Analysis and optimisation of plant biomass degrading enzyme production in *Aspergillus*

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Analysis and optimisation of plant biomass degrading enzyme production in *Aspergillus*

Analyse en optimalisatie van de productie van planten biomassa afbrekende enzymen in *Aspergillus* (met een Nederlandse samenvatting)

Proefschrift

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door

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geboren op 3 april 1986 te Wexford, Ireland

Promotor: Prof. Dr. ir. R.P. de Vries

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For my parents and family

The Aspergillus niger image on the cover was kindly provided by; Dr. Nick Reid, Professor of Fungal Cell Biology, Director, Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester, CTF Building, Grafton Street, Manchester M13 9NT.

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Chapter 1

General Introduction

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Filamentous Fungi

Fungi form a large and diverse group of eukarvotic organisms. To date, about 80,000 fungal species have been described, with the actual number world-wide being estimated at 1.5 million [57]. Fungi have the ability to grow in many diverse and often harsh environments, including extreme heat [73], extreme acidity [107], high pressure [96] and radiation [27]. The major recognized fungal groups are Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota and Basidiomycota [70], with the majority of described species belonging to the latter two phyla. Fungi can be unicellular (yeast) or multicellular (filamentous), but some species have the ability to switch between both forms (dimorphic fungi) [14]. Filamentous fungi are chemoheterotrophic and depend on the presence of organic material for their carbon and energy source. To facilitate this, filamentous fungi grow by means of extending hyphae which branch sub-apically to form a network of hyphae called a mycelium. This hyphal mode of growth supplies a large surface area for the uptake of nutrients but also enables the fungus to colonize its substrate in an efficient manner [52]. The organic material, which acts as a substrate for most fungal species is generally composed of large, complex molecules which need to be broken down before they can serve as a source of nutrition. To this end, filamentous fungi secrete diverse enzymatic mixtures, tailormade to the polysaccharide which they encounter.

The genus Aspergillus

The genus Aspergillus is a group of ascomycete filamentous fungi, consisting of more than 250 species. Of these species, the majority are saprotrophic fungi that live off dead or decaying organic matter but there are some e.g. A. fumigatus and A. chevalieri, which are opportunistic human pathogens [67, 79] and others e.g. A. niger and A. flavus which are phytopathogens [16, 48]. Some Aspergillus species e.g. A. flavus and A. parasiticus, are known to produce mycotoxins [25, 58], others e.g. A. niger and A. oryzae, are extensively used in industry for the production of enzymes and metabolites [52] while A. nidulans is routinely used as a model organism for lower eukaryotes [50]. The diversity of topics that are relevant to Aspergillus has resulted in it becoming one of the most widely studied groups of filamentous fungi. Combined with the fact that many Aspergilli have good fermentation capabilities and high levels of protein secretion [30], it is of no surprise that this fungus has been applied in industries such as food [94] and feed [13], pulp and paper [22], biofuels, biodegradable plastics, textiles [9] and as hosts for heterologous protein production [28]. As common soil fungi, found in many different environments, Aspergilli are also found to be capable of producing an extensive set of enzyme mixes that degrade a very broad range of polysaccharides [23].

Plant biomass degrading enzymes and their substrates

Plant biomass is the most abundant and universally used carbon source by most fungal species on earth. Primarily consisting of plant cell walls, it contains a complex structure of polysaccharides, proteins and lignin which differ greatly in monomeric composition and linkage. Fungi, including Aspergillus, degrade these polysaccharides extracellularly by secreting enzymes which release oligo- and monosaccharides that can then be utilized by the fungal cell [52]. Plant polysaccharides can be divided into two main groups; plant cell wall polysaccharides (cellulose, hemicelluloses [xyloglucans, xylan, galacto(gluo)mannan] and pectin) and storage polysaccharides (e.g. starch and inulin.) The plant cell wall consists of the primary and secondary cell wall, which both differ in composition and function. The primary cell wall is rich in polysaccharides (~90% polysaccharides (cellulose, hemicelluloses and pectin) and $\sim 10\%$ proteins) and is formed during growth [3]. The secondary cell wall is deposited on the primary layer after cell elongation stops and consists mainly of cellulose. hemicelluloses and lignin [10]. These cell wall polysaccharides interact with each other as well as the aromatic polymer, lignin, to form a network of linkages and hydrogen bonds that give the plant cell wall its rigidity [34].

Due to the diverse and complex structure of plant biomass, fungi are required to produce a large range of enzyme activities to degrade these polysaccharides into their monomeric components [23, 34]. These enzymes can be divided into families based on the catalytic modules present in their corresponding amino acid sequence i.e. glycoside hydrolases (GH), glycosyl transferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and carbohydrate binding modules (CBM). The resulting Carbohydrate-Active enzyme database (CAZy-http://www.cazy.org) has become a powerful tool in providing insight into the carbohydrate potential of a fungus and supports the identification and prediction of the function of novel genes [17, 23]. A full description of the enzyme activities involved in plant polysaccharide degradation are detailed below and corresponding CAZy families and EC numbers are presented in Table 1.

Cellulose

Cellulose is the most abundant plant cell wall polysaccharide. It exists as highly ordered linear polymers of β -1,4-linked D-glucose residues which are bundled together in microfibrils via hydrogen bonds (Fig. 1) [45]. This polymeric structure gives the plant cell wall its strength and definition [34]. Four enzymes groups are involved in the biodegradation of cellulose: endoglucanases, cellobiohydrolases, β -glucosidases and *exo*-glucanases. *Endo*-glucanases and cellobiohydrolases hydrolyze cellulose into gluco-oligosaccharides and cellobiose, respectively. These oligosaccharides are then further degraded into D-glucose molecules by the action of β -glucosidases and *exo*-glucanases (Fig. 1, Table 1).

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Figure 1: Representation of cellulose and its hydrolysis by cellulose degrading enzymes. The enzymes involved in the complete biodegradation of cellulose are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Hemicelluloses

The hemicellulose group of polysaccharides represent the second most abundant polysaccharide in plant cell walls and form cross-links with cellulose through hydrogen bonds to provide extra rigidity and protection against plant pathogens, such as fungi [20, 127]. Hemicellulose is present in three main forms in the plant cell wall: xylans, xyloglucan and mannans.

Xylan

The xylan group of polysaccharides consists of a backbone of β -1,4-linked D-xylose residues which can be acetylated and/or substituted with either single residues or short side chains of α -1,2- or α -1,3-linked L-arabinose (arabinoxylan) and/or single α -1,2-linked D-glucuronic acid residues (glucuronoxylan) [42, 125]. D-xylose can be found attached to the L-arabinose residues while feruloyl and *p*-coumaroyl residue substitutions can be found in the terminal L-arabinose residues. The D-xylose attached to the L-arabinose residues may be acetylated while methylation is only found in the D-glucuronic acid residues [106, 124] (Fig. 2). The main xylan present in cereals is arabinoxylan while hardwood contains mainly glucuronoxylan [34]. Due to its variable composition, the enzymatic mixture produced to break down xylan can be highly

varied [8, 15, 60, 103]. *Endo*-xylanases cleave the xylan backbone into smaller oligosaccharides which act as a substrate for degradation by β -xylosidases to D-xylose [93]. The L-arabinose side chain residues are hydrolyzed by α -arabinofuranosidases and arabinoxylan arabinofuranohydrolases, and the glucuronic acid residues by α -glucuronidases. The acetyl, feruloyl and *p*-coumaroyl are removed by the action of xylan acetylesterases, feruloyl esterases and *p*-coumaroyl esterases, respectively [93] (Fig. 2, Table 1).



Figure 2: Representation of xylan and its hydrolysis by xylan degrading enzymes. The enzymes involved in the complete biodegradation of xylan are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Xyloglucan

Xyloglucan consists of β -1,4-linked D-glucose residues substituted by D-xylose. Two major groups of xyloglucans have been identified [119]. The XXXG-type xyloglucan contains repeating units of three β -1,4-linked D-glucose residues which are substituted with D-xylose residues through an α -1,6-linkage and are separated by an unsubstituted D-glucose residue (Fig. 3). The XXGG-type xyloglucan consists of two D-glucose residues substituted with α -1,6-linked D-xylose separated by two unsubstituted Dglucose residues [99]. The structural features of these structures have been discussed in detail by Vincken *et. al.* (1997) [119]. The D-xylose residues in xyloglucan can be substituted with D-galactose via β -1,2-linkages and in some instances D-galactose has been further decorated with L-galactose or L-fucose via an α -1,2-linkage [46]. The Dxylose and unsubstituted D-glucose residues may also be substituted with L-arabinose via α -1,2-linkages, which in turn may be further decorated with β -1,2-linked D-xylose [59, 61, 129] (Fig. 3). As the basic backbone of xyloglucan is similar to that of cellulose, the same *endo*-glucanases and β -glucosidases can be responsible for hydrolyzing both polysaccharides although *endo*-glucanases which are specific to the xyloglucan backbone have also been reported (e.g. from *A. aculeatus*) [85]. Accessory enzymes for xyloglucan degradation include: α -xylosidases, α -/ β -galactosidases and α fucosidases. As with xylan, the L-arabinose side chain residues of xyloglucan can be removed by α -L-arabinofuranosidases. Xyloglucan acetylesterases are active only if one or more of the D-galactose residues are acetylated [34, 52] (Fig. 3, Table 1).



Figure 3: Representation of both the XXGG and XXXG forms of xyloglucan and its hydrolysis by xyloglucan degrading enzymes. The enzymes involved in the complete biodegradation of xyloglucan are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Mannan (galacto(gluco)mannan)

Mannan or galacto(gluco)mannan, consists of a β -1,4-linked D-mannose backbone, which can be interrupted by varying amounts of β -1,4-linked D-glucose residues and substituted with α -1,6-linked D-galactose residues. These D-galactose residues were found to be further decorated with β -1,2-linked D-galactose residues in the galacto(gluco)mannan from Nicotiana plumbaginifolia, although this is not common [105]. Water-soluble galactoglucomannan contains acetyl groups attached to the mannan backbone and has a higher degree of D-galactose substitution than waterinsoluble galactoglucomannan [110] (Fig. 4). The backbone of galactoglucomannan is hydrolyzed to manno-, gluco-manno-, and galacto-gluco-mannooligosaccharides by β endomannanases and β -mannosidases [75]. The complete hvdrolvsis galactoglucomannan requires the additional action of β -glucosidases, α -galactosidases and galactomannan acetyl esterases [34, 74] (Fig. 4, Table 1).



Figure 4: Representation of the water-soluble galactomannan structure and its hydrolysis by galactomannan degrading enzymes. The enzymes involved in the complete biodegradation of galactomannan are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Pectin

Pectin is also responsible for rigidity and definition and plays an important role in the physiology of the plant cell wall including porosity, pH, ion balance and surface charge [52, 126]. It contains several substructures: homogalacturonan (HGA), xylogalacturonan (XGA), rhamnoglacturonan I (RG-I) and rhamnogalacturonan II (RG-II) [126].

Homogalacturonan (HGA)

HGA or pectin's "smooth" region, consists of a linear chain of α -1,4-linked D-galacturonic acid residues which can be acetylated at O-2 or O-3 or methylated at O-6 [127] (Fig. 5). Pectin methyl and acetyl esterases act on this substrate to de-esterify the backbone and make it suitable for cross-linking to calcium molecules, thus forming a type of gel which plays an important role in intracellular adhesion [77]. HGA is cleaved by *endo-* and *exo*-polygalacturonases and/or pectin and pectate lyases while the acetyl and methyl groups are removed by pectin acetyl and methyl esterases [26] (Fig. 5, Table 1).



Figure 5: Representation of the homogalacturonan (HGA) structure and its hydrolysis by pectin degrading enzymes. The enzymes involved in the complete biodegradation of homogalacturonan (HGA) are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Xylogalacturonan (XGA) and Rhamnogalacturonan I (RG-I)

XGA and RG-I together make up the "hairy" region of pectin [34, 52]. XGA, like HGA, contains an α -1,4-linked D-galacturonic acid backbone which can be substituted with β -1,3-linked D-xylose [101], which in turn can be further decorated with β -1,2- or β -1,4-linked D-xylose (Fig. 6) [69, 80, 131]. RG-I contains an alternating backbone of α -1,4-linked D-galacturonic acid and α -1,2-linked L-rhamnose residues, the latter with long side chains of L-arabinose (arabinan), D-galactose (galactan) or mixture of both (arabinogalactan) [68] (Fig. 7 and 8). The main chain may also have ester-linked acetyl groups attached to the D-galacturonic acid residues [100, 102] (Fig. 7). The arabinan side chains consist of α -1,5-linked L-arabinose residues which can be substituted with α -1,3-linked L-arabinose and/or feruloyl residues [63]. The galactan chains exist as a linear chain of β -1,4-linked D-galactose residues which also can be substituted with feruloyl residues [63]. The arabinogalactan substituted with L-arabinose residues or β -1,3-linked galactan which can be substituted with L-arabinose residues or β -1,3-linked galactan substituted with L-arabinose residues or β -1,3-linked galactan substituted with L-arabinose residues or β -1,3-linked galactan which can be substituted with L-arabinose residues or β -1,3-linked galactan substituted with L-arabinose residues or α -1,3-, α -1,5- and α -1,6-linked L-arabinose residues [63] (Fig. 8).

While many of the same enzyme activities act on HGA and XGA, some xylogalacturonases which are specific to XGA may also be present [113]. The β -linked D-xylose side chains of XGA are removed by β -xylosidases (Fig. 6, Table 1). RG-I is cleaved by endorhamnogalacturonan hydrolases, exorhamnogalacturonan hydrolases, rhamnogalacturonan lyases and α -rhamnosidases [34] while the acetyl groups are removed by rhamnogalacturonan acetyl esterases [33] (Fig. 7, Table 1). The side-chains of RG-I are hydrolyzed by endo-/exo-arabinases, arabinofuranosidases, *endo-/exo*-galactanases and β -galactosidases [34] (Fig. 8, Table 1).



Figure 6: Representation of the xylogalacturonan (XGA) structure and its hydrolysis by pectin degrading enzymes. The enzymes involved in the complete biodegradation of xylogalacturonan (XGA) are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].



Figure 7: Representation of the rhamnogalacturonan I (RG-I) structure and its hydrolysis by pectin degrading enzymes. RG-I contains an alternating backbone of α -1,4-linked Dgalacturonic acid and α -1,2-linked L-rhamnose residues, the latter to which long side chains of Larabinose (arabinan), D-galactose (galactan) or mixture of both (arabinogalactan) can be attached (Fig. 8).The enzymes involved in the complete biodegradation of rhamnogalacturonan I (RG-I) are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

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Figure 8: Representation of the rhamnogalacturonan I (RG-I) arabinan and arabinogalactan side chains and their hydrolysis by pectin degrading enzymes. The enzymes involved in the complete biodegradation of rhamnogalacturonan I (RG-I) are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Rhamnogalacturonan II (RG-II)

RG-II is a complex polysaccharide, consisting of approximately 30 monosaccharide units [34] with a backbone of eight or more D-galacturonic acid residues [118] and is substituted with up to five different side-chains [118]. These decorations may be of mono- or oligosaccharide structure and can consist of the following residues; Lrhamnose, D-galactose, L-arabinose, D-glucuronic acid, L-fucose, D-apiose, L-aceric acid, 2-O-methyl L-fucose, 2-O-methyl D-xylose, L-galactose, 2-keto-3-deoxy-D-lyxoheptulosaric acid (Dha) and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO) [118] (Fig. 9). Due to its extremely complex structure, enzymatic degradation of pectin requires a large set of activities (Table 1). The identification of the enzymes involved in the degradation of RG-II residue however, thus far remain unclear (Fig. 9).



