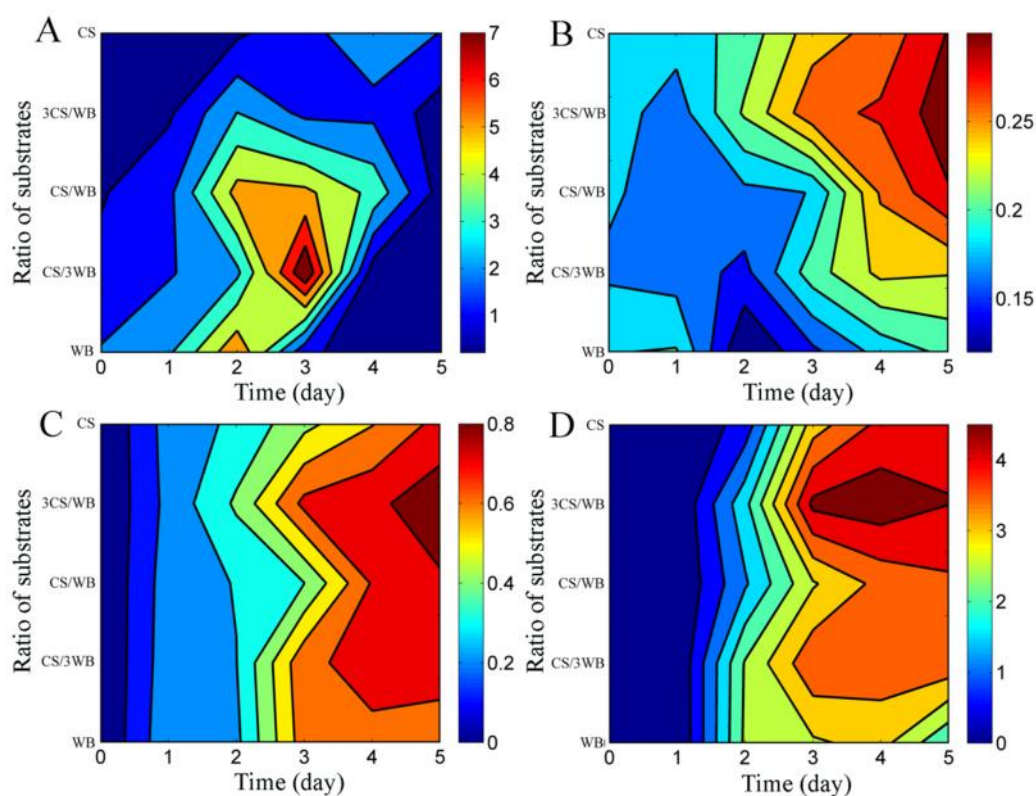


Figure S4 Dynamic changes of concentration of extracellular reducing sugars and proteins as well as the glycoside hydrolase activities of *A. fumigatus* AF14 during the 5 days solid-state fermentation under different ratios (CS/WB:1:0, 3:1, 1:1, 1:3, 0:1, respectively) of corn straw (CS) and wheat bran (WB) as substrates. A, reducing sugar concentration; B, extracellular protein concentration; C, endoglucanase activities; D, xylanase activities.

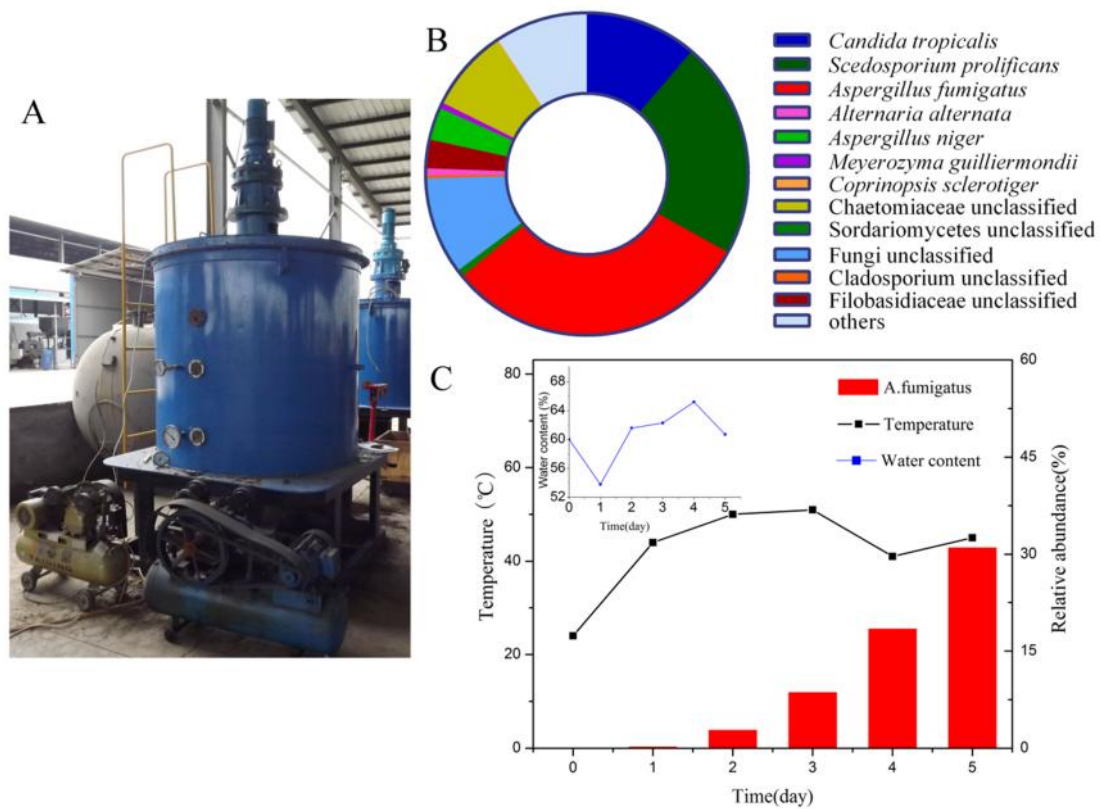


Figure S5 Fermentation cylinder (A) and microbial diversity in the bottom layer (0.15 m) of the fermentor during 5 days composting (B); dynamic changes of *A. fumigatus* relative abundance and compost temperature as well as water content of samples were also illustrated (C)

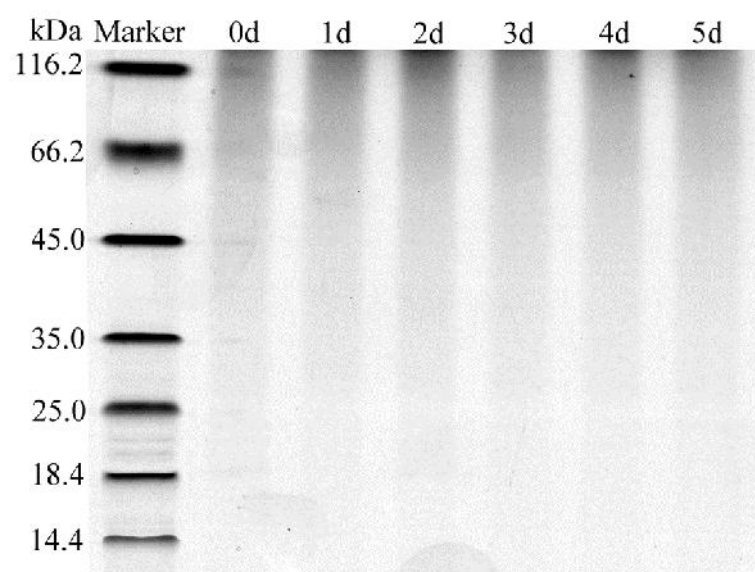


Figure S6 The SDS-PAGE of sample from control group. In experiment, the samples were concentrated 10 times.

Table S1 Number of enzymes related to protein degradation in *A. fumigatus* genome and secretome.

	AA	Type	Genome	Secretome
Exopeptidase	18	Ampeptidase	15	14
		Carboxypeptidase	16	16
		Oligopeptidase	10	10
Endopeptidase	9	Metallopeptidase	14	10
		Endopeptidase	21	8
Others	15	Peptidase	17	10

Pink, the number of genes encoding the protein is equal to that detected in secretome; blue, the amount of proteins in secretome is almost equal to the protein-coding genes in the genome; green, number of protein detected in the secretome is much smaller than the number of the protein-coding genes in the genome.