Figure 9: Representation of the rhamnogalacturonan II (RG-II) structure. A-E represent rhamnogalacturonan II (RG-II) side chains, with two possible positions for 'E' being indicated. The enzymes involved in complete hydrolysis of this polysaccharide remain elusive, and are therefore not included in the figure. The figure is based on [91].

| Substrate | Enzyme Class | Enz. | CAZy families | EC no. |
|---------------|---|------|---------------|-----------|
| Cellulose | β -1,4-D-glucosidase* | BGL | GH1,3 | 3.2.1.21 |
| Cellulose | Cellobiohydrolase | CBH | GH6,7 | 3.2.1.91 |
| Cellulose | β -1,4-D-endoglucanase | EGL | GH5,7,12,61 | 3.2.1.4 |
| Xylan | α-L-arabinofuranosidase* | ABF | GH43,51,54 | 3.2.1.55 |
| Xylan | α-glucuronidase | AGU | GH67,115 | 3.2.1.139 |
| Xylan | acetyl xylan esterase | AXE | CE1 | 3.1.1.72 |
| Xylan | arabinoxylan arabinofuranohydrolase | AXH | GH62 | 3.2.1.55 |
| Xylan | β -1,4-D-xylosidase* | BXL | GH3,43 | 3.2.1.37 |
| Xylan | feruloyl esterase* | FAE | CE1 | 3.1.1.73 |
| Xylan | β -1,4-D-endoxylanase | XLN | GH10,11 | 3.2.1.8 |
| Xyloglucan | a-L-arabinofuranosidase* | ABF | GH43,51,54 | 3.2.1.55 |
| Xyloglucan | α-L-fucosidase | AFC | GH29,95 | 3.2.1.51 |
| Xyloglucan | α-1,4-D-galactosidase* | AGL | GH27,36 | 3.2.1.22 |
| Xyloglucan | α-D-xylosidase | AXL | GH31 | 3.2.1 |
| Xyloglucan | β -1,4(1,2)-D-galactosidase* | LAC | GH2,35 | 3.2.1.23 |
| Xyloglucan | xyloglucan-active β-1,4-D- endoglucanase | XEG | GH12,74 | 3.2.1.151 |
| Xyloglucan | xyloglucan acetylesterase | XGAE | - | - |
| Xyloglucan | β -xylosidase* | BXL | GH3,43 | 3.2.1.37 |
| Galactomannan | α-1,4-D-galactosidase* | AGL | GH27,36 | 3.2.1.22 |
| Galactomannan | β -1,4-D-glucosidase* | BGL | GH1,3 | 3.2.1.21 |
| Galactomannan | galactomannan acetyl esterase | GMAE | - | - |
| Galactomannan | β -1,4-D-endomannanase | MAN | GH5,26 | 3.2.1.78 |
| Galactomannan | β -1,4-D-mannosidase | MND | GH2 | 3.2.1.25 |
| Pectin | α-L-arabinofuranosidase* | ABF | GH43,51,54 | 3.2.1.55 |
| Pectin | endo-arabinanase | ABN | GH43 | 3.2.1.99 |
| Pectin | exo-arabinanase | ABX | GH93 | 3.2.1 |
| Pectin | β -xylosidase* | BXL | GH3,43 | 3.2.1.37 |
| Pectin | feruloyl esterase* | FAE | CE1 | 3.1.1.73 |
| Pectin | β -1,4-endogalactanase | GAL | GH53 | 3.2.1.89 |
| Pectin | β -1,3/-1,6-endogalactanase | GLN | GH5 | 3.2.1.164 |
| Pectin | β -1,4(1,2)-D-galactosidase * | LAC | GH2,35 | 3.2.1.23 |

Table 1: CAZymes involved in plant biomass degradation. Adapted from [23].

| Pectin | pectin acetyl esterase | PAE | CE12 | 3.1.1 |
|--------|--|------|---------|-----------|
| Pectin | pectin lyase | PEL | PL1 | 4.2.2.10 |
| Pectin | rhamnogalacturonan lyase | RGL | PL4,11 | 4.2.2 |
| Pectin | endo-polygalacturonase | PGA | GH28 | 3.2.1.15 |
| Pectin | exo-polygalacturonase | PGX | GH28 | 3.2.1.67 |
| Pectin | pectate lyase | PLY | PL1,3,9 | 4.2.2.2 |
| Pectin | pectin methyl esterase | PME | CE8 | 3.1.1.11 |
| Pectin | rhamnogalacturonan acetyl esterase | RGAE | CE12 | 3.1.1 |
| Pectin | rhamnogalacturonan galaturonohydrolase/exorhamnogalactur onase | RGX | GH28 | 3.2.1.40 |
| Pectin | α-rhamnosidase/rhamnogalacturonan rhamnohydrolase | RHA | GH78 | 3.2.1.40 |
| Pectin | rhamnogalacturonan hydrolase/endorhamnogalacturonase | RHG | GH28 | 3.2.1 |
| Pectin | d-4,5-unsaturated glucuronyl hydrolase | UGH | GH88 | 3.2.1 |
| Pectin | unsaturated rhamnogalacturonan hydrolase | URH | GH105 | 3.2.1 |
| Pectin | β -1,4-exogalactanase | XFG | - | - |
| Pectin | β -1,6-exogalactanase | XSG | - | - |
| Pectin | β -1,3-exogalactanase | XTG | GH43 | 3.2.1.145 |
| Starch | Isoamylase | ISA | GH31 | 3.2.1.68 |
| Starch | a-amylase | AMY | GH13 | 3.2.1.1 |
| Starch | Glucoamylase | GLA | GH15 | 3.2.1.3 |
| Inulin | endo-inulinase | INU | GH32 | 3.2.1.7 |
| Inulin | <i>exo-</i> inulinase | INX | GH32 | 3.2.1.80 |
| Inulin | invertase/fructofuranosidase | SUC | GH32 | 3.2.1.26 |

*indicates enzyme activities which are involved in the degradation of more than one different substrate.

Storage polysaccharides: Starch and Inulin

In addition to the cell wall polysaccharides outlined above, plants also consist of storage polysaccharides such as starch and inulin [52]. The formation of these polysaccharides allows the plant to store simple sugars such as glucose and fructose.

Starch is located in the plastids and is comprised of a branched α -linked D-glucose backbone that is degraded by the enzymatic action of α -amylase which is an *endo*-acting enzyme that hydrolyses starch into gluco-oligosaccharides. These gluco-oligosaccharides are then further degraded into D-glucose by the action of glucoamylases and isoamylase [112] (Fig. 10, Table 1).

Inulin is found in the roots and rhizomes of many plants and is composed of a linear polymer of β -2,1-linked D-fructose, connected to a terminal sucrose residue [86]. This polysaccharide is hydrolyzed by *endo-* and *exo-*inulinases to D-fructose [40], while the sucrose disaccharide is broken down into D-glucose and D-fructose by the action of fructofuranosidase (invertase) [120] (Fig. 10, Table 1).



Figure 10: Representation of the starch and inulin structures and their hydrolysis by starch/inulin degrading enzymes. The enzymes involved in the complete biodegradation of starch/inulin are indicated. Abbreviations of enzyme names are explained in Table 1. The figure is modified from [5].

Plant polysaccharide degradation by Aspergillus

Plant polysaccharide degradation has been studied in detail in *Aspergillus* [34, 37]. To date, the genome sequences of *A. niger* [6, 90], *A. nidulans* [47], *A. oryzae* [72], *A. fumigatus* [81], *A. flavus* [87], *A. sojae* [98], *A. clavatus* [128], *A. terreus* [128], *A. aculeatus*, *A. carbonarius*, *A. kawachii*, *Neosatorya fischeri*, *A. sydowii*, *A. versicolor*, *A. brasiliensis*, *A. tubingensis*, *A. glaucus*, *A. zonatus*, *A. acidicus* and *A. wentii* are all available; (http://www.ncbi.nlm.nih.gov/bioproject, http://genome.jgi.psf.org/programs /fungi/genome-releases.jsf). Comparative analysis of these genomes increases our understanding of the biology of these species and helps us realize their full potential in industrial applications but also in plant biomass degradation [52].

One such comparative study compared the plant polysaccharide degrading potential of *A. nidulans* to that of two industrially relevant strains: *A. niger* and *A. oryzae* [23]. Comparison of the CAZy content of the genomes of these fungi demonstrated significant differences (Table 2). The *A. oryzae* genome contained a significantly higher number of xylan- and pectin-related genes than the other two species, while this was the case for galactomannan-related genes in *A. nidulans* and for inulin-related genes in *A. niger*. Differences in the genome content don't always correlate with the growth profiles of these species found in the Fung-Growth website (www.fung-growth.org). While a good correlation can be found for inulin this is less clear for the other polysaccharides (Fig. 11, Table 2).



Figure 11: Growth comparison of three Aspergilli on four plant polysaccharides relative to growth on glucose.

 Table 2: Comparison of the number of putative genes involved in plant biomass

 degradation of three Aspergilli. Modified from [23].

| Polysaccharide | CAZy families | A. nidulans | A. niger | A. oryzae |
|----------------|--|-------------|----------|-----------|
| cellulose | GH1,3,5,6,7,45,61 | 37 | 30 | 33 |
| xylan | GH3,10,11,43,62,67,115; CE1,15 | 20 | 20 | 37 |
| xyloglucan | GH12,29,31,74,95 | 6 | 7 | 8 |
| galactomannan | GH2,5,26,27,36 | 19 | 12 | 12 |
| pectin | GH2,28,35,43,51,53,54,78,88,93,10 5; PL1,3,4,9,11; CE1,8,12 | 73 | 66 | 92 |
| starch | GH13,15 | 16 | 15 | 21 |
| inulin | GH32 | 2 | 5 | 4 |

Chapter 1

Efficient degradation of polysaccharides not only relies on the production of a specific set of enzymes but also requires synergistic interactions between the enzymes themselves. Synergy between enzymes in Aspergilli is a well-accepted phenomenon and one which has been shown to enhance its degrading abilities [34]. Addition of acetylxylan esterase increased the release of xylose and associated oligosaccharides for the *endo*-xylanses by up to a factor of 4.4 (*endo*-xylanase I), 14.7 (*endo*-xylanase II) and 16.3 (*endo*-xylanae III) [65]. The action of endo-xylanase and β -xylosidase on the xylan backbone increased 2- and 2.5-fold through the cooperation of arabinoxylan arabinofuranohydrolase and α -L-arabinofuranosidase, respectively [33]. Similarly, in the degradation of pectin, the action of β -galactosidase was shown to support the activity of feruloyl esterase A by increasing the amount of ferulic acid released by over two-fold [33].

Regulatory systems of Aspergillus related to plant biomass degradation

Due to the complexity of plant biomass and its large variety depending on species, tissue and season, efficient degradation by fungi requires a fine-tuned production of diverse enzyme sets, tailored to the available biomass. This is organized through a set of transcriptional regulators that activate or repress the expression of genes encoding plant biomass degrading enzymes. Regulation of plant biomass degradation has been best studied in Aspergillus and will be discussed in the next sections and is summarised in Table 3. The general model for regulation of plant biomass utilization is depicted in Fig. 12 (de Vries, unpublished data). When a monomeric component of a polysaccharide is sensed by the fungus, a signalling pathway results in the activation of a transcription factor (regulator) that enters the nucleus and binds to the promoter of its target genes. These genes are then expressed and produce enzymes involved in metabolism of the compound to enable the cell to grow. As monomeric compounds are rare in the environment, the presence of it suggests the presence of the polymeric compound it originates from. Therefore, genes encoding extracellular polysaccharide degrading enzymes are also expressed resulting in liberation of more of the monomeric compound.

The amylolytic regulator AmyR

AmyR controls the genes involved in starch degradation, such as glucoamylase (*glaA*), alpha-amylase (*amyA*, *amyB*) and α -glucosidase (*agdA*) [92, 109]. AmyR contains a Zn₂Cys₆ DNA-binding domain and belongs to the Gal4 regulator-family [83]. AmyR can bind to CGGN₈CGG and CGGAAATTTAA sequences in the promoter of its target genes in the presence of specific compounds (inducers), such as starch and maltose [92]. Maltose was long considered the true inducing compound of the system but recent studies have questioned this. In *A. oryzae*, isomaltose has been shown to be

the best inducer [78], while glucose has also been shown to induce activation of AmyR in *A. niger* and *A. oryzae* [19, 117]. In *A. niger*, AmyR also regulates genes encoding β -glucosidases and α - and β -galactosidases [117].

The (hemi-)cellulolytic regulator XlnR

XlnR is also a member of the Gal4 (Zn₂Cys₆) family of regulators. While this regulator was originally described as a xylanolytic regulator [115], later studies demonstrated that is also controls genes involved in the degradation of cellulose and xyloglucan [31, 49, 114] as well as several genes of the pentose catabolic pathway [11, 12, 29, 55, 116]. XlnR binds to GGCTAR sequences in the promoter of its target genes in the presence of its inducer, xylose [36]. While *xlnR* itself is constitutively expressed, the protein gets activated in the presence of xylose by C-terminal proteolytic cleavage that results in transport of XlnR to the nucleus [56].

XlnR has also been studied in *A. oryzae* and *A. nidulans* where it performs a similar role, although the set of target genes are not identical in the three species. However, approximately 20 genes were shown to be consistently regulated by XlnR in these three species [4].

The arabinanolytic regulator AraR

After the availability of the *A. niger* genome sequence [90] three genes with amino acid homology to XlnR were identified and analysis of these genes demonstrated that the closest homolog encoded AraR also contains a Zn_2Cys_6 DNA binding domain [12]. AraR activates the expression of genes encoding enzymes releasing arabinose from arabinoxylan and pectin, as well as genes encoding other pectinases and genes of the pentose catabolic pathway. The binding site of AraR has not yet been determined, but *araR* itself is induced in the presence of arabinose [12], suggesting autoregulation. Analysis of the function of AraR in *A. nidulans* revealed differences in its effect on specific target genes, such as *ladA* (encoding L-arabitol dehydrogenase) [11].

The inulinolytic regulator InuR

InuR was identified through analysis of the surrounding region of genes encoding inulin-degrading enzymes (invertase, *endo-* and *exo-*inulinase) in *A. niger* [130], based on the gene organization for *amyR* in *Aspergillus* [92]. Interestingly, InuR has sequence similarity to AmyR and is likely to have originated from a common ancestor, similar to XlnR and AraR.

InuR is also a member of the Zn_2Cys_6 family of transcriptional regulators, but its DNA binding site has not yet been determined. The inducer of InuR has been suggested to be sucrose [130].

The galactose-related regulators GalR and GalX

GalR and GalX form a two-factorial regulatory system for galactose utilization in *A. nidulans* [21]. GalR was identified by homology to XlnR and is also a member of the Zn₂Cys₆ family of transcriptional regulators. Detailed analysis demonstrated a second gene encoding a Zn₂Cys₆ regulator (GalX) next to GalR in the genome of *A. nidulans*. GalX regulates the expression of GalR and some D-galactose metabolic genes, while the majority of the metabolic genes as well as at least one α -galactosidase encoding gene are under control of GalR. GalR is unique to *A. nidulans*, while GalX is found in several Aspergilli as well as other filamentous ascomycetes [21]. In *A. niger* GalX regulates the oxido-reductive D-galactose catabolic pathway [53].

The pectinolytic regulator RhaR

In *A. niger* the presence of a general galacturonic acid responsive regulator that controls most pectinases was suggested, with additional regulators responding to rhamnose and arabinose (AraR, see above) [35]. Analysis of micro array data on a pectin-related compound identified several candidate regulator encoding genes that were specifically expressed in the presence of rhamnose and rhamnose-containing substrates. One was located next to genes of a recently discovered L-rhamnose catabolic pathway [123] and disruption of this gene (*rhaR*) resulted in reduced growth on L-rhamnose as well as reduced α -rhamnosidase activity compared to the wild [52]. RhaR affects the expression of not only α -rhamnosidase encoding genes, but also genes encoding other pectinolytic enzymes, such as rhamnogalacturonases, rhamnogalacturonan lyases, rhamnogalacturonan and pectin acetyl esterases and β -galactosidases [52]. Most of these genes are related to degradation of the rhamnogalacturonan I.



Figure 12: Model for the utilization of plant polysaccharides by fungi.

The mannanolytic regulator ManR

ManR is also a member of the Zn_2Cys_6 family of transcriptional regulators and was identified in *A. oryzae* [82]. ManR was shown to regulate genes encoding *endo*-mannanase, β -mannosidase, α -galactosidase, acetylmannan esterase and β -glucosidase and disruption of this regulator resulted in a significant reduction in growth on galactomannan [82].

The carbon catabolite repressor CreA

CreA was originally discovered in A. nidulans as a glucose-repressor that suppresses the expression of its target genes in the presence of glucose [41] and its DNA-binding site was established to be SYGGRT [66]. Later studies demonstrated that CreA suppresses the expression of many genes in the present of sufficient amounts of easily metabolizable carbon sources, such as glucose, but also xylose, mannose, glucuronic acid and others [97]. Its role seems to be in conserving energy by turning off genes for secondary carbon sources when sufficient amount of a primary carbon source is present. This not only includes genes of alternative metabolic pathways, but also genes involved in the release of monomeric carbon sources, such as those encoding plant biomass degrading enzymes [97]. Several studies demonstrated significant increases in the production of plant polysaccharide degrading enzymes by fungi when creA is deleted [32, 62, 108]. One of the best cellulose producing strains is the Trichoderma reesei RUT-C30 strain, which has been shown to be a cre1 deletion strain [104]. It was also suggested that a bigger effect on protein production can be achieved by removing the repression on the positively acting regulator (e.g. AmyR) than on its target genes (e.g. amylase-encoding genes) [2].

Interaction between regulators

In recent studies, the interaction between several regulators was examined and demonstrated. XlnR and AraR both regulate the genes associated with the common steps of the L-arabinose and D-xylose catabolic pathways [12]. In addition, they act antagonistically with respect to their target genes. In a wild type strain, XlnR-regulated genes are expressed on xylose while AraR-regulated genes are expressed on arabinose. In a *xlnR*-knockout however, AraR-regulated genes are also expressed on xylose while in an *araR*-knockout, XlnR-regulated genes are also expressed on arabinose [12]. The mechanism of this interaction has not yet been elucidated.

XlnR and CreA, together, determine the expression level of xylanolytic genes in the presence of D-xylose. In a wild type strain, xylose induces the genes through the action of XlnR, but increasing levels of xylose result in a reduction of the expression of these genes [32]. This did not occur in a CreA-mutant, demonstrating that this modulation of the expression level occurs via CreA [32]. In a recent study this data has

been confirmed for a larger group of xylanolytic genes, although the strength of the CreA effect differed for sub-groups of these genes [71].

| Regulator | Function | Inducer | Reference | | |
|------------------------------|--|---|------------------|--|--|
| Positively acting regulators | | | | | |
| AmyR | Starch degradation, glucose/galactose release | D-glucose, isomaltose | [78, 92, 117] | | |
| AraR | L-arabinose release and catabolism | L-arabitol | [11, 12] | | |
| GalR | D-galactose catabolism | D-galactose | [21] | | |
| GalX | D-galactose catabolism | D-galactose | [21, 53] | | |
| InuR | Inulin degradation | Sucrose | [130] | | |
| ManR | (galacto-)mannan degradation | Mannobiose | [82] | | |
| XlnR | (hemi-)cellulose degradation, D-xylose catabolism | D-xylose | [114, 115] | | |
| Negatively acting regulators | | | | | |
| CreA | Carbon catabolite repression | Sufficient levels of monomeric carbon sources | [41, 97] | | |

Table 3: Regulators involved in plant biomass utilisation in Aspergillus.

Improved enzyme production

The initial focus of protein production in filamentous fungi was to increase homologous expression. Examples of such studies are the development of an *A. niger* strain in which secretion of glucoamylase went from 0.5 g/L to levels of up to 30 g/L through classical mutation-selection techniques, media development and fermentation optimizations, over a 20 year period. Finkelstein (1987) showed that recombinant technology could be used as a viable alternative to the classical methods of strain improvement [43] and thus created an interest in these fungi as possible production hosts for heterologous proteins.

The high yields achieved with homologous protein production have not yet been realized with heterologous proteins [51]. Apart from protease activity, which is one of the leading problems for heterologous protein production, this may be due to a number of factors including transcription, translation, secretion and host strain physiology. To improve recombinant protein production in fungi, several strategies have been developed. Genetic strategies include the introduction of multi-copies of the target protein gene, the use of strong promoters and effective secretion signals, gene fusions to genes associated with well-expressed and secreted proteins and development of

protease-deficient host strains [95, 121]. Some success has also been obtained using a bioprocessing approach of optimizing fungal morphology, mycelia immobilization and culture conditions [1, 18].

Transcriptional control

Studying gene expression in filamentous fungi has increased our understanding of the molecular mechanisms controlling transcription initiation and/or regulation along with the selection of strong promoters. The promoter regions of the *Aspergillus* amylase genes consist of four highly conserved regions, which work together to enhance expression levels [76, 122]. A sequence of CCAAT, present in the *amdS* promoter region of *A. nidulans* and responsible for high-level expression of acetamidase, was also found in many other promoter regions in *A. nidulans* [84, 122]. Furthermore, the enolase gene (*enoA*) which is one of the most highly expressed genes in *A. oryzae*, was found to contain a 15-bp sequence, essential for transcriptional regulation of that gene [111, 122].

A number of other studies have shown transcriptional control to be a contributor in production levels of heterologous proteins in filamentous fungi. One such analysis, carried out by Archer *et al.* (1994) showed that hen egg-white lysozyme (HEWL) mRNA levels under the control of the *glaA* promoter in *A. niger* correlated well with HEWL protein production, but comparable HEWL and glucoamylase mRNA levels did not lead to comparable secreted protein levels [7].

Protein synthesis and secretion

A general model for protein synthesis and secretion in fungi was proposed [44]. During synthesis, proteins are transported to the endoplasmic reticulum where folding and glycosylation are initiated. In Aspergilli, folding and maturation of secretory proteins is assisted by protein disulfide isomerase (Pdi). SNARE proteins facilitate vesicle-mediated transport of the completed proteins to the more-porous hyphal tip for extracellular secretion while those which did not fold or glycosylate correctly are sent to the proteosomes or vacuoles for degradation. It has been hypothesized that over-production of recombinant proteins into the ER has the potential of congesting the folding, assembly and secretion pathways of the fungal hosts. The over-production of foldases and chaperones, which catalyze and mediate the folding of emerging polypeptides into functional proteins, could alleviate this bottleneck [122]. After secretion, recombinant proteins often face the risk of degradation by homologous proteases produced by many filamentous fungi. Different heterologous proteins are subject to degradation by different proteases [7] although careful consideration of species such as *A. vadensis* [38] or the use of protease deficient mutants can limit this.

Bioprocessing strategies

Optimization of the yield of secreted heterologous proteins also relies on the response of protein secretion to growth conditions. Alterations to the growth medium, including agitation and dissolved oxygen [24], have been shown to relate directly to the specific responses of a given promoter but also to fungal morphology, protease production [39] and cell viability [7]. Ambient pH directly regulates the level of proteases produced in *A. niger* [39] while carbon and nitrogen sources can have either repressing or inducing effects on enzyme production [88, 89]. Carbon and nitrogen sources can also effect protease activity with most extracellular proteases being inhibited under conditions of high glucose or ammonium in the medium [121]. Furthermore, some studies have shown that gene expression and protein secretion might be quite different in solid-state fermentation than that observed in submerged fermentation e.g. in submerged cultures some enzyme activities are found in the cell wall of the mycelia while in solid-state cultures they are found to be secreted into the media [54, 64].

Aim and outline of this thesis

Much research over the past 25 years has been applied to the development of filamentous fungi, most notably *Aspergillus*, as hosts for recombinant protein production. Their inherent abilities to grow at high rates and to high biomass densities and their exceptional capacity to secrete high levels of homologous product are well recognized. Despite there being many advances made in the hyper-production of heterologous proteins in filamentous fungi, their ability to produce and secrete homologous proteases along with different native protein glycosylation still requires further strain improvement to efficiently produce a wide range of heterologous proteins. The aim of this thesis was to develop an efficient fungal expression system for the production of recombinant proteins, in particular those involved in plant biomass degradation. Due to its potential in biomass degradation and with its favourable fermentation capabilities, *Aspergillus* was chosen for this study.

In **Chapter 2** eight different *Aspergillus* species were compared with respect to their genomic ability to degrade plant cell wall biomass. While all tested Aspergilli had a similar potential to degrade plant biomass, results showed that even in closely related species, their strategies differed markedly. Combining the approaches from different species is likely to result in better enzyme mixtures for industrial applications, such as the saccharification of plant biomass for biofuel production.

In **Chapter 3**, the molecular and phenotypical differences between *A. vadensis* and six other species of black Aspergilli were examined. Growth on varying carbon sources and extracellular enzyme profiles when grown on maltose/starch indicated significant and unique differences between *A. vadensis* and the other black Aspergilli. Further analysis of the partial genome of *A. vadensis* genome, combined with gene

expression data when grown on maltose indicate that its aberrant phenotype is likely caused by the low expression of prtT and amyR regulators or their associated genes and not a mutation or deletion as was originally concluded.

In **Chapter 4** six novel constitutive promoters from *A. niger* (pef1 α , ptktA, pef1 β , ptal1, pcetA and ppgkA) and a further five from *A. vadensis* (pef1 α , prps31, pgpdA, pubi1 and poliC) were tested in *A. vadensis* using a gene encoding a secreted arabinofuranosidase from *Fusarium oxysporum* as a reporter for heterologous protein production. Of the promoters tested, 3 from *A. niger* (pef1 α > ptal > ppgkA) and 3 from *A. vadensis* (pef1 α > poliC > prps31) all resulted in higher ABF activity than for that of the commonly used gpdA promoter from *A. nidulans*.

In **Chapter 5** the potential of *A. vadensis* as an expression host was tested by successfully expressing an α -L-arabinofuranosidase (*abfB*) (GH54) and an *endo*-1,4- β -D-glucanase (*eglA*) (GH12) from *A. vadensis* under the control of the *gpdA* promoter from *A. vadensis*.

In **Chapter 6** an evolutionary screening method was used to improve the inulin degradation potential of *Aspergillus oryzae* through the upregulation of *exo*-inulinase. As an organism with no predicted *endo*-inulinase function, improved inulin degradation would be largely dependent on the overproduction of this enzyme. Subsequent generation growth of *Aspergillus oryzae* (Rib40) on inulin for 9 weeks successfully resulted in *exo*-inulinase overproducing mutants.

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Chapter 2

Closely related fungi deploy diverse enzymatic strategies to degrade plant biomass

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Abstract

Plant biomass is a major carbon source for many fungi and also an important industrial substrate for the production of food, textiles, fuels and chemicals. Degradation of plant biomass by fungi is a highly complex process that involves a large number of extracellular enzymes as well as non-hydrolytic proteins, whose production is controlled by a set of transcriptional regulators. Aspergillus species form one of the best studied fungal genera, largely due to their applications in biotechnology and relevance in human health. In this study we have compared the genomic content and the enzymes produced by eight Aspergilli for the utilization of plant biomass. While all tested Aspergilli have a similar genomic potential to degrade plant biomass, their approaches differ markedly in the overall activities as well as the specific enzymes they employ. An exception is A. clavatus that has a strongly reduced pectinolytic ability. Many of the genes have orthologs in (nearly) all tested species, but only very few of the corresponding enzymes are produced by all species during growth on wheat bran or sugar beet pulp. In addition, significant differences were observed between the enzyme sets produced on these feedstocks, largely correlating with their polysaccharide composition. These data demonstrate that Aspergillus species employ significantly different approaches to degrade plant biomass. Combining the approaches from different species is expected to result in improved enzyme mixtures for industrial applications, such as the saccharification of plant biomass for biofuel production.

Introduction

Plant biomass is the predominant carbon source for most fungi and consists largely of polymeric compounds, of which polysaccharides are the main components [15, 18]. In addition, lignin encrusts the polysaccharides and acts as a physical barrier that impedes fungal enzymes from gaining access to them. Fungi cannot take up intact polysaccharides, but need to degrade them extracellularly to monomeric and oligomeric compounds using diverse enzymatic mixtures [18, 55]. Plant polysaccharide degradation by fungi has been a topic of study for many decades due to its relevance in many industrial applications, such as paper & pulp, food & feed, beverages, textiles and detergents. More recently, the increasing interest in the production of alternative fuels and chemicals from plant biomass has provided an even greater push for research into fungal decomposition of plant biomass.

Analysis of an increasing number of fungal genome sequences has demonstrated the fundamental differences in the plant polysaccharide degrading machinery of fungi [2, 5, 8, 10, 19, 22]. In addition, the regulatory systems that control plant biomass degradation also differ strongly among fungi, although they are largely conserved among different *Aspergillus* species [29, 53]. Results from a previous study on the utilization of polysaccharides by three Aspergilli [10] suggest that related fungal species may have developed different approaches to plant biomass degradation. In nature, biomass-degrading fungi live in community with other microorganisms. It can be expected that different species target distinct components of the substrate and degrade them using dissimilar enzyme combinations. An enhanced understanding of these strategies will not only increase our knowledge of fungal biodiversity, but will help in the design of efficient industrial enzyme mixtures for plant biomass degradation. In this study, we compared the plant biomass degradation potential and approaches of eight *Aspergillus* species: *A. clavatus*, *A. fischeri*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. oryzae* and *A. terreus* (Suppl. Table 1).

Materials and methods

Media and growth conditions

The fungal strains used in this study are listed in Suppl. Table 1. *Aspergillus* minimal medium was described previously [17]. All monomeric and oligomeric carbon sources were added to a final concentration of 25 mM, while pure polymeric substrates and crude substrates were added to a final concentration of 1% and 3%, respectively. The pH of the medium was adjusted to 6.0. For plate growth, the centre of the plates was inoculated with 2 μ l of a suspension of 500 spores/ μ l and plates were incubated for 5 days at 30°C. All eight species were grown on minimal medium with 35 carbon sources including crude plant biomass, pure plant polysaccharides, oligosaccharides, monosaccharides and control substrates (casein, lignin) (Suppl. Fig. 1, www.fung-

growth.org). To confirm that the detected differences were species specific, a second isolate of each species was examined along with the sequenced strain. Growth on 25 mM D-glucose was used as a reference because the tested strains grow at different rates and D-glucose, among the monosaccharides, supported the fastest growth for all species. Growth on the other substrates relative to growth on D-glucose was then compared among the species. Growth on plates was analyzed by visual inspection by two authors independently after which these were compared and discussed.

Liquid cultures were inoculated with 10⁶ spores/ml (final concentration) and incubated at 250 rpm for 3 days. All cultures were incubated at 30°C and performed in duplicate. Two to three strains of all species were grown in liquid cultures with 1% wheat bran or 1% sugar beet pulp. Culture filtrates after three days of cultivation were analyzed for the presence of free monomeric sugars, but no glucose, xylose, galacturonic acid, rhamnose or fructose was detected. SDS-PAGE analysis of the extracellular proteins revealed nearly identical profiles for strains of the same species (Suppl. Fig. 6), indicating that enzyme production is highly conserved within a species. Detailed analysis of the produced enzymes was therefore only performed on a single strain.

Chemicals and media

Glucose, maltose, sucrose, inulin, beechwood xylan, Guar gum, apple pectin and all *p*nitrophenyl-substrates were from Sigma–Aldrich. Soluble starch was from Difco. Red Debranched Arabinan (S-RDAR), Azo-CM-cellulose (S-ACMC), Azo-galactan (S-AGALP) and AZ-rhamnogalacturonan (S-AZRH), Azo-wheat arabinoxylan (S-AWAXP) and polygalacturonic acid (PGA) were from Megazyme International Ireland.

Composition analysis of plant biomass substrates

Sugar composition was determined by analyzing the sugars as their alditol acetate derivatives using GC-FLD as described previously [30].

CAZy annotation

The identification step of CAZymes followed the procedures previously described [10] where sequences are subject to BlastP analysis [1] against a library composed of modules derived from the CAZy database, the positive hits are then subjected to a modular annotation procedure that maps the individual modules onto the peptide using hits against libraries of catalytic and carbohydrate models derived from CAZy using BlastP or Hidden Markov models [1, 20]. The functional annotation step involves BlastP comparisons against a library of modules derived from biochemically characterized enzymes [10].

Orthology and synteny analysis

Genome scale protein ortholog clusters were constructed using OrthoMCL [34] by inflation factor 1, E-value cutoff 1E-3, percentage match cutoff 60% as for identification of distant homologs [9]. The orthologs clusters were further split according to the synteny detected by the Sybil algorithm [12] at www.aspgd.org. Sequences of genes were manually double checked by multiple sequence alignments with MAFFT[28] and potential errors of gene models were corrected.

Enzyme assays

All exo-acting CAZy enzyme activities were performed in micro titer plates. Reactions were carried out in 100 µL volumes containing 25 mM sodium acetate (pH 5), 0.01% substrate and suitably diluted culture filtrate. The mixture was incubated at 30°C for 2 h and the reaction was terminated by the addition of 100 µL 250 mM sodium carbonate. Enzyme activities (α -arabinofuranosidase, cellobiohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, glucoamylase (α -maltosidase), β -mannosidase, α -rhamnosidase and β -xylosidase) were determined spectrophotometrically at 405 nm by measuring the release of *p*-nitrophenol (*p*NP) from their appropriate *p*NP-substrates and standardized against a known concentration of *p*-nitrophenol (*p*NP). Activities were expressed as nmol *p*NP/mL sample/min.