Table S2. Binding sites and cleavage sites of proteases secreted by *A. fumigatus* and *A. niger* under solid fermentation identified by LTQ-orbitrap.

Type	Accession number	Protein name	Cleavage site	PSM	Binding site								
					-4'	-3'	-2'	-1'	1	2	3	4	
<i>A.fumigatus</i>													
exopeptidase	Q4WPF0	aminopeptidase	Arg +NHNap	61				Arg	NAP				
	Q4WES0	aminopeptidase I	Leu +NHPhNO2	57				Leu	NAN				
	A1CHL4	aminopeptidase	Arg +NHNap	35				Arg	NAP				
	B0XYT4	Gly-Xaa carboxypeptidase	Z-Gly+Leu	24				Gly	Leu				
	B0XRV0	dipeptidyl-peptidase 5	Ala-Ala +NHPhNO2	21				Ala	AMC				
	Q4WNL2	dipeptidyl-peptidase III		21									
	A1CY57	aminopeptidase Y	Ala +NHMec	15				Ala	AMC				
	A1D0J8	carboxypeptidase OcpA	Bz-Arg +OMe	14			Bz	Arg	OMe				
	B0Y6C5	dipeptidyl peptidase 4	Ala-Ala +NHPhNO2	11									
	B0Y3V7	aminopeptidase P1	Ala+Pro	9				Ala	Pro				
endopeptidase	A1DB21	carboxypeptidase OcpB	Serine	7									
	Q4WX56	aspartyl aminopeptidase	Glu1+His -peptide	48			Glu	His	Phe	Arg	Trp		
	B0Y4X9	acid metalloproteinase	Boc-Val-Leu-Lys-NHMec	38	Boc	Val	Leu	Lys	AMC				
	Q4WZD5	acid prolyl endopeptidase	Serine	38									
	O42630	aspartic proteinase		30									
	P97996	oligopeptidase MepB	Pz-Pro-Leu+Gly -Pro-D-Arg	24		Pz	Pro	Leu	Gly	Pro	DAr		
	B0XT80	acid prolyl endopeptidase		13			Ala	Ala	NAN				
	B0XXT6	M28B peptidases		10									
	unassigned	A1CWF3	allergen Asp fl 1		22				Ala	Ala	NAN		
		Q70GH4	grifolisin	Ala-Ala-Phe +NHPhNO2	6			Ala	Ala	Phe	NAN		

Type	Accession number	Protein name	Cleavage site	PSM	Binding site							
					-4'	-3'	-2'	-1'	1	2	3	4
<i>A.niger</i> AN76												
exopeptidase	G3Y926	Aap1' aminopeptidase	Arg +NHNap	20					Arg	NAP		
	A2QZF1	dipeptidyl-peptidase 5	Ala- Ala +NHPhNO2	21				Ala	Ala	NAN		
	A2QAF7	methionyl aminopeptidase 1	Met 1+ Val -peptide	7				Met	Val	Gln	Ser	Ser
endopeptidase	A2QR21	acid prolyl endopeptidase		15								
unassigned	P52718	Mername-AA083 peptidase	Thr-Thr- Met 211+ Pro	21			Thr	Thr	Met	Pro		
	G3YDG9	subfamily A1A peptidases		1								
	A5AB21	Mername-AA083 peptidase	Thr-Thr- Met 211+ Pro	20			Thr	Thr	Met	Pro		
	A2QMZ7	subfamily S8A peptidases		11								
	A2QH12	unassigned peptidases	Asp- Arg 35+ Val	33				Asp	Arg	Val		
	A2QDI4	saccharopepsin	peptide- Asn 111+ Lys -peptide	28	Leu	Arg	Val	Asn	Lys	Ile	Lys	Asp
	A2QAJ5	family M19 peptidases		22								