Endoarabinanase, *endo*-1,4- β -glucanase (cellulase), *endo*-1,4- β -galactanase and rhamnogalacturonanase activities were measured using 20 mg/mL of Red Debranched Arabinan (S-RDAR), Azo-CM-cellulose (S-ACMC), Azo-galactan (S-AGALP) and AZ-rhamnogalacturonan (S-AZRH), respectively. Endo-1,4-β-xylanase activity was measured using 10 mg/mL Azo-wheat arabinoxylan (S-AWAXP). 100 µL reactions were carried out containing equal volumes of buffered substrate (pH 4.5) and suitably diluted culture filtrate which were then incubated at 40°C for 1 h in the case of the endoarabinanase, *endo*-1.4- β -glucanase and *endo*-1.4- β -xylanase activities and 16 h for the *endo*-1.4- β -galactanase and rhamnogalacturonan activities. *Endo*-arabinanase reactions were terminated with the addition of 400 μ L 95% ethanol, endo-1.4- β galactanase, rhamnogalacturonanase and *endo*-1,4- β -xylanase reactions with 250 μ L 95% ethanol and *endo*-1,4- β -glucanase reactions with a 250 μ L solution of sodium acetate trihydrate (40 mg/mL) and zinc acetate (4 mg/mL) in 76% ethanol. Precipitated reactions were then centrifuged at 1000×g for 10 min and optical density of supernatants was measured at 590 nm. Endoarabinanase reactions were measured at 520 nm. Endo-acting enzyme activities are expressed as amount of dye released (absorbance change)/mL sample/min.

Pectate lyase activity was assayed using polygalacturonic acid (PGA). Reaction mixtures contained equal volumes of 50 mM N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) (pH 10.0) and 2.5 mg/mL PGA, to which suitably diluted culture filtrate

was added. Changes in absorbance at 235 nm were measured for approximately 30 min at 40 $^{\circ}\mathrm{C}.$

Laccase activity was assayed using 2,2'-azino-di-(3-ethylbenzothiazoline-6sulphonic acid) (ABTS). Reaction mixtures contained 700 μ L H₂O, 100 μ L 0.5 M glycine-HCl (pH 3.0), 100 μ L culture filtrate and 100 μ L 14 mM ABTS. The reaction was monitored by measuring the change in absorption at 436 nm at 30°C. The extinction coefficient of 29,300 M⁻¹·cm⁻¹ was used for oxidized ABTS. Activity is expressed as is in nmol/min/ml.

Feruloyl esterase activities were determined spectrophotometrically (Shimadzu PharmaSpec UV-1700) at 37°C in 100 mM MOPS (3-(*N*-morpholino)propanesulfonic acid buffer (pH 6.0). Methyl caffeate (MC), methyl ferulate (MF), methyl *p*-coumarate (MpC) and methyl sinapate (MS) (1.18 mM stock solutions in 100 mM MOPS, pH 6.0) were used as substrates. Reaction mixture contained 100 µL of culture liquid, 870 µL MOPS buffer and the reaction was started by the addition of 30 µL substrate. Absorbance was monitored for 5 min at 308 nm for MpC ($\varepsilon_{308} = 20,390 \text{ M}^{-1} \text{ cm}^{-1}$), 320 nm for MF ($\varepsilon_{320} = 29,680 \text{ M}^{-1} \text{ cm}^{-1}$) and MS ($\varepsilon_{320} = 15,890 \text{ M}^{-1} \text{ cm}^{-1}$), and 322 nm for MC ($\varepsilon_{322} = 14,720 \text{ M}^{-1} \text{ cm}^{-1}$). FAE activities were expressed as nkat/1 (10⁻⁹ mol/s/L).

Determination of monomeric sugars in the cultures

Presence of monomeric sugars in wheat bran and sugar beet pulp liquid cultures was measured by using Megazyme's Assay kits for D-glucose & D-fructose (K-FRUGL), D-xylose (K-XYLOSE), D-glucuronic acid (K-URONIC) and L-rhamnose (K-RHAMNOSE) using the provider's instruction. All measurements were done with two biological replicates.

SDS-PAGE

Protein profiles were obtained by combining 25 μ L of culture supernatant supplemented with 5 μ L of 5 x Laemmli Loading Buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 0.1 M dithiothreitol, 0.2 mg/mL Bromophenol Blue) and separating this on 12% SDS-PAGE gels. Proteins were visualized by silver staining and a PageRuler Unstained Protein Ladder (Thermo Scientific) was used as protein marker.

Proteomics analysis

Proteins from 3 ml of culture filtrate were precipitated with cold TCA/acetone and the amount of protein recovered was determined using the RCDC kit assay (BioRad, Mississauga, Ont). Five micrograms of protein were digested with trypsin and an aliquot analyzed by LC-MS/MS as previously described [44] on a Velos LTQ-Orbitrap mass spectrometer (Thermo-Fisher, San Jose, CA). MS/MS data were processed using

Proteome Discoverer Quant 1.3 (Thermo-Fisher) and spectral data were searched against *Aspergillus* protein databases downloaded from the Aspergillus Genome Database (AspGD). Search parameters used were 0.80 Da for fragment ion tolerance and 10.0 ppm for parent ion tolerance, fixed iodoacetamide cysteine modification and variable methionine oxidation. Protein and peptide identification confidence filters were applied to satisfy a 1% false discovery rate at the Peptide and Protein level. Protein grouping was applied so as to satisfy the principles of parsimony.

Hierarchical clustering and correlation analysis

Matrix files of presence/absence and activity of CAZy genes and protein production measured by proteomics experiments were generated. The hierarchical clustering of CAZy genes were created by R [48] using the Euclidean distance with complete linkage and visualized by iTOL [31, 32]. The dendrogram and heatmaps of protein abundance were created and visualized using Genesis [50] with Pearson's correlation and complete linkage.

Phylogenetic analysis

Sequences of the RPB1, RPB2 (RNA polymerase II genes), Tsr1 (putative ribosome biogenesis protein), Cct8 (putative chaperonin complex component TCP-1) and AguA (α -glucuronidase) genes were downloaded from the full genome data sets and aligned using the Muscle software in the MEGA5 package [52]. After aligning, the data sets were combined and maximum likelihood analysis was performed using RAxML version 7.2.8 [49]. Each locus was treated as a separate partition. The number of bootstrap replicates was set on 1000 replicates. Sequences of *Penicillium chrysogenum* Wisconsin 54-1255 were used as outgroup.

Results

Genomic potential of the studied Aspergilli related to plant biomass utilization

Based on the Carbohydrate-Active enZymes (CAZy) [35] annotation pipeline, total numbers of Glycoside Hydrolases (GH), Polysaccharide Lyases (PL) and Carbohydrate Esterases (CE) vary among the species (Fig. 1). The percentage of GH genes related to plant polysaccharide degradation (PPD) is 58-66% for all genomes, except that *A. clavatus* has 20-30% less GH genes than the others (Fig. 1), largely due to a reduction in pectinases (GH28, GH54, GH78, GH88) (Suppl. Table 2). *A. clavatus* also contains the lowest percentage of PPD-related PL genes (71% as compared to >86%), which are also all related to pectin degradation. When the genomes were compared for individual CAZy families, significant differences in numbers of genes were observed (Suppl. Table 3A&B). Variations in gene numbers are particularly obvious in certain CAZy families involved in the degradation of mannan (GH26),

pectin (GH28, GH53, GH78, GH88, GH93, PL1, PL3, CE8 and CE12), xyloglucan (GH29 and GH74), starch (GH31), sucrose/inulin (GH32), cellulose (GH45 and GH61), and xylan (GH115 and CE15). Genes encoding lignin peroxidase, manganese peroxidase or versatile peroxidase are not present in any of these genomes, but significant differences are found in the number of laccases and other oxidoreductase enzymes, which may play a role in lignin or polysaccharide degradation (Suppl. Table 3). *A. niger* is richest in laccases (13 in its genome), while the other species have 2-9. The variations in CAZy content are relatively small compared to previous studies with a more diverse set of fungal species [2, 5, 8, 10, 19, 22]. This can be explained by their close phylogenetic relationships and their similar habitats, which would push genome evolution in a similar direction.

Orthologous clustering of the CAZymes showed that only 14.7% of the genes encoding hydrolytic enzymes are shared by all species (Suppl. Table 4A). In contrast 27.5% of the genes are unique to a single species, with the largest number in *A. nidulans*, *A. niger* and *A. terreus*. For the oxidative enzymes, 10.8% of the genes are shared by all species, while 40.8% of the genes are unique to a single species, with again the largest number in *A. nidulans*, *A. niger* and *A. terreus*. For the oxidative enzymes, 10.8% of the genes are shared by all species, while 40.8% of the genes are unique to a single species, with again the largest number in *A. nidulans*, *A. niger* and *A. terreus* (Suppl. Table 4B). In general, the CAZyme distribution among the species follows their phylogenetic relationship. In total, this means that only 70 genes are shared by all species, while the number of unique genes differs strongly by species (Fig. 1).

Growth on plant biomass related substrates

Major differences of these eight species grown on mono- oligo- and polysaccharides were observed (Suppl. Fig. 1), indicating different carbon source preferences. Two isolates per species were tested to check that the differences are species specific and not strain specific. These carbon source preferences can be partially linked to their diverse genomic content of CAZyme-encoding genes. For instance, growth of *A. clavatus* was particularly poor on pectin which correlates well with its low number of 30 pectin-targeting genes, as compared to 55-92 for the other species (Suppl. Table 2). Good growth on inulin was observed for *A. niger* ATCC 1015 and *A. fischeri*, while for all other species growth was reduced on inulin, but not on sucrose. This does not correlate with the number of putative inulin/sucrose-targeting genes in the genomes, as *A. fischeri* has only two, while *A. niger* has four, fewer than the six genes for *A. terreus* which grows poorly on inulin (Suppl. Table 2).

All species grew well on wheat bran and also, with the exception of *A. clavatus*, on sugar beet pulp (Suppl. Fig. 1). These substrates were therefore selected to analyze their enzymatic ability in more detail. Composition analysis (Suppl. Table 5) showed that wheat bran contains mainly of cellulose and (arabino)xylan, with xyloglucan and



species is indicated behind their name in the taxonomic tree.

pectin as minor components. In contrast, sugar beet pulp contains mainly cellulose, xyloglucan and pectin, which explains the reduced growth of *A. clavatus*.

Enzyme profiles during growth on wheat bran and sugar beet pulp

A preliminary test demonstrated that at Day 3 of cultivation, activities for eight plant biomass degrading enzymes were maximal for all fungi (data not shown). This time point was therefore selected for the full enzymatic analysis. Nineteen extracellular, lignocellulose-active enzyme activities of the liquid cultures were measured (Suppl. Fig. 2-4). Comparison of these profiles demonstrated strong differences among the species, not only in the quantities of the activities, but also in the induction of specific enzymes. For instance, the highest activity levels for most enzymes of A. terreus were observed during growth on sugar beet pulp, while wheat bran resulted in higher levels of most enzymes for A. flavus (Suppl. Fig. 2). When the individual activities were compared across the species, specific differences became noticeable. Wheat bran consists mainly of cellulose and arabinoxylan and the main regulator controlling degradation of these polysaccharides is XlnR, which is present in all Aspergilli [7]. Endoxylanase and β -xylosidase were mainly produced on wheat bran, and levels were particularly high for A. niger (Suppl. Fig. 2). Endoarabinanase, α -rhamnosidase, pectate lyase and endogalactanases, all related to pectin degradation, were mainly produced on sugar beet pulp, but rarely were all four activities produced by one species. Sugar beet pulp contains mainly cellulose and pectin and therefore pectinases and cellulases would be expected to be the main enzymes produced on this substrate, which is confirmed by our data.

Mass spectrometric analysis of the extracellular proteins confirmed the activity measurements with respect to the enzymes that were detected (Suppl. Table 6A-6D). Figure 2 and Suppl. Fig. 5 show the presence of orthologous enzymes involved in the degradation of different polysaccharides in wheat bran and sugar beet pulp. This analysis demonstrates the high degree of diversity among the species in the production of orthologous enzymes. Only a few orthologous enzymes are produced by all or most species and in most cases they are produced on both wheat bran and sugar beet pulp (Fig. 2) although often with significantly different levels (Suppl. Table 6A-6D). These data highlight the different enzymatic approaches used by the eight species to degrade plant biomass.

Correlation of CAZy profiles, taxonomy and enzyme activity of the eight Aspergilli Figure 3 shows the correlation of the species for genome content, enzyme activity and production of individual enzymes. Correlating the number of genes per CAZy family demonstrated that with respect to genome content, closely related species (A. oryzae – A. flavus, A. fischeri – A. fumigatus) cluster together (Fig. 3A). This indicates that the





evolution of their genome content related to plant biomass degradation follows the evolutionary history of the species. However, more distantly related species, *A. nidulans* and *A. terreus*, can display similar CAZy content. A possible explanation for this finding is that natural habitat exerts a stronger influence on genome evolution than phylogenetic relatedness.

No clear correlation was observed between the enzyme activities produced in response to complex substrates and evolutionary relatedness (Fig. 3B), possibly due to the range of non-plant substrates some species are known to consume (e.g. collagen for A. terreus, A. flavus and A. nidulans and insect larvae for A. clavatus), resulting in a varying biotope range and dependence on plant biomass. The composition of wheat bran and sugar beet pulp is different and they should elicit different activity profiles. For six of the tested species, the wheat bran and sugar beet pulp activity profiles diverge strongly. Unexpectedly, the sugar beet pulp and wheat bran activity profiles clustered together for A. flavus and A. oryzae. Two of the three tested A. niger strains (N402 and ATCC 1015) clustered together for both substrates, while the third (CBS 513.88) was strongly divergent in the enzyme activity profile. These results show that strains of the same species (CBS 513.88 and ATCC 1015) with near identical genomic content can use dramatically different sets of enzymes to hydrolyze complex biomass. It should be noted that genome sequence analysis suggests that strains ATCC 1015 and N402 are likely descended from the same isolate (A. Tsang and co-workers, unpublished data), which explains the clustering of their activity profiles.

Correlation of the proteomics data did not follow the activity correlation (Fig. 3B and 3C), which can be explained by the production of non-orthologous enzymes for the same general activity by different species (Suppl. Table 6A-6D). This adds an additional dimension to the highly divergent strategies of these Aspergilli. Considering the fairly similar genome content of these species, we conclude that the differences in their plant biomass degrading strategies are mainly at the regulatory level. More detailed studies into the regulation of orthologous CAZy-genes in several species could reveal whether this is due to different sets of target genes of the main regulators or whether additional unknown regulators modulate the influence of the main regulators.

Figure 3: Correlation analysis of the genome (A), enzyme activity (B) and proteomics data (C). Continued on next page.



Figure 3 Cont: Correlation analysis of the genome (A), enzyme activity (B) and proteomics data (C). (Please see pdf file to enlargen text).



Discussion

In this study we compared eight Aspergilli with respect to plant polysaccharide degradation. The variations in CAZy content between these species was relatively low compared to previous studies in which a more diverse set of fungal species was compared [2, 5, 8, 10, 19, 22]. This can be explained by the close phylogenetic relationships and/or by and the highly similar habitats of these Aspergilli, which would direct genome evolution in a similar direction. Human use of and/or interaction with the species differs markedly; with *A. niger* and *A. oryzae* being widely used industrial fungi, *A. fumigatus* one of the most significant opportunistic fungal human pathogens, and *A. flavus* a plant pathogen. However, all these species are common inhabitants of soil and stored agricultural products, and their spores are widespread in both indoor and outdoor environments. Although some of the sequenced strains are domesticated and not recent natural isolates, the comparison to a second strain that is a natural isolate showed that growth on 35 carbon sources is identical for two strains of the same species. This demonstrates that the sequenced isolates have maintained their natural ability to use the tested carbon sources.

Hierarchical clustering of the plant polysaccharide degrading enzymes of these species demonstrated that in general the species with the most similar CAZy content are also taxonomically close.

The number of unique genes (Suppl. Table 2) per species also correlates well with the phylogenetic distance of the species. The lowest number was found for *A. oryzae*, *A. flavus*, *A. fischeri* and *A. fumigatus*. As the first two and the last two species, respectively, are closely related, their high similarity explains this low number. The more distant species (*A. nidulans*, *A. terreus*, *A. niger*, *A. clavatus*) have higher numbers of unique genes.

A high level of variation was detected in the enzyme activities of the tested species during growth on sugar beet pulp and wheat bran. While all species grew well on these substrates, with the exception of somewhat less growth of *A. clavatus* on sugar beet pulp, the enzyme profiles of the species showed strong differences. Wheat bran consists mainly of cellulose and arabinoxylan, while sugar beet pulp contains mainly cellulose and pectin. Enzymes able to degrade these different combinations of polysaccharides would therefore be expected to be prominent in the culture filtrate of all species grown on these substrates. Our study confirmed this as nearly all enzymes activities detected on wheat bran in all species are involved in xylan or cellulose degradation, while mainly pectinolytic and cellulolytic enzymes were detected on sugar beet pulp. The main regulator controlling the production of xylanolytic and cellulolytic enzymes in *Aspergillus* is XlnR, which has been studied in detail in *A. niger, A. oryzae* and *A. nidulans* [14, 27, 38, 39, 43, 51, 56, 57]. XlnR activates the expression of xylanolytic and cellulolytic genes in response to the presence of xylan or

xylose, the latter being the actual inducer. Indications for similar regulation have been reported for the other species [4, 13, 21, 25, 40, 45]. Regulation studies in A. niger have previously demonstrated that pectinolytic genes are induced by galacturonic acid, rhamnose, polygalacturonic acid or pectin [16, 26, 37]. Differences in pectinolytic gene content between A. niger, A. nidulans and A. oryzae may be influenced by the pH of their natural habitat [46]. An acidic pH favors pectin hydrolases, while a neutral to alkaline pH favors pectin lyases, supported by the finding that all fungal GH28 pectin hydrolases have activity optimum between pH 2 and pH 5, while pectin lyases have optimum between pH 7 and pH 10 (https://mycoclap.fungalgenomics.ca) [41]. The pH of most samples was 7 except for A. *nidulans* on sugar beet pulp (pH = 8), A. *niger* on wheat bran (pH = 5.5) and sugar beet pulp (pH = 4.5), and A. *clavatus* on sugar beet pulp (pH = 6). The pH in the sugar beet pulp cultures correlates well with the pectin hydrolase and lyase activities and with the proteomics results (Suppl. Table 6A-6D). Therefore, the differences in enzyme levels are likely caused by regulatory variation. Since the major regulators are shared by all tested species [53], their function or range of target genes in the tested Aspergilli is different and/or additional non-shared regulators are involved in the utilization of complex biomass. A difference in the function of the arabinanolytic regulator AraR in A. niger and A. nidulans was recently described [6], and the inducers for activation of AmyR also appear to differ between A. niger, A. nidulans and A. oryzae [58]. More detailed analysis of the set of target genes, and function and mechanism of the polysaccharide related regulators in the other species will be required to understand the mechanism responsible for these differences. Interestingly, the production of several cellulases appears to be conserved among the species, suggesting that this may be a core-activity for all species. In contrast, the production of hemicellulases is highly varied, suggesting specific adaptations of the species in their biomass degrading approach.

Laccase activity was detected for most species, with the highest activity on wheat bran for *A. flavus* and on sugar beet pulp for *A. fumigatus*. This does not correlate with the numbers of putative laccases detected in the genomes, suggesting significantly different regulation of the production of these enzymes among the species. Induction of laccase-encoding genes was mainly studied in basidiomycetes in which transcription is modulated by metal ions (Cu^{2+} , Ag^+ , Mn^{2+}), aromatic compounds, nitrogen and carbon sources (nature and ratio) [47]. In ascomycetes, regulatory elements such as HSE (Heat shock elements), MRE (Metal response elements) and nitrogen metabolite regulation elements (NIT-2 like) were identified in the promoter region of laccase-like multicopper oxidase [33]. In addition, laccases are also involved in other biological processes, such as spore pigment formation [54], and not only in lignin degradation, so the total number of laccases likely does not reflect the number of laccases which play a role in plant biomass degradation.

Although the fungi tested in this study produce diverse enzyme sets, they all grow well on the crude plant biomass substrates. This suggests that different strategies for the degradation of plant biomass may be equally efficient (as measured by fungal growth). In biotechnological applications, such as biofuel production, complete hydrolysis of the plant biomass is difficult to achieve with currently available enzyme cocktails. This may in part be explained by the absence of specific activities in these mixtures. The data obtained in this study show the existence of distinctly different enzymatic approaches to degrade biomass. A judicious mix of these approaches is likely to result in improved enzyme cocktails for biomass hydrolysis. Recently it was shown that addition of *Podospora anserina* hydrolases increases the efficiency of a *Trichoderma reesei* enzyme mixture [11]. In this study we provide indications that similar results could be obtained with more closely related fungi. The advantage of using enzymes from other Aspergilli to improve enzyme cocktails of *A. niger* or *A. oryzae* is that heterologous production of these enzymes is not likely to cause problems due to the high similarity in gene structure of these species.

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Closely related fungi deploy diverse enzymatic strategies to degrade plant biomass

Supplementary Figure 1: Growth profiling of eight Aspergilli on plant-biomass related carbon sources. Growth of the eight Aspergillus species was evaluated on 35 plant biomass related carbon sources (full profiles are available at www.fung-growth.org). Minimal medium (MM) [17] was used supplemented with 25 mM of mono- or disaccharide. 1% polysaccharide or 3% crude plant biomass. Strains were grown for five days after which pictures were taken immediately. A second strain was also assessed for each species to confirm that the differences were species specific. The general growth speed differed between the strains of a species, but for most species no significant carbon source related differences were observed between the strains (data not shown). An exception to this is A. niger CBS513.88 that grew poorly on all pure carbon sources and was shown to have an amino acid auxotrophy (unpublished data), which explains this phenotype. Apparently, both wheat bran (WB) and sugar beet pulp (SBP) contain sufficient protein/amino acids to supplement this deficiency. All other strains grew well on MM + glucose and glucose was therefore used as an internal reference to compare the strains, to avoid misleading differences caused by general differences in growth speed between the species. Growth on the other substrates relative to growth on D-glucose was then compared between the species.

Growth on pure cellulose was zero to very poor for all species. Most fungi had similar growth on glucose, maltose, starch and xylan, with the exception of *A. nidulans*, for which poor growth on maltose was observed for one strain. Growth on sucrose was similar to growth on glucose for nearly all strains, but strong differences were observed on inulin. Good growth on this substrate was observed for *A. niger* ATCC1015 and *A. fischeri*, while for all other species growth was reduced compared to sucrose. This does not correlate with the number of putative inulin-targeting genes in the genomes as *A. fischeri* has only two (Table 2), while *A. niger* has six just like *A. terreus*, which grows poorly on inulin. This suggests that not all GH32 proteins may be involved in inulin degradation. Good growth compared to the other species was observed for *A. niger*, *A. nidulans*, *A. fumigatus* and *A. flavus* on guar gum (galactomannan). While *A. nidulans* has the highest number of galactomannan-targeting genes. Growth of *A. clavatus* was particularly poor on pectin which correlates well with its very low number of pectin-targeting genes (only 30 putative genes), which was less than half of the other species (Suppl. Table 2).

The monomeric composition of the plant biomass substrates was determined and the polysaccharides present in the substrates were estimated based on this analysis (Suppl. Table 5). Wheat bran and cotton seed pulp contain mainly cellulose and (arabino)xylan, with xyloglucan and pectin as minor components of cotton seed pulp. In contrast, sugar beet pulp contains mainly cellulose, xyloglucan and pectin. (Continued on next page).



Supplementary Figure 2: Hydrolytic enzyme activity profiles of the eight species. Enzyme profiles of the eight *Aspergillus* species during growth on sugar beet pulp (SBP, red) and wheat bran (WB, blue). Samples were taken after three days and are identical to the samples used for proteomics. Vertical lines separate the activities related to the same substrate; from left to right: cellulose (C), xylan (X), galactomannan (G), starch (S), pectin (P). BGL = β -glucosidase, CBH = cellobiohydrolase, EGL = endoglucanase, BXL = β -xylosidase, XLN = endoxylanase, MND = β -mannosidase, AGL = α -galactosidase, AGD = α -glucosidase, GLA = glucoamylase, PLY = pectate lyase, RHG = endorhamnogalacturonase, RHA = α -rhamnosidase, ABN = endoarabinanase, ABF = α -arabinofuranosidase, GAL = endogalactanases, LAC = β -galactosidase.

Activity units were: For all *exo*-acting enzyme activities (ABF, CBH, AGL, LAC, AGD, BGL, GLA, MND, RHA, BXL) are expressed as nmol *p*NP released/ml sample/min. *Endo*-acting enzyme activities (ABN, EGL, GAL, XLN and RHG) are expressed as amount of dye released (absorbance change)/ml sample/min. Pectate lyase (PLY) activity is expressed as absorbance change/ml sample/min.

Significant differences can be observed between the profiles both with respect to the relative activity of the different enzymes as well as to the production of the different activities on SBP and/or WB. Related species do not have high similarity with respect to enzyme activity profiles. Significant differences were observed between *A. oryzae* and *A. flavus*, and between *A. fumigatus* and *A. fischeri*. The reduction of pectinase encoding genes in the *A. clavatus* genome is reflected in very low pectinolytic enzyme activity. (Continued on next page).



Supplementary Figure 3: Laccase activity of the eight species. Laccase activity of the eight *Aspergillus* species during growth on sugar beet pulp (SBP, red) and wheat bran (WB, blue). Samples were taken after three days and are identical to the samples used for proteomics. Laccase activity is in nmol/min/ml. *A. niger* N402 was used for these assays.

The highest laccase activity was observed for *A. flavus* during growth on WB and for *A. fumigatus* during growth on SBP. Activities in the other species were significantly lower.



Supplementary Figure 4: Differences in feruloyl esterase production. Fungal strains were grown in 50 ml liquid minimal medium [17] with 1% wheat bran (WB) or sugar beet pulp (SBP) in 250 ml Erlenmeyer flasks. Culture filtrate samples were taken on day 3 and used for enzyme assays. Feruloyl esterase activities were determined spectrophotometrically at 37 °C in 100 mM MOPS buffer (pH 6) using methyl caffeate (MC), methyl ferulate (MF), methyl *p*-coumarate (MpC) and methyl sinapate (MS) as substrates. Absorbance was monitored for 5 min at 308 nm for MpC ($\varepsilon_{308} = 20,390 \text{ M}^{-1} \text{ cm}^{-1}$), 320 nm for MF ($\varepsilon_{320} = 29,680 \text{ M}^{-1} \text{ cm}^{-1}$) and MS ($\varepsilon_{320} = 15,890 \text{ M}^{-1} \text{ cm}^{-1}$), and 322 nm for MC ($\varepsilon_{322} = 14,720 \text{ M}^{-1} \text{ cm}^{-1}$).

Strongly divergent FAE activity profiles were observed for the studied Aspergilli. Differences were observed with respect to the carbon source that induced the activities as well as the substrate that was converted in the assays. The highest FAE activities were detected in *A. nidulans* WB cultures, but no activity was observed against MS. Both *A. terreus* and *A. niger* CBS513.88 strains produced FAE activity only in SBP cultures with MpC as substrate. In contrast, *A. niger* N402 and *A. clavatus* produced FAE activity only in the WB cultures with MS as a substrate. While no activity was detected in the SBP cultures of *A. niger* ATCC1015, it produced activities against MF, MpC and MS in WB cultures. FAE activity against MC was detected in both SBP and WB cultures of *A. oryzae*, *A. fischeri* and *A. nidulans*. For other substrates, the activity profiles differed between these strains. As it is unlikely that the substrate specificity of orthologous enzymes would differ this much with respect to these four substrates, the data implies that different feruloyl esterases are produced by the strains.



Supplementary Figure 5: Proteomic analysis of the presence of CAZy enzymes during growth on wheat bran and sugar beet pulp. Proteomics data of the eight *Aspergillus* species during growth on sugar beet pulp (SBP, purple) and wheat bran (WB, orange). Samples were taken after three days and are identical to the samples used for activity assays. The proteins are plotted using the ortholog clusters (Suppl. Table 4). The dendrogram was created with the presence/absence pattern of orthologous genes by R using Euclidean distance with complete linkage. The resulting tree was visualized by iTOL. Presence of the gene in a genome is depicted by a grey box in the circle corresponding to the species/strain. An orange colour indicates that the protein was detected during growth on wheat bran, while a purple colour indicates presence in sugar beet cultures.

These figures demonstrate the high diversity of the *Aspergillus* species, not only with respect to presence/absence of orthologs, but also with respect to the production of orthologous enzymes during growth on plant biomass. (Continued on next page; Please see pdf file to enlargen text).


Chapter 2

Supplementary Figure 6: Conserved SDS-PAGE profiles for isolates of the same species. SDS-PAGE profiles of the strains used in this study. Extracellular culture samples from the wheat bran and sugar beet cultures were separated by SDS-PAGE and the gels were stained using silver staining. Two or three isolates per species were analysed, which demonstrated high conservation of the extracellular protein profile within a species. Larger differences were visible between the species. The profiles of more closely related species (*A. oryzae – A. flavus* and *A. fumigatus – A. fischeri*) were more similar to each other than to the profiles of the other species.



| Species | Strain number | Alternative strain number | Genome sequence reference |
|--------------|------------------|----------------------------------|------------------------------|
| A. nidulans | FGSC A4 | ATCC 38163 | [24] |
| A. nidulans | | DTO 131-G5 | n/a |
| A. niger | CBS 513.88 | | [46] |
| A. niger | ATTC 1015 | CBS 113.46, NRRL 328, FGSC A1144 | [3] |
| A. niger | N402 | | n/a |
| A. terreus | NIH 2624 | FGSC A1156 | Unpublished |
| A. terreus | | DTO 8-G3 | n/a |
| A. oryzae | RIB 40 | ATCC 42149 | [36] |
| A. oryzae | | DTO 26-C3 | n/a |
| A. flavus | NRRL 3357 | CBS 128202, ATCC 200026 | [59] |
| A. flavus | | DTO 52-B6 | n/a |
| A. clavatus | NRRL 1 | CBS 513.65, ATCC 1007 | [23] |
| A. clavatus | | DTO 27-C2 | n/a |
| A. fischeri | NRRL 181 | CBS 544.65, ATCC 1020 | [23] |
| A. fischeri | | DTO 3-E7 | n/a |
| A. fumigatus | Af293 | FGSC A1435 | [42] |
| A. fumigatus | | DTO 26-B5 | n/a |

Supplemental Table 1: Strains used in this study.

n/a = not available

| Species | Cellulose* | Xyloglucan | Xylan | Galactomannan | Pectin | Starch | Inulin | |
|--------------------|--------------------------|---------------------------|-------------------|---------------|--|----------------------|--------|--|
| | GH1, GH12 ¹ , | GH12 ² , GH29, | CE14, CE15, GH35, | GH27, GH58, | CE8, CE12, GH2 ⁹ , GH28, | GH13 ¹¹ , | GH32 | |
| | GH5 ¹ , GH6, | GH31 ³ , GH74, | GH10, GH11, | GH26, GH27, | GH35, GH43 ¹⁰ , GH51, GH53, | GH15, | | |
| | GH7, GH45, | GH95 | GH436, GH62, | GH36, | GH54, GH78, GH88, GH93, | GH3112 | | |
| | AA9 | | GH67, GH115 | | GH105, PL1, PL3, PL4, PL9, | | | |
| | | | | | PL11 | | | |
| A. nidulans | 22 (36) | 7 | 29 | 19 | 71 | 21 | 2 | |
| A. niger ATCC1015 | 19(33) | 8 | 14 | 12 | 64 | 15 | 4 | |
| A. terreus | 30(43) | 11 | 33 | 18 | 55 | 19 | 6 | |
| A. oryzae | 22 (39) | 7 | 34 | 14 | 89 | 23 | 4 | |
| A. flavus | 22 (39) | 7 | 34 | 14 | 92 | 22 | 4 | |
| A. clavatus | 22 (28) | 4 | 21 | 11 | 30 | 23 | 1 | |
| A. fischeri | 30 (44) | 8 | 29 | 14 | 66 | 24 | 2 | |
| A. fumigatus Af293 | 26(37) | 9 | 28 | 14 | 65 | 22 | 4 | |
| | | | | | | | | |

Supplemental Table 2: Comparison of the polysaccharide degradation potential of eight Aspergilli based on their genome content. The potential per polysaccharide was determined by adding up the number of genes per polysaccharide-related (sub-)family

¹⁰Only In brackets the numbers including putative GH3 BGLs are given. BGLs are also involved in other processes than cellulose degradation and their high number in the genomes could hide the real difference in gene numbers related to cellulose degradation between the species. ¹Only endoglucanases of this family. ²Only xyloglucan-active endoglucanases of this family. ³Only a-xylosidases of this family. ⁶Only acetyl xylan esterases of this family. ⁵Only β -xylosidases of this family. ⁶Only β -xylosidases and α -arabinofuranosidases of this family. ⁷Only β -mannosidases of this family. ⁸Only endomannaneses of this family. ⁹Only β -galactosidases of this family. endoarabinanases of this family. ¹²Only α -galactosidases of this family.