Type	Accession number	Protein name	Cleavage site	PSM	Binding site							
					-4'	-3'	-2'	-1'	1	2	3	4
<i>A.niger</i> ATCC1015												
exopeptidase	Q96VT2	prolyl aminopeptidase 2	Hyp +NHNAp	4					Hyp	NAP		
	G3Y926	Aap1’ aminopeptidase	Arg +NHNAp	11					Arg	NAP		
	G3Y2V9	carboxypeptidase O	Thr-Thr- Met 211+ Pro	16			Thr	Thr	Met	Pro		
	A2QZF1	dipeptidyl-peptidase 5	Ala- Ala +NHPhNO2	14				Ala	Ala	NAN		
endopeptidase	G3Y3K2	aspartic proteinase	peptide- Asn 111+ Lys -peptide	51	Leu	Arg	Val	Asn	Lys	Ile	Lys	Asp
	A2QR21	acid prolyl endopeptidase		30								
	Q96UQ4	family M1 peptidases		6								
unassigned	P52718	Mername-AA083 peptidase	Thr-Thr- Met 211+ Pro	15			Thr	Thr	Met	Pro		
	G3YDG9	subfamily A1A peptidases		3								
	G3Y013	subfamily A1A peptidases		1								
	A5AB21	Mername-AA083 peptidase	Thr-Thr- Met 211+ Pro	24			Thr	Thr	Met	Pro		
	A2QH12	unassigned peptidases	Asp- Arg 35+ Val	54				Asp	Arg	Va		
	A2QAJ5	family M19 peptidases		24								

Table S3 Binding sites and cleavage sites of proteases identified in *A. fumigatus* genome

Accession	Protein name	PSM Value	binding site							
			-4	-3	-2	-1	1	2	3	4
Q5AZT7	Lon protease homolog									
Q4WP08	Zinc metalloprotease									
A1CXL2	Zinc metalloprotease									
B0Y340	Aminopeptidase									
A1D432	Zinc metalloprotease									
Q4WPF0	Aminopeptidase									
B0XYK8	Dipeptidyl-aminopeptidase B									
A1D7R6	Dipeptidyl-aminopeptidase B									
Q5BH58	Lon protease homolog 2									
A1CHL4	Aminopeptidase									
A1DCT3	Metallopeptidase MepB									
Q4WNL2	Dipeptidyl peptidase									
B0Y6C5	Dipeptidyl peptidase 4									
A1CX29	Dipeptidyl peptidase 4									
Q5B612	Glutamate carboxypeptidase				Pro	Leu	Gly	Pro		
P97996	MepB	23			Pro	Leu	Gly	Pro		
Q4WGR2	Metallopeptidase MepB	46								
B0Y9Z2	Oligopeptidase family protein				Ala	Ala				
B0XRVO	Dipeptidyl-peptidase 5	18			Gly	Phe				
					His	Ser				
					Lys	Ala				
					Lys	Ala				

					Ser	Phe Tyr				
A1DGJ8	Secreted dipeptidyl peptidase									
A1C4J8	Peptidase									
B0Y3K2	Dipeptidyl peptidase III									
A1DJJ3	Proteinase			Gln	Arg	Arg				
B0Y870	Alkaline serine protease AorO	7		Gln	Gly	Arg				
				Gly	Arg	Arg				
				Gly	Lys	Arg				
				Leu	Arg	Arg				
				Leu	Gly	Arg				
				Leu	Lys	Arg				
				Leu	Thr	Arg				
			Phe	Leu	Arg	Arg	Ile	Arg	Pro	Lys
			Asp	Asp	Asp	Lys	Ile	Val	Gly	Gly
A1DF95	Carboxypeptidase S1				Asp	Arg	Val			
P46075	Extracellular metalloproteinase mep	31		Asp	Arg	Val	Tyr			
			Asp	Arg	Val	Tyr	Ile			
			Arg	Val	Tyr	Ile	His			
			Val	Tyr	Ile	His	Pro			
			Ile	His	Pro	Phe	His			
			His	Pro	Phe	His	Leu			
			Tyr	Ile	His	Pro	Phe			
			Pro	Pro	Gly	Phe	Ser			