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Supplemental Table 3B: Numbers of putative genes per Plant Polysaccharide Degradation-related CAZy family for the 10 genomes addressed in this study. Colour coding is meant to visualize the relative numbe rof genes in the species and families. Supplemental Table 4A: Orthology clusters of feruloyl esterase (SF), glycoside hydrolase (GH), carbohydrate esterase and polysaccharide lyase (PL) families.

Colour codes: Yellow = at least one of the orthologs has biochemical support

Green = only present in one species

Orange = present in all species

Blue = present in only one strain of this species

The number of species in which an ortholog is present is listed after the orthologous cluster and a sum of this is given at the bottom.

The number of unique genes per species is given at the bottom of the species column.

(Continued on next 6 pages).

| species | | | | - | | - | | - | | | - | | | | - | | | | | | | - | | | - | - | | | | | | | | - | | | | | | - | |
|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-----------|-----------|-------------|-----------|-----------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|
| species. | | | | | - | | | | | - | | | | | | - | | - | | | | | - | - | | | - | 2 | | | | | | | | | | | | | |
| pecies 2 | | | | | | | - | | | | | | | | | | | | | | | | | | | | | | | | - | | | | | - | | | | | |
| ecies 3 s | - | | | | | | | | | | | | | - | | | | | | | | | | | | | | | | | | | - | | | | - | | | | - |
| acies 4 sp | | | - | | | | | | | | | - | | | | | | | | | | | | | | | | | - | | | | | | | | | | - | | |
| cies 5 sp | | | | | | | | | | | | | | | | | | | - | | | | | | | | | | | - | | | | | | | | | | | |
| cies 6 spe | | | | | | | | | | | | | | | | | | | | | - | | | | | | | | | | | | | | | | | - | | | |
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| us Bis 8 spec | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| orthologo cluste | ister 001 | ister 002A | ster 002B | ster 003A | ister 003B | ister 004 | ister 005 | ster 006 | ister 007 | ister 008 | ister 009 | ster 010 | ister 011 | ister 012A | ster 012B | ister 013 | ister 014 | ister 015 | ister 016 | ister 017 | ister 018 | ster 019 | ster 020 | ster 021 | ster 022 | ster 023 | ster 024 | ster 025 | ster 026 | ster 027 | ster 028 | ister 029 | ster 030 | ster 031 | ister 032 | ster 033 | ster 034 | ster 035 | ster 036 | ster 037 | ster 038 |
| jatus | Clu | 040 Clu | 150 Clu | Clu | Cli | Clu | Clu | Clu | 570 Clu | 510 Clu | Clu | 380 Clu | 530 Clu | Clu | Clu | 40 Clu | 360 Clu | Clu | 520 Clu | SEO CIU | 380 Clu | Clu | CIU | 250 Clu | Clu | CIU | CIU | 180 Clu | 250 Clu | 270 Clu | CIU | 510 Clu | 390 Clu | Clu | 710 Clu | 100 Clu | 500 Clu | 970 Clu | S00 Clu | Clu | CIP |
| A. fumi AF293 | | 0 Afu6g09 | 0 Afu6g00 | | | | | | 0 Afu8g06 | Afu1g17 | | 0 Afu7g02 | 0 Afu2G14 | | | 0 Afu2g09 | 0 Afu5g09 | | 0 Afu8g01 | 0 Afu3g07 | 0 Afu8g06 | | | 0 Afu8g07. | | | | Afu8g06 | 0 Afu2g17: | 0 Afu2g17. | | 0 Afu3g14 | 0 Afu6g14 | | 0 Afu1g14 | 0 Afu1g16 | 0 Afu6g14 | 0 Afu8g06 | 0 Afu3g12 | | |
| fumigatus 163 | | UB_07499 | 70760_BU | | | | | | UB_08120 | | | UB_08895 | UB 03015 | | | UB_02530 | UB 05742 | | UB_08509 | UB 04145 | UB 08089 | | | UB_08050 | | | | | UB_03290 | UB_03292 | | UB_03472 | UB_00037 | | UB_01426 | UB_01574 | UB_00017 | UB_08082 | UB_03658 | | |
| heri A1 | | 54700 AF | 47590 AF | | | | | | 99230 AF | | 84920 | 15130 AF | 89720 AF | | | ΑF | 77450 AF | | 95020 AF | 69500 AF | 99600 AF | | | 00100 AF | | | | 99110 | 92640 AF | 92660 AF | | 62750 AF | 60260 AF | | 10690 AF | 09040 AF | 60550 AF | 99670 AF | 64710 AF | | |
| A. fisc | | 60 NFIA_0 | NFIA_0 | | | | | | 20 NFIA_0 | | NFIA_0 | 30 NFIA_1 | 50 NFIA 0 | 20 | | 80 | 70 NFIA 0 | | 40 NFIA_0 | 10 NFIA 0 | NFIA 0 | | | NFIA_1 | 10 | 80 | | NFIA_0 | NFIA_0 | NFIA_0 | | 70 NFIA_0 | 20 NFIA_0 | | 60 NFIA_0 | 80 NFIA_0 | NFIA_0 | NFIA_0 | 20 NFIA_0 | 00 | |
| . clavatus | | CLA_0833 | | | | | | | CLA_0812 | | | CLA_0651 | CLA 0550 | CLA 0615 | | CLA_0174 | CLA 0128 | | CLA_0442 | CLA 0356 | | | | | CLA_0599 | CLA_0599 | | | | | | LCL_04197 | CLA_0875. | | CLA_0206 | CLA_0191 | | | CLA_0404 | CLA_0642 | |
| us A | 07436 | 09228 A | 07310 | | 09470 | | 11725 | | 05471 A | | | 00922 A | 04047 A | 06446 A | | A | 00281 A | 01578 | 03618 A | 10916 A | 09467 | | 08528 | | A | A | 01797 | | | 09543 | 08631 | 06177 A | • | | 02496 A | • | 08626 | 08111 | A | A | 05262 |
| A. flav | 7 AFL2G | 2 AFL2G | 6 AFL2G | en 19 | 4 AFL2G | | 3 AFL2G | | 5 AFL2G | | | 5 AFL2G | 8 AFL2G | 4 AFL2G | | | 7 AFL2G | 4 AFL2G | 9 AFL2G | 4 AFL2G | 0 AFL2G | | 9 AFL2G | | | | 8 AFL2G | | 2 | 1 AFL2G | 5 AFL2G | 6 AFL2G | | | 7 AFL2G | | 8 AFL2G | 5 AFL2G | | | AFL2G |
| zae | 000100020 | 000100058 | 00010000 | 01020001 | 1020001 | | 001000057 | | 001100074 | | | 000500094 | 002300015 | 070100088 | | | 00500027 | 00300151 | 01200074 | 02000040 | 1020001 | | 011300003 | | | | 000300126 | | 01020000 | 01020000 | 011300015 | 070100055 | | | 000300049 | | 011300014 | 12000007 | | | |
| A. or | 907 A009 | 563 AO09 | 212 A009 | A009 | A009 | 115 | A009 | | 343 A009 | | | A009 | 112 A009 | 544 A009 | 914 | | 138 AO09 | A009 | A009 | 704 AO09 | A009 | | A009 | | | | A009 | | 016 AO09 | A009 | A009 | 511 A009 | 945 | | 587 AO09 | | A009 | 557 AO09 | 135 | | 745 |
| A. terreus | ATEG 08 | ATEG_06 | ATEG_02 | | | ATEG_02 | | | ATEG_091 | | | | ATEG 08 | ATEG 06 | ATEG_01! | | ATEG 06 | | | ATEG 01 | | | | | | | | | ATEG_10 | | | ATEG_039 | ATEG_00! | | ATEG_00 | | | ATEG_028 | ATEG_04 | | ATEG 09 |
| A. niger TCC 1015 | 51662 | 51478 | | | | | 190471 | 43194 | 211544 | | | | 43785 | | | | 53315 | | 44585 | 174365 | 214857 | | | | | | | | 51400 | 190025 | | 189254 | | 140573 | 213437 | | | 131747 | | | 189620 |
| 1.88 A | 0120 | 0390 | | | | | 2780 | 5350 | 5010 | | | | 2550 | | | | 3100 | | 6310 | 2505 | 9690 | | | | | | | | 9360 | 2270 | | 2160 | | 2100 | 3740 | | | 3170 | | | 2410 |
| s CBS51: | An09g0 | An12g1 | | | | | An04g0 | An0990 | An12g0 | | | | An12g0 | | | | An07g0 | | An03g0 | An02g1 | An04g0 | | | | | | | | An04g0 | An05g0 | | An09g0 | | An11g0 | An03g0 | | | An04g0 | | | An05g0 |
| 4. nidular | | M1772 | | | | | | | N6093 | AN8320 | | | AN5267 | | | | N8782 | | | AN4860 | 0666NM | 9967NM | | | | | | | | AN7240 | M2834 | M2528 | | | N10124 | | | M9183 | M10375 | | M3200 |
| CAZY | faeA | SF1 | SF1 | SF3 | SF3 | SF3 | SF4 | SF4 | CE 1 | CE 8 | CE 8 | CE 8 | CE 8 | CE 8 | CE 8 | CE 8 | CE 8 | CE 8 | CE 12 | CE15 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 2 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | a | a | a | a | a | a | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | lyl esteras | | | | | | | | | |
| | se | 56 | se | se | e | se | se | e | sterase | sterase | sterase | se | se | e | 9 | 9 | | esterase | esterase | esterase | esterase | esterase | esterase | esterase | esterase | esterase | ronan ace | terase | se | se | se Se | 86 | 86 | se | 80 | lase |
| tion | oyl esterat | yl xylan et | yl xylan et | yl xylan et | oyl esterat | | in methyl | in methyl | in methyl v | in methyl | in methyl | in methyl v | in methyl v | in methyl | in methyl v | nnogalactu | nnogalactu | nnogalactu | nnogalactu | nnogalactu | nnogalactu | uronoyl es | glucosida | n glucosida | a glucuronia |
| /me e | SF7 feru | SF1 feru | SF1 ferul | SF3 ferul | SF3 ferul | SF3 ferul | SF4 ferul | SF4 ferul. | acet | acet | acet | SF5 ferul | SF5 feul | SF5 feul | SF5 feul. | SF5 feul. | | pect | pect | pect | pect | pect | pect | pect | pect | pect | E man | gluc | beta | beta | beta | beta | beta | beta | beta | beta |
| enz | FAE | AXE | AXE | AXE | FAE | FAE | FAE | FAE | FAE | ~ | PME | PME | PME | PME | PME | PME | PME | PME | PME | RGA | RGA | RGA | RGA | RGA | RGA | 띵 | BGL | BG | BGL | BGL | BGL | BGL | BG | ŝ |

| 3H), carbohydrate | |
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| lycoside hydrolase (| |
| lloyl esterase (SF), gl | ed on next 5 pages). |
| ogy clusters of feru | .) families. (Continue |
| le 4A Cont: Orthol | accharide lyase (PI |
| Supplemental Tab | esterase and polys: |