B0Y3N9 Carboxypeptidase S1

8

Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe
Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Gly	Asp	Ala	Ser
Gly	Asp	Ala	Ser	Gly
Asp	Ala	Ser	Gly	Glu
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Arg	Arg	Pro	Tyr	Ile
	Trp	Met	Asp	Phe
Arg	Pro	Tyr	Ile	Leu
Pro	Arg	Arg	Pro	Tyr
Phe	Ile	Gly	Leu	Met
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His

Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His
His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe
Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Gly	Asp	Ala	Ser
Gly	Asp	Ala	Ser	Gly
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu

			Trp	Met	Asp	Phe
Q4WPI7	Aspartic-type endopeptidase					
A1D9T2	Tripeptidyl-peptidase (TppA)					
Q70J59	Tripeptidyl-peptidase sed2					
Q70GH4	Tripeptidyl-peptidase sed3					
Q4WZD5	Extracelular serine carboxypeptidase					
A1D7F7	Tripeptidyl peptidase SED3					
A1D806	Vacuolar carboxypeptidase Cps1					
B0YB72	Serine protease				Gly	Leu
B0XYT4	Vacuolar carboxypeptidase Cps1	17		Asp	Arg	Val
A1DB21	Carboxypeptidase S1	7	Asp	Arg	Val	Tyr
			Asp	Arg	Val	Ile
			Arg	Val	Tyr	Ile
			Val	Tyr	Ile	His
			Ile	His	Pro	His
			His	Pro	Phe	His
			Tyr	Ile	His	Leu
			Pro	Pro	Phe	Pro
			Pro	Gly	Phe	Pro
			Gly	Phe	Ser	Pro
			Phe	Ser	Pro	Phe
			Trp	Ala	Gly	Arg
			Ala	Gly	Gly	Asp
			Gly	Gly	Asp	Ala
			Asp	Ala	Ser	Glu

Q4WCK3 Carboxypeptidase S1

16

Gly	Asp	Ala	Ser	Gly
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His
Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His
His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe

A1DP75 Carboxypeptidase Y homolog A

10

Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Asp	Ala	Ser	Gly
Gly	Gly	Asp	Ala	Ser
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His
Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His

His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe
Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Asp	Ala	Ser	Gly
Gly	Gly	Asp	Ala	Ser
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val

B0XM76 Carboxypeptidase CpyA/Prc1

14	Asp	Arg	Val	Tyr
	Asp	Arg	Val	Ile
	Arg	Val	Tyr	His
	Val	Tyr	Ile	Pro
	Ile	His	Pro	His
	His	Pro	Phe	Leu
	Tyr	Ile	His	Phe
	Pro	Pro	Gly	Ser
	Pro	Gly	Phe	Pro
	Gly	Phe	Ser	Phe
	Phe	Ser	Pro	Arg
	Trp	Ala	Gly	Asp
	Ala	Gly	Gly	Ala
	Gly	Asp	Ala	Gly
	Gly	Gly	Ala	Ser
	Asp	Ala	Ser	Glu
	Phe	Ile	Gly	Met
		Phe	Met	Phe
			Gln	Tyr
		Gln	Leu	Glu
	Gln	Leu	Tyr	Asn
	Leu	Tyr	Glu	Lys
	Tyr	Glu	Asn	Pro
	Glu	Asn	Lys	Arg
	Asn	Lys	Pro	Arg
	Lys	Pro	Arg	Pro

A1CUJ5 Carboxypeptidase Y homolog A

5

Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His
Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His
His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe
Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Asp	Ala	Ser	Gly
Gly	Gly	Asp	Ala	Ser
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn

			Leu	Tyr	Glu	Asn	Lys
			Tyr	Glu	Asn	Lys	Pro
			Glu	Asn	Lys	Pro	Arg
			Asn	Lys	Pro	Arg	Arg
			Lys	Pro	Arg	Arg	Pro
			Pro	Arg	Arg	Pro	Tyr
			Arg	Arg	Pro	Tyr	Ile
			Arg	Pro	Tyr	Ile	Leu
				Trp	Met	Asp	Phe
Q70J58	FuSed4 protease				Asp	Arg	Val
Q4WL26	Carboxypeptidase S1,	17		Asp	Arg	Val	Tyr
			Asp	Arg	Val	Tyr	Ile
			Arg	Val	Tyr	Ile	His
			Val	Tyr	Ile	His	Pro
			Ile	His	Pro	Phe	His
			His	Pro	Phe	His	Leu
			Tyr	Ile	His	Pro	Phe
			Pro	Pro	Gly	Phe	Ser
			Pro	Gly	Phe	Ser	Pro
			Gly	Phe	Ser	Pro	Phe
			Phe	Ser	Pro	Phe	Arg
			Trp	Ala	Gly	Gly	Asp
			Ala	Gly	Gly	Asp	Ala
			Gly	Asp	Ala	Ser	Gly
			Gly	Gly	Asp	Ala	Ser

Q5VJG7 Carboxypeptidase 5

	Asp	Ala	Ser	Gly	Glu	
	Phe	Ile	Gly	Leu	Met	
		Phe	Met	Arg	Phe	
			Gln	Leu	Tyr	
		Gln	Leu	Tyr	Glu	
	Gln	Leu	Tyr	Glu	Asn	
	Leu	Tyr	Glu	Asn	Lys	
	Tyr	Glu	Asn	Lys	Pro	
	Glu	Asn	Lys	Pro	Arg	
	Asn	Lys	Pro	Arg	Arg	
	Lys	Pro	Arg	Arg	Pro	
	Pro	Arg	Arg	Pro	Tyr	
	Arg	Arg	Pro	Tyr	Ile	
	Arg	Pro	Tyr	Ile	Leu	
		Trp	Met	Asp	Phe	
		Thr	Thr	Met	Pro	
11	Thr	Thr	Met	Pro	Leu	
	Thr	Met	Pro	Leu	Trp	
	Ser	Ala	Glu	Glu	Arg	
	Asn	Ser	Ala	Glu	Glu	
	Met	Glu	Asp	Ile	Lys	
	Glu	Asp	Gln	Ala	Met	-
	Asp	Gln	Ala	Met	Glu	
	Gln	Ala	Met	Glu	Asp	
	Ala	Met	Glu	Asp	Ile	
	Gln	Gly	Leu	Pro	Gln	

Tyr	Phe	Tyr	Pro	Glu
Pro	Glu	Leu	Phe	Arg
Glu	Leu	Ala	Tyr	Phe
Leu	Ala	Tyr	Phe	Tyr
Ala	Tyr	Phe	Tyr	Pro
Pro	Glu	Val	Phe	Gly
Glu	Val	Phe	Gly	Lys
Val	Ala	Pro	Phe	Pro
Ala	Pro	Phe	Pro	Glu
Pro	Phe	Pro	Glu	Val
Phe	Pro	Glu	Val	Phe
Gly	Leu	Pro	Gln	Glu
Leu	Asn	Glu	Asn	Leu
Glu	Val	Leu	Asn	Glu
Phe	Tyr	Pro	Glu	Leu
Asn	Gln	Glu	Leu	Ala
Leu	Pro	Gln	Glu	Val
His	Gln	Gly	Leu	Pro
	His	Gln	Gly	Leu
Pro	Gln	Glu	Val	Leu
Gln	Glu	Val	Leu	Asn
Val	Leu	Asn	Glu	Asn
Asn	Glu	Asn	Leu	Leu
Glu	Asn	Leu	Leu	Arg
Gln	Glu	Leu	Ala	Tyr
Tyr	Pro	Glu	Leu	Phe

			Arg	
			Leu	
			Lys	
			Phe	
			Phe	Ala
			Phe	
			Ala	Lys
Arg	Ala	Gln	Asp	Phe
Ala	Gln	Asp	Phe	Val
Phe	Val	Gln	Trp	Leu
Trp	Leu	Met	Asn	Thr
Gln	Trp	Leu	Met	Asn
Val	Gln	Trp	Leu	Met
Asp	Phe	Val	Gln	Trp
Gln	Asp	Phe	Val	Gln
Arg	Arg	Ala	Gln	Asp
Ser	Arg	Arg	Ala	Gln
Asp	Ser	Arg	Arg	Ala
Leu	Asp	Ser	Arg	Arg
Tyr	Leu	Asp	Ser	Arg
Lys	Tyr	Leu	Asp	Ser
Ser	Lys	Tyr	Leu	Asp
Tyr	Ser	Lys	Tyr	Leu
Asp	Tyr	Ser	Lys	Tyr
Ser	Asp	Tyr	Ser	Lys
Leu	Val	Cys	Gly	Glu