| GUS | beta glucuronidase | GH 2 AN2395 | An02g00610 | 52111 ATEG_01031 AO | 090023000053 | AFL2G_03956 | NFIA_0 | 39700 AFUB_030140 Afu2g1 | 4520 Cluster 039 | | | | | |
|--------|---|--------------|--------------|----------------------|---|----------------|-------------------|----------------------------|-------------------|---|---|---|---|---|
| GUS | beta glucuronidase | GH 2 AN5361 | An01g01260 | 46827 ATEG_10255 AO | 00000880000 | AFL2G_08828 | | | Cluster 040 | | - | | | |
| LAC | beta galactosidase | GH 2 | | ATEG_04784 | | | | | Cluster 041A | | | | | - |
| LAC | beta galactosidase | GH 2 AN2463 | | ATEG_00712 | | | | | Cluster 041B | | | | - | |
| LAC | beta galactosidase | GH 2 AN3201 | | ATEG_10243 AO | 0090012000389 | AFL2G_03296 | NFIA_0 | '2410 AFUB_062230 Afu6g1 | 4550 Cluster 041C | | - | | | |
| LAC | beta galactosidase | GH 2 AN6388 | | | | | | | Cluster 042 | | | | | - |
| LAC | beta galactosidase | GH 2 AN1107 | | | | | NFIA_0 | 72890 AFUB_061780 Afu6g1 | 4090 Cluster 043 | | | | - | |
| QNW | beta mannosidase | GH 2 AN1742 | An11g06540 | 138876 ATEG_06636 AO | 090001000556 | AFL2G_09201 A | CLA_083570 NFIA_0 | 3490 AFUB_074800 Afu6g0 | 8840 Cluster 044 | - | | | | |
| QNW | beta mannosidase | GH 2 AN11680 | An01g06630 | 172687 ATEG_07339 AO | 090003001410 | AFL2G_01666 A | CLA_078510 NFIA_0 | 16260 AFUB_100920 Afu4g0 | 0390 Cluster 045 | - | | | | |
| QNW | beta mannosidase | GH 2 | | ATEG_09890 AC | 090005000740 | AFL2G 00728 | | | Cluster 046A | | | | - | |
| QNW | beta mannosidase | GH 2 AN3368 | An12g01850 | 212893 ATEG 08684 AC | 090010000208 | AFL2G 11464 A | CLA_066240 NFIA_1 | 14030 AFUB_087900 Afu7g0 | 1320 Cluster 046B | - | | | | |
| BGL | beta glucosidase | GH 3 | | ATEG_10274 | | | | | Cluster 047A | | | | | - |
| BGL | beta glucosidase | GH 3 | An14g01770 | 210981 ATEG 02806 AC | 090003001511 | ALF2G 01582 | NFIA_0 | 95760 AFUB_084510 Afu8g0 | 2100 Cluster 047B | | - | | | |
| BGL | beta glucosidase | GH 3 AN7396 | An11q00200 | 179266 ATEG 10320 AO | 090012000135 | AFL2G 03066 | NFIA 0 | 77920 AFUB 016780 Afu1q1 | 7410 Cluster 047C | | | | | |
| BGL | beta glucosidase | GH 3 | | AO | 090103000127 | AFL2G 12245 | | | Cluster 047D | | | | - | |
| BGL | beta glucosidase | GH 3 | | AO | 090701000841 | AFL2G 06408 A | CLA 007810 | | Cluster 047E | | | | - | |
| BGL | beta glucosidase | GH 3 | An07q07630 | 139037 | | | | | Cluster 048 | | | | | - |
| BGL | beta glucosidase | GH 3 | An07g09760 | 39613 | | | | | Cluster 049A | | | | | - |
| BGL | beta olucosidase | GH 3 AN7915 | An08a08240 | 38077 AG | 090001000266 | AFL2G 07497 | | | Cluster 049B | | | - | | |
| BGL | beta diucosidase | GH 3 AN10482 | An11a06080 | 208871 ATEG 06617 AC | 090001000544 | AFL2G 09187 A | CLA 083710 NFIA 0 | 4350 AFUB 074660 Afu6a0 | 8700 Cluster 050A | | | | | |
| BG | beta olicosidase | GH 3 AN4102 | An18n03570 | 56782 ATEG 03047 AC | 9350006000600 | AFI 2G 10322 A | CLA 028810 NFIA 0 | 18950 AFUB 006160 Afri100 | 5770 Cluster 050B | | | | | |
| BG | beta discosidase | GH 3 AN6652 | An15n01890 | 182309 ATEG 07121 AC | 090009000554 | AFI 2G 10164 A | CLA 096980 NFIA 0 | 50080 AFUB 094720 Afti6d0 | 3570 Cluster 050C | | | | | |
| | heta discosidase | CH3 | 200.06 | ATEG 02724 AC | 200000000000000000000000000000000000000 | AFI 2G DURRA | | | Cluster 061 | | | | | |
| 200 | tota di contraso | | 000-00-V | | 100000000000000000000000000000000000000 | | | | Cluster DCDA | | | | | |
| | nera dincosinase | 200 | neconficnity | 07044 | tennoni nen | | | | | | | | | |
| BGL | beta glucosidase | GH 3 AN3903 | | ATEG_04069 AO | 090166000090 | AFL2G_09413_A | CLA_087610 NFIA_0 | 50370 AFUB_000280 Afu6g1 | 4490 Cluster 052B | - | | | | |
| BGL | beta glucosidase | GH 3 AN2227 | An17g00520 | 129891 ATEG_09329 AO | 090701000244 | AFL2G_05886 A | CLA_010450 NFIA_0 | 0070 AFUB_054750 Afu5g0 | 7190 Cluster 052C | - | | | | |
| BGL | beta glucosidase | GH 3 AN2612 | | | | | | AFUB_086800 Afu7g0 | 0240 Cluster 052D | | | | - | |
| BGL | beta glucosidase | GH 3 AN7865 | | | | | NFIA_0 | 57590 AFUB_077900 Afu6g1 | 1910 Cluster 052E | | | | - | |
| BGL | beta glucosidase | GH 3 | | AQ | 0090012000003 | AFL2G_02949 | | | Cluster 053 | | | | - | |
| BGL | beta glucosidase | GH 3 AN1804 | | AO | 090026000123 | AFL2G_07119 | | | Cluster 054 | | | | - | |
| BGL | beta glucosidase | GH 3 | | AO | 090038000223 | AFL2G_09023 | | | Cluster 055A | | | | - | |
| BGL | beta glucosidase | GH 3 AN5976 | An15g04800 | 181816 ATEG 02713 AO | 090038000425 | AFL2G_07763 | NFIA_10 | 00430 | Cluster 055B | | - | | | |
| BGL | beta glucosidase | GH 3 | | | | | NFIA_0 | 8520 | Cluster 055C | | | | | - |
| BGL | beta glucosidase | GH 3 | An06g02040 | 176601 AC | 090166000048 | AFL2G_09452 | NFIA_1 | 2660 | Cluster 056 | | | - | | |
| BGL | beta glucosidase | GH 3 | | ATEG_07931 | | | | | Cluster 057A | | | | | - |
| BGL | beta glucosidase | GH 3 AN2828 | | ATEG_07419 | | | NFIA_0 | 27390 AFUB_091720 Afu7g0 | 6140 Cluster 057B | | | - | | |
| BGL | beta glucosidase | GH 3 AN0712 | | | | | | | Cluster 058 | | | | | - |
| BGL | beta glucosidase | GH 3 AN3949 | | | | | | | Cluster 059 | | | | | - |
| BGL | beta glucosidase | GH 3 | | 129779 ATEG_00157 | | | | | Cluster 060 | | | | - | |
| BGL | beta glucosidase | GH 3 | | | | | NFIA_0 | 0750 AFUB_048210 Afu3g0 | 0230 Cluster 061 | | | | | |
| BGL | beta glucosidase | GH 3 | | | | | NFIA 0 | 7910 AFUB 077990 Afu6g1 | 2010 Cluster 062A | | | | - | |
| BGUB | 1. beta glucosidase/beta xylosidase | GH 3 AN0479 | | | | | | | Cluster 063 | | | | | - |
| BXL | beta xylosidase | GH 3 AN2359 | An01g09960 | 205670 ATEG 05106 AO | 9860002000600 | AFL2G 00957 | | AFUB 016310 Afu1g1 | 6920 Cluster 064A | | - | | | |
| BXL | beta xylosidase | GH 3 | | ATEG 07383 AO | 090011000140 | AFL2G 04928 A | CLA 018590 | | Cluster 064B | | | - | | |
| BXU/AB | F beta xvlosidase/alpha arabinofuranosidase | GH 3 AN8401 | | ATEG 09052 A0 | 090103000120 | AFL2G 12252 A | CLA 062400 NFIA 0 | 3180 AFUB 046310 Afu3d0 | 2090 Cluster 064C | | | | | |
| BXL/AB | F beta xylosidase/alpha arabinofuranosidase | GH 3 AN2217 | An17a00300 | 50997 ATEG 09314 AO | 090701000274 | AFL2G 05912 A | CLA 010340 NFIA 0 | 0180 AFUB 054640 Afu5a0 | 7080 Cluster 065A | - | | | | |
| BXL/AB | F beta xylosidase/alpha arabinofuranosidase | GH 3 | | ATEG 08027 | | | | | Cluster 065B | | | | | - |
| ~ | | GH 3 AN1416 | An16g09090 | 45461 ATEG_00018 AO | 090103000019 | AFL2G_12338 A | CLA_057390 NFIA_0 | 96770 AFUB_083500 Afu8g0 | 4060 Cluster 066A | - | | | | |
| ~ | | GH 3 | | | | | | AFUB_097970 Afu1g0 | 0540 Cluster 066B | | | | | - |
| ~ | | GH 3 AN3360 | An06g02460 | 37673 ATEG_04963 | | A | CLA_052760 NFIA_1 | 12600 AFUB_070710 Afu4g1 | 3770 Cluster 067 | | - | | | |
| | | CH 3 ANDR70 | An02007500 | 100101 ATEC 01700 AC | 112000000000000000000000000000000000000 | AEI 20 02070 A | CLA DROCO NELA O | TTTTA ACI ID 027440 A6-244 | 100 01-1-1 0C0 | • | | | | |

| (GH), carbohydrate | |
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| lycoside hydrolas | |
| l esterase (SF), gl | n next 4 pages). |
| usters of feruloy | lies. (Continued c |
| ont: Orthology cl | le lyase (PL) fam |
| ital Table 4A Co | d polysaccharid |
| Supplemer | esterase an |

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|-------------|-------------|-------------|--------------|--------------|--------------|-------------|-------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-----------------|-------------------|-------------------|------------|--------------|----------------|----------------|-------------|-------------|-------------|-------------|--------------|--------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|----------------|----------------|--------------|--------------|
| | | | | | | | | - | | | | | | | | | | | | | | | • | | | | | | | | | | - | | | | | | | | | | | | - | | | | |
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| | | - | | | | | | | | | - | - | | | | | - | ~ | | | | | | | | | | | | - | | | | | - | | | | | - | | | | | | - | | | _ |
| Cluster Ub9 | Cluster 070 | Cluster 071 | Cluster 0724 | Cluster 072E | Cluster 073 | Cluster 074 | Cluster 075 | Cluster 076 | Cluster 0774 | Cluster 077E | Cluster 078 | Cluster 079 | Cluster 080 | Cluster 081 | Cluster 082 | Cluster 083 | Cluster 0844 | Cluster 084E | Cluster 085 | Cluster 086 | Cluster 087 | Cluster 088 | Chister 089 | Cluster 090 | Cluster 001 | Cluster 091 | | Cluster 033 | Cluster 0845 | Cluster 0940 | Cluster 095 | Cluster 096 | Cluster 097 | Cluster 098 | Cluster 0994 | Cluster 099E | Cluster 100 | Cluster 101 | Cluster 1024 | Cluster 102E | Cluster 103/ | Cluster 104/ | Cluster 104E | Cluster 105 | Cluster 106 | Cluster 107 | Cluster 108 | Cluster 1094 | Cluster 109E |
| | | Afu3g08820 | Afu6g11610 | | Afu5g01830 | Afu6g07480 | Afu2g09520 | | | | Afu6q09250 | Afu1a03600 | Afu7g05610 | | Afu2g09350 | Afu5a14560 | Afu7g01070 | | Afu5a00550 | Afu8a07030 | | | | | A4+1-00640 | Afri2c01010 | a cinfont | A6.6~11600 | And the second | Afu6a07070 | Afu7a01540 | Afu6a01800 | 0 | Afu6a13610 | Afu4g09480 | | Afu6g11820 | Afu3g15210 | | Afu3q00320 | Afu3g00470 | Afu6g12210 | | | | Afu1g04730 | | Afu7g06150 | |
| | | FUB 040280 | FUB 077620 | | FUB 050360 | FUB 073450 | FUB 025390 | | | | FUB 075220 | FUB 004010 | FUB 091190 | | FUB 025210 | FUB 062240 | FUB 087650 | | FUB 049020 | FUB 080770 | | | | | | ELIE MAGAN | | FLIB 077620 | | FUB 073010 | FUB 088110 | FUB 096550 | | FUB 001130 | FUB_066600 | | FUB 077830 | FUB 034010 | | FUB 048130 | FUB 047990 | FUB 078210 | | | | FUB_005070 | | FUB_091730 | |
| | | A 068300 A | IA 057290 A | IA_095570 | IA 040280 A | IA 053150 A | IA 085010 A | IA 056040 | | IA 113230 | A 054930 A | A 021060 A | IA 026860 A | | IA 084850 A | IA 072400 A | IA 113780 A | | IA 041960 A | A 07760 A | | | | | 101460 | 10 002000 A | A DAFFORD | 1A 057290 A | 1A 000570 | A 052720 A | A 114250 A | A 047960 A | | A 059570 A | IA 106540 A | | IA 057510 A | IA 061880 A | | IA 000850 A | IA 001010 A | IA 058160 A | | IA 055240 | | A_020020 A | | IA_027400 A | |
| | | A 036770 NF | A 085250 NF | N | N | A 081650 NF | A 081310 NF | IN | | NF | A 083150 NF | A 031040 NF | A 007330 NF | | N | UN N | A 066420 NF | | R | A 044470 NF | 1 | | | | an a | A DEPERT ME | | A DREAGO MF | | A 088870 NF | A 066030 NF | A 098940 NF | | A 086910 NF | A_048770 NF | | N | N | | A 063140 NF | L N | A 085410 NF | A 064270 | μN | | A_029940 NF | | A_007820 NF | |
| G_U8686 | | G 01298 ACI | ACI | | G_01726 | G 01447 ACI | G_05447 ACI | | | G 03782 | G 09249 ACI | G 02039 ACI | G 00412 AC | G 10308 | G 05484 | G 02982 | G 11381 ACI | | G 02951 | G 07781 ACI | | | | | | AC. | 04 97770 0 | 0 07674 ACI | | G 03805 AC | G 11497 AC | AC | G 07437 | G 12071 ACI | G 06449 ACI | G_11983 | | | | G 08066 ACI | | G 07347 ACI | ACI | G 07138 | G 12233 | G_02120 ACI | | G_07140 ACI | |
| AFI2 | | 01389 AFL2 | | | 01341+, AFL2 | 01553 AFL2 | 00715 AFL2 | | | 00917 AFL2 | 00604 AFL2 | 00990 AFL2 | 00423 AFL2 | 00373 AFL2 | 00757 AFL2 | 00046 AFL2 | 00122 AFL2 | | 00006 AFL2 | 00444 AFL2 | | | | | | | 110 10000 | 00248 AFL2 | | 00941 AFL2 | 00314 ALF2 | | 00208 AFL2 | 00326 AFL2 | 00887 AFL2 | 00423 AFL2 | | | | 00026 AFL2 | | 00111 AFL2 | | 00103 AFL2 | 00141 AFL2 | 00905 AFL2 | | 00102 AFL2 | |
| | | 2 A00900050 | ~ | | 2 A00900030 | A00900050 | A00900110 | | • | A00900120 | 3 A00900010 | A00900030 | A00900050 | A00900090 | A00900110 | 2 A00900120 | 1 A00900100 | | 2 A00900120 | A00900380 | | | | | | | 0000000 | | | 7 A00900120 | A00900100 | | A00900010 | A00901030 | A00907010 | 5 A00901030 | | 0 | | A00901200 | | 3 A00900010 | | A00900260 | A00901030 | 5 A00900030 | | A00900260 | - |
| | | ATEG 01592 | ATEG 05003 | | ATEG_09802 | | ATEG_04390 | ATEG 08371 | ATEG 07715 | | ATEG 06686 | ATEG 03845 | ATEG 06365 | ATEG 03062 | ATEG 09844 | ATEG 10242 | ATEG 08654 | ATEG 02665 | ATEG 10292 | | | | ATEG 0999 | | ATEC: 01374 | ATEG 07403 | ATTO DAMO | ATEG 05003 | | ATEG 03727 | ATEG 08700 | ATEG 08705 | | ATEG 03410 | ATEG 00805 | ATEG_08906 | | ATEG_07190 | | ATEG 07461 | | ATEG 04943 | | | | ATEG_03756 | | ATEG_07420 | ATEG 05519 |
| | | 210716 | 205580 | | | 209376 | 214608 | | 175759 | 52811 | 123981 | 202490 | | 42805 | | 194447 | 50378 | | | | | | | | 124012 | 124312 | 000007 | 64773 | | 53159 | | | | | 57436 | | 50977 | | | 52071 | | 171269 | | 183088 | | 52011 | 191511 | 211053 | |
| | | An16g02100 | An01g11670 | | | An07g08950 | An16g06800 | | An06g02060 | An08g01100 | An11q07660 | An18a04100 | | An18g03330 | | An03a01050 | An05a01320 | | | | | | | | An11n06040 | An12c0220 | 02270 00-V | An0101011660 | Book Book | An07a09330 | | | | | An03g00940 | | | | | An01q00780 | An14g07390 | An01g14600 | | An15g04550 | | An01g03340 | An03g05530 | An14g02760 | |
| ~ | 3 AN2599 | 5 AN3013 | 5 AN1285 | 10 | 5 AN8068 | 5 AN5214 | 10 | 10 | 5 AN8947 | 5 AN1332 | 5 AN7633 | 5 AN4052 | 10 | 10 | 5 AN3777 | 5 AN9166 | 5 AN3358 | 5 | 10 | 5 AN7639 | 5 AN2709 | 5 AN3297 | 5 AM6427 | AN9276 | | CBC3MA 2 | ANIANTO A | VIDADA C | | 7 AN5176 | 7 AN3418 | | | 0 AN7401 | 0 AN1818 | | D AN2356 | | 1 AN3613 | 1 AN9365 | | | | | | 2 AN0452 | ~ | ~ | 2 |
| 5 | 풍 | B | B | B | B | ЭH | НÐ | B | HB | н | В | нg | ß | R | Э | B | B | R | 뮹 | B | H | 풍 | 5 | B | 1 | 5 | 5 | 5 3 | 5 8 | 5 5 | B | æ | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1 | GH 1: | GH 1: | GH 1 | GH 1 | GH 15 | GH t | GH 1 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ucanase | ucanase | | |
| | | enase | mase | nase | anase | anase | inase | alactanase | alactanase | alactanase | alactanase | alactanase | alactanase | alactanase | lucanase | alactanase | anase | anase | anase | anase | anase | anase | | anase Janase | | lanase drolaeo | divided o | drolase | drolano | drolase | astu | nase | 1350 | ase | lase | ase | lase | ase | ase | ase | lase | ase | 1350 | 1356 | ase | n active endog | n active endog | anase | anase |
| | | endogluca | endogluca | endoglucs | endoglucs | endogluca | endogluca | exo 1,3 g | exo 1,3 gi | exo 1,3 g | exo 1,3 q | exo 1.3 g | exo 1,3 ge | exo 1,3 g | beta 1,6 g | exo 1.6 g | endoman | endoman | endoman | endoman | endomane | endomane | endoman | endoman | and on the second | endomant | | Cellobiony | Collabia | cellabiohy | endodluca | endogluca | endoxvlar | endoxylar | endoxylar | endoxylar | endoxylar. | endoxylar | endoxylar | endoxylar | endoxylar | endoxylar | endoxylar | endoxylar | endoxylar | . xylogluca. | L xylogluca | L. endogluca | L endogluca |
| ~ | ~ | EGL | EGL | EGL | EGL | EGL | EGL | EXG | EXG | EXG | EXG | EXG | EXG | EXG | EXG | GLN | MAN | MAN | MAN | MAN | MAN | MAN | MAN | MAN | NAM I | MMM | | 5 5 | 100 | B | EG | EG | XLN | XLN | XLN | XLN | XLN | XLN | XLN | XLN | XLN | NUX | XLN | XLN | XLN | XG EG | XG EG | XG EG. | XG EG |

Supplemental Table 4A Cont: Orthology clusters of feruloyl esterase (SF), glycoside hydrolase (GH), carbohydrate esterase and polysaccharide lyase (PL) families. (Continued on next 3 pages).