			Phe	Tyr	Thr	Pro	Lys
			Tyr	Thr	Pro	Lys	Ala
			Val	Cys	Gly	Glu	Arg
			Gly	Glu	Arg	Gly	Phe
						Ala	Arg
						Ala	Ile
						Ala	Leu
						Ala	Lys
						Ala	Met
B0XT80	Serine peptidase				Asp	Arg	Val
A1DKU1	Serine carboxypeptidase (CpdS)	5		Asp	Arg	Val	Tyr
			Asp	Arg	Val	Tyr	Ile
			Arg	Val	Tyr	Ile	His
			Val	Tyr	Ile	His	Pro
			Ile	His	Pro	Phe	His
			His	Pro	Phe	His	Leu
			Tyr	Ile	His	Pro	Phe
			Pro	Pro	Gly	Phe	Ser
			Pro	Gly	Phe	Ser	Pro
			Gly	Phe	Ser	Pro	Phe
			Phe	Ser	Pro	Phe	Arg
			Trp	Ala	Gly	Gly	Asp
			Ala	Gly	Gly	Asp	Ala
			Gly	Asp	Ala	Ser	Gly
			Gly	Gly	Asp	Ala	Ser

Q5VJG8 Carboxypeptidase 4

20

Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His
Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His
His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe

B0YDY3 Serine carboxypeptidase (CpdS)

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Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Asp	Ala	Ser	Gly
Gly	Gly	Asp	Ala	Ser
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
		Asp	Arg	Val
	Asp	Arg	Val	Tyr
Asp	Arg	Val	Tyr	Ile
Arg	Val	Tyr	Ile	His
Val	Tyr	Ile	His	Pro
Ile	His	Pro	Phe	His

His	Pro	Phe	His	Leu
Tyr	Ile	His	Pro	Phe
Pro	Pro	Gly	Phe	Ser
Pro	Gly	Phe	Ser	Pro
Gly	Phe	Ser	Pro	Phe
Phe	Ser	Pro	Phe	Arg
Trp	Ala	Gly	Gly	Asp
Ala	Gly	Gly	Asp	Ala
Gly	Asp	Ala	Ser	Gly
Gly	Gly	Asp	Ala	Ser
Asp	Ala	Ser	Gly	Glu
Phe	Ile	Gly	Leu	Met
	Phe	Met	Arg	Phe
		Gln	Leu	Tyr
	Gln	Leu	Tyr	Glu
Gln	Leu	Tyr	Glu	Asn
Leu	Tyr	Glu	Asn	Lys
Tyr	Glu	Asn	Lys	Pro
Glu	Asn	Lys	Pro	Arg
Asn	Lys	Pro	Arg	Arg
Lys	Pro	Arg	Arg	Pro
Pro	Arg	Arg	Pro	Tyr
Arg	Arg	Pro	Tyr	Ile
Arg	Pro	Tyr	Ile	Leu
	Trp	Met	Asp	Phe
			Ala	

Q4WIY5	Aminopeptidase	14	Arg	Arg
			Arg	
			Asn	
			Cys	
			Gln	
			Hyp	
			Leu	
			Lys	Ala
			Lys	
			Met	
			Phe	
			Pro	
			Ser	
			Tyr	
			Leu	
A1CZR9	Aspartyl aminopeptidase	5	Leu	
Q4WES0	Vacuolar aspartyl aminopeptidase Lap4	31	Ala	
A1CY57	Aminopeptidase	11	Arg	Arg
			Arg	
			Asn	
			Cys	
			Gln	
			Hyp	
			Leu	
			Lys	Ala
			Lys	

			Met				
			Phe				
			Pro				
			Ser				
			Tyr				
Q4WX56	Aspartyl aminopeptidase	35	Glu	His	Phe	Arg	Ile
			Asp	Arg	Val	Tyr	
			Asp	Ala			
			Asp	Ala	Pro		
			Asp	His			
			Asp	Leu			
			Asp				
			Asp	Phe			
			Asp	Ser			
			Glu				
B0XN37	Xaa-Pro aminopeptidase	6	Arg				
			Lys				
			Met				
			Ala	Ala			
			Ala	Arg			
			Ala	Asn			
			Ala	Asp			
			Ala	His			
			Ala	Ile			
			Ala	Lys			
			Ala	Met			

			Ala	Phe
			Ala	Val
			Glu	Tyr
			Arg	
B0Y1L0	Carboxypeptidase S1	49	Lys	
			Met	
			Ala	Ala
			Ala	Arg
			Ala	Asn
			Ala	Asp
			Ala	His
			Ala	Ile
			Ala	Lys
			Ala	Met
			Ala	Phe
			Ala	Val
			Glu	Tyr
A1D7L8	Aspartyl aminopeptidase		Arg	
A1D0J8	Carboxypeptidase S1	28	Lys	
			Met	
			Ala	Ala
			Ala	Arg
			Ala	Asn
			Ala	Asp
			Ala	His

						Ala	Ile			
						Ala	Lys			
						Ala	Met			
						Ala	Phe			
						Ala	Val			
						Glu	Tyr			
B0XW47	Xaa-Pro aminopeptidase pepP									
Q4WDN4	Aspartic-type endopeptidase opsB									
Q4WHL2	Proline iminopeptidase									
B0YDC1	Aspartic endopeptidase (AP1)									
A1DM79	Carboxypeptidase									
Q4WMK3	Zinc metallopeptidase									
O42630	Vacuolar protease A					Tyr				
B0Y708	Alkaline protease 1	136				Arg				
			Ala	Ala	Pro	Leu				
						Ala				
B0XZL3	D-aminopeptidase	2				Ala				
						Ala	His			
				Val	Leu	Lys				
B0Y4X9	Neutral protease 2 homolog	18				Phe	Val	Asn	Gln	His
			Phe	Val	Asn	Gln	His	Leu	Cya	Gly
					Gly	Ser	His	Leu	Val	Glu
				Gly	Glu	Arg	Gly	Phe	Phe	Tyr
						Phe	Val	Asn	Gln	His
			Phe	Val	Asn	Gln	His	Leu		

			Ser	His	Leu	Val	Glu	Ala	Leu	Tyr
			Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala
				Phe	Val	Asn	Gln	His	Leu	
				Phe	Val	Asn	Gln	His	Leu	
			His	Leu		Gly	Ser	His	Leu	Val
			His	Leu		Gly	Ser	His	Leu	Val
			Gly	Ser	His	Leu	Val	Glu	Ala	Leu
			His	Leu	Val	Glu	Ala	Leu	Tyr	Leu
			His	Leu	Val	Glu	Ala	Leu	Tyr	Leu
			Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val
				Gly	Ser	His	Leu	Val	Glu	Ala
			Glu	Ala	Leu	Tyr	Leu	Val	Cya	Gly
			Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala
			Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val
			Glu	Ala	Leu	Tyr	Leu	Val	Cya	Gly
						Tyr				
O74265	Alkaline protease	217				Arg				
			Ala	Ala	Pro	Leu				
						Tyr				
Q64HZ1	Alkaline protease	1				Arg				
			Ala	Ala	Pro	Leu				
M5A798	Extracellular alkaline protease									
A1CZ35	Alkaline serine protease									
A1CEW8	Alkaline serine protease					Ala	Pro			
B0Y3V7	Xaa-Pro aminopeptidase P	12				Arg	Pro			

Arg	Pro			
Arg	Pro	Pro		
Tyr	Pro	Phe	Pro	Gly
Tyr	Pro	Phe	Pro	Gly
Arg	Pro	Pro	Gly	Phe
Arg	Pro	Pro	Gly	Phe
Gly	Pro	Arg	Pro	
Gly	Pro			
His	Pro	Val		
Lys	Pro			
Tyr	Pro	Ser	Lys	Pro
Phe	Pro	Ala		
Phe	Pro			
Arg	Pro	Lys	Pro	
Tyr	Pro	Phe		
Tyr	Pro	Leu	Gly	

A1D1S6	Xaa-Pro aminopeptidase
A1DER5	Autophagic serine protease Alp2
B0Y1V8	Aspartic protease pep1