| | GH 27 | | ATEG_01905 | 5 | | | | Cluster 142 | | | - |
|---|---------------|------------|-------------------|------------------|-------------|--------------------------------|-------------------|--------------|---|---|---|
| 5 | 27 | | ATEG 03427 | 7 | | NFIA 048850 AFUB 0 | 195770 Afu6g02560 | Cluster 143 | | - | |
| G | H 27 | An06900170 | 37736 ATEG_04382 | 2 A0090005000217 | AFL2G_00225 | ACLA_016820 NFIA_073100 | Afu5g13830 | Cluster 144 | - | | |
| σ | H 27 AN0022 | An01g01320 | 172232 | | | NFIA_023390 AFUB_0 | 079600 Afu1g01200 | Cluster 145 | | - | |
| σ | H 27 AN7624 | An02g11150 | 207264 ATEG_09830 | 0 AO090003001305 | AFL2G_01765 | ACLA_003130 NFIA_039990 AFUB_0 | 050660 Afu5g02130 | Cluster 146 | | | |
| Ö | H 27 | An11g06330 | 39180 | | | | | Cluster 147 | | | - |
| Ö | H 27 AN7152 | An14g01800 | 41606 ATEG_02160 | 0 A0090023000151 | AFL2G_04039 | NFIA_029860 AFUB_0 | 199470 Afu4g03580 | Cluster 148 | - | | |
| O | H 27 | | | | | ACLA_097900 | | Cluster 149 | | | - |
| G | H 28 AN8327 | An01g14670 | 46255 ATEG_07748 | 00 | | NFIA_008150 AFUB_0 | 016610 Afu1g17220 | Cluster 150 | | - | |
| 0 | SH 28 | An05g02440 | 43957 | | | | | Cluster 151 | | | - |
| | GH 28 | An15g05370 | 182156 | | | | | Cluster 152 | | | - |
| | GH 28 | An16g06990 | 214598 | | | | | Cluster 153 | | | - |
| Ŭ | SH 28 AN6666 | An09g03260 | 50161 | A0090005000186 | AFL2G_00201 | NFIA_099410 | | Cluster 154 | | - | |
| - | GH 28 | An02g12450 | 172944 ATEG_01601 | 1 AO090005001400 | AFL2G_01310 | ACLA_036670 NFIA_068440 AFUB_0 | 040410 Afu3g08680 | Cluster 155 | F | | |
| | GH 28 AN4372 | An01g11520 | 141677 ATEG 04991 | 1 AO090023000401 | AFL2G_04252 | ACLA_052860 NFIA_102450 AFUB_0 | 070830 Afu4g13920 | Cluster 156 | | | |
| | GH 28 | An02g04900 | 52219 | A0090023000161 | AFL2G_04049 | NFIA_095620 AFUB_0 | 384640 Afu8g01970 | Cluster 157 | | - | |
| | GH 28 | | | A0090138000086 | | | | Cluster 158 | | | - |
| | GH 28 | | | | | AFUB_0 | 181060 Afu8g06730 | Cluster 159 | | | - |
| | GH 28 | | | | AFL2G 08764 | NFIA 023290 AFUB 0 | 179470 Afu1g01320 | Cluster 160 | | - | |
| | GH 28 | An11g04040 | 178172 | | | | | Cluster 161 | | | - |
| | GH 28 AN8891 | An03g06740 | 191158 ATEG 10357 | 7 A0090010000753 | AFL2G 11892 | ACLA 043100 NFIA 096340 AFUB 0 | 183960 Afu8g02630 | Cluster 162 | | | |
| | GH 28 AN8761 | An12g07500 | 42184 ATEG_07152 | 2 AO090026000784 | AFL2G_06533 | NFIA_049320 AFUB_0 | 195310 Afu6g02980 | Cluster 163 | - | | |
| | GH 28 AN9045 | | | | | | | Cluster 164 | | | - |
| | GH 28 | | | | | NFIA 100120 | Afu8g07265 | Cluster 165 | | | - |
| | GH 28 | An01g14650 | 172236 | AO090001000133 | AFL2G_07371 | | | Cluster 166 | | - | |
| | GH 28 | An18g04810 | 42917 | AO090009000470 | AFL2G_10228 | NFIA_018590 AFUB_0 | 106520 Afu1g06140 | Cluster 167 | | - | |
| | GH 28 AN10274 | An03g02080 | 194461 ATEG 06408 | 8 AO090102000139 | AFL2G 09582 | NFIA 027700 AFUB 0 | 191970 Afu7g06410 | Cluster 168 | - | | |
| | GH 28 | | ATEG_09025 | 5 A0090113000199 | AFL2G_08671 | | | Cluster 169 | | - | |
| | GH 28 | | | A0090138000066 | AFL2G_03102 | | | Cluster 170 | | | - |
| | GH 28 AN11626 | | | | | | | Cluster 171 | | | - |
| | GH 28 | | | A0090138000067 | AFL2G_08746 | | | Cluster 172 | | | F |
| | GH 28 | An11g06320 | 178393 | | | | | Cluster 173 | | | - |
| | GH 28 | An07g01000 | 180922 | AO090003000524 | AFL2G_02475 | AFUB_1 | 100610 Afu4g00100 | Cluster 174 | | - | |
| | GH 28 | | | A0090005000600A | AFL2G_00087 | | | Cluster 175 | | | ÷ |
| | GH 28 | An06g02070 | 123651 | A0090026000252 | AFL2G_06999 | | | Cluster 176 | | - | |
| | GH 28 | An11g08700 | 39337 | A0090124000009 | AFL2G_08037 | | | Cluster 177 | | - | |
| | GH 28 | | | A0090026000120 | AFL2G_07122 | | | Cluster 178 | | | ÷ |
| | GH 28 AN3389 | An04g09700 | 46065 | A0090102000011 | AFL2G_09468 | NFIA_099610 AFUB_0 | 180880 Afu8g06890 | Cluster 179 | - | | |
| | GH 29 | An13g02110 | 44822 ATEG_08111 | - | | | | Cluster 180 | | | - |
| | GH 29 | | ATEG_05691 | - | | | | Cluster 181 | | | - |
| | GH 31 AN2017 | An04g06920 | 214233 ATEG_00723 | 3 A0090003001209 | AFL2G_01842 | ACLA_049370 NFIA_105900 AFUB_0 | 067270 Afu4g10150 | Cluster 182A | | | |
| | GH 31 | | ATEG_08278 | 80 | | NFIA_060440 | | Cluster 182B | | | - |
| | GH 31 | | ATEG_08472 | ~ | | | | Cluster 182C | | | |
| | GH 31 AN0280 | An01g04880 | 55419 ATEG_02528 | 8 A0090005000767 | AFL2G_00750 | ACLA_031260 NFIA_021450 AFUB_0 | 103550 Afu1g03140 | Cluster 183A | | | |
| | GH 31 AN7120 | | | | | | | Cluster 183B | | | |
| | GH 31 AN0941 | An01g10930 | 119858 ATEG 05177 | 7 A0090005001084 | AFL2G_01038 | ACLA 019300 NFIA 009180 AFUB 0 | 115590 Afu1g16250 | Cluster 184A | | | |
| | GH 31 AN8953 | | | AO090038000471 | AFL2G_07812 | | | Cluster 184B | | - | |
| | GH 31 AN7345 | | | | | | | Cluster 184C | | | |
| | GH 31 AN10935 | | | A0090026000111 | AFL2G_07131 | | | Cluster 185 | | - | |
| | GH 31 AN3504 | An18g05620 | 49940 ATEG_02966 | 6 AO090023000288 | AFL2G_04152 | NFIA_018130 AFUB_0 | 006940 Afu1g06560 | Cluster 186A | - | | |
| Č | GH 31 | | | AO090023000290 | | | | Cluster 186B | | | - |



| | AO09771100558 AFI 2C 06180 |
|--------------------------------|---|
| NFIA 032680 | AO090701000558 AFL2G_06180 A0090701000539 AFL2G_0639 AFL2G_0539 NFIA_032680 |
| AAA4475 | |
| AFU NFIA 041980 AFU | AFU NFIA 041980 AFU |
| | D |
| ACI A 194550 NEIA 133540 45 | AODBNZ01000400 AEI 2G 06028 ACI A 094660 NEIA 033640 AE |
| | |
| NFIA_051560 AI | AO090701000038 AFL2G_05693 NFIA_051560 AI |
| | A CODOM OF AN A 10707 |
| | |
| | A0090103000043 AFL2G 12317 |
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| | |
| | AO090001000259 AFL2G_07489 |
| ACLA_088440 NFIA_052310 AF | A0090003000042 AFL2G_02901 ACLA_088440 NFIA_052310 AF |
| NFIA 008690 A | A0090120000158 AFL2G 08182 NFIA 008690 A |
| AULA_UZIZ60 NFIA_011250 AF | AUUSUUIZUUU445 AFLZG U3352 AULA UZIZGU NFIA UTIZSU AF AONAN12000746 AFL3C 03546 AULA UZIZGU NFIA 000040 AF |
| | A0090701000770 |
| | AFL2G 06343 |
| NFIA_041490 / | A0090023000076 AFL2G_03977 NFIA_041490 A |
| ACLA_018830 | ACLA_018830 |
| ADI A DALEDO META DOLEDO A | AFL2G_12387 |
| NULA 044920 111 14 024200 | W0000 10000004 HL LKG 11074 W019 044920 14 9704930 |
| ACLA 044560 NFIA 094540 | A0090011000063 AFL2G 04865 ACLA 044560 NFIA 094540 |
| ACLA 047600 NFIA 107920 | A0090023000743 AFL2G 04555 ACLA 047600 NFIA 107920 |
| NFIA_032900 | A0090012000356 AFL2G_03265 NFIA_032900 |
| | AO090005000065 AFL2G_00086 |
| NFIA_002750 | A0090701000838 AFL2G_06400 NFIA_002750 |
| NFIA_078690 | NFIA_078690 |
| ACLA_072730 NFIA_089980 | A0090026000804 AFL2G_06513 ACLA_072730 NFIA_089980 |
| ACLA 037080 NFIA 068020 | A0090005001320 AFL2G 01244 ACLA 037080 NFIA 068020 |
| | A0090005000064 AFL2G_00085 |
| ACLA_018180 NFIA_008030 / | ACLA_018180 NFIA_008030 / |
| ACLA_098980 NFIA_047920 A | A0090138000055 AFL2G_08737 ACLA_098980 NFIA_047920 A |
| ACLA 073460 NFIA 089320 A | A0090023000165 AFL2G_04053 ACLA_073460 NFIA_089320 A |
| | |
| ACLA_042100 NFIA_062660 AF | A0090701000481 AFL2G_06106 ACLA_042100 NFIA_062660 AF |
| ACLA 090090 NFIA 081240 A | A0090003000239 AFL2G 02739 ACLA 090090 NFIA 081240 A |
| NFIA_027350 A | A0090005000476 AFL2G 00456 NFIA 027350 A |
| ACLA_087680 NFIA_060490 / | A0090103000268 AFL2G 12122 ACLA 087680 NFIA 060490 A |
| V(ACLA_058100/ NFIA_097490/(AF | A0090005000698 AFL2G_00689/(ACLA_058100/NFIA_097490//AF |
| | |
| | |

Supplemental Table 4A Cont: Orthology clusters of feruloyl esterase (SF), glycoside hydrolase (GH), carbohydrate esterase and polysaccharide lyase (PL) families. (Continued on next page).

| R | beta xylosidase | GH 43 | | ATEG_07 | 784 AO090010000494 | AFL2G_11656 | NFIA_0233 | 160 AFUB_079570 Aftu1g01230 | Cluster 223 | | - | | |
|----------|--|----------------|--|----------------|----------------------|---------------|------------------------|-----------------------------|---------------|---|---|---|---|
| 2 | beta xylosidase | GH 43 AN7313 | An08g10780 | 122978 ATEG_01 | 1188 AO090010000562 | AFL2G_11714 | ACLA_043260 NFIA_0962 | 340 AFUB_084070 Afu8g02510 | Cluster 224 | - | | | |
| ų | beta xylosidase | GH 43 AN2664 | | ATEG_06 | 3107 AO090012000350 | AFL2G_03257 | NFIA_0727 | *80 AFUB_061880 Afu5g14190 | Cluster 225 | - | | | |
| J | beta xylosidase | GH 43 AN8477 | | ATEG_01 | 1292 | AFL2G_06974 | ACLA_018160 | | Cluster 226A | | - | | |
| J | beta xylosidase | GH 43 | An08g01900 | 47677 ATEG_01. | 1292 AO090102000331 | AFL2G_09750 | ACLA_072000 NFIA_0883 | 370 AFUB_028830 Afu2g13190 | Cluster 226B | - | | | |
| J | beta xylosidase | GH 43 AN7864 | | | AO090113000059 | | | | Cluster 227 | | | - | |
| 5 | beta xylosidase | GH 43 | | ATEG_07 | 517 | | | | Cluster 228 | | | | - |
| 5 | beta xylosidase | GH 43 AN2633 | | | | | | | Cluster 229 | | | | - |
| | beta xylosidase | GH 43 AN1043 | | | | | | | Cluster 230 | | | | - |
| ಕ | endoglucanase | GH 45 AN6786 | | | | | NFIA_0280. | 120 AFUB 092320 Afu7g06740 | Cluster 231 | | | - | |
| 5 | beta xylosidase | GH 43 | | ATEG 10 | 193 | | | | Cluster 233A | | | | - |
| | beta xylosidase | GH 43 AN7276 | | ATEG_06 | 643 AO090701000886 | AFL2G_06448 | ACLA_078600 NFIA_0332 | 720 AFUB 018010 Afu2g00930 | Cluster 233B | - | | | |
| # | alpha arabinofuranosidase | GH 51 AN1277 | An08g01710 | 38549 ATEG 00 | 1198 | AFL2G 07796 | ACLA 025610 NFIA 0157 | 730 AFUB 009340 Aft 1909900 | Cluster 234 | - | | | |
| ۳. | alpha arabinofuranosidase | GH 51 | | 50979 ATEG 03. | 1540 | | | | Cluster 235A | | | | |
| ж. | alpha arabinofuranosidase | GH 51 AN2541 | An01g00330 | 206387 ATEG_02 | 882 AO090124000023 | AFL2G_08019 | ACLA_099110 | | Cluster 235B | - | | | |
| <u>۳</u> | alpha arabinofuranosidase | GH 51 AN9439 | An09g00880 | 131891 | | | | | Cluster 235C | | | | |
| L. | alpha arabinofuranosidase | GH 51 | | | A0090020000712 | AFL2G 10643 | | | Cluster 236 | | | | |
| ц. | alpha arabinofuranosidase | GH 51 | | ATEG 07 | 7868 AO090012000298 | AFL2G 03217 | ACLA 074120 NFIA 0904 | 10 AFUB 030820 Afu2q15160 | Cluster 237 | - | | | |
| 7 | endogalactanase | GH 53 | An16a06590 | | | | | | Cluster 238 | | | | - |
| 1 | endocalactanase | GH 53 AN5727 | An18a05940 | 187227 ATEG 02 | 927 AO090001000492 | AFL2G 09135 | NFIA 0177 | 780 AFUB 007290 Afri1a06910 | Cluster 239 | • | | | |
| - | endonalactanase | 6H5 | | | | AFI 2G 07488 | | | Chieter 240 | | | | - |
| | errogenecientese alpha arahinofuranosidase | CH 54 AN1571 | An15a02300 | 200605 ATEG 07 | 939 AO09002300001 | AFI 2G 03901 | ACLA 066470 NFIA 0606 | 30 Ahi6n14620 | Cluster 241 | - | | | |
| | arahimoodan arahimofurannhudrolase | CH R2 | and a state of the | ATEG 00 | 1186 ACIG01103000088 | AFI 2G 12281 | ACI A 071560 NFIA 0879 | 00 AFLIB 028400 Afri2d12770 | Chister 256 | • | | | |
| ; ; | arabinovjen arabinovana murulase | CILICO ANDEOD | U200000000V | CC12C ATEC 40 | 100 00000100000 0010 | VELOC 1247 | ACLA 061610 MELA 0227 | 10 AEIE 018000 Af-200000 | Cluster 200 | • | | | |
| 5 2 | arabinoxylari arabinofuranohydrolase | CH E2 AV7000 | nosonBcnuw | 00100 VIEG_10 | | M_L2(0_00441 | AULA_UGIDIU NEIA_U332 | | Chicker 200 | - | | | |
| 5 | al autroxylari al autrour artoriyorolase | 00E MAY 20 LIO | | AT A AN | 1 | | | | Ciuster 200 | | | | |
| = | arabinoxylan arabinofuranohydrolase | GH 62 | | ATEG_10 | 3/9 | | | | Cluster 259 | | | | - |
| 2 | alpha glucuronidase | GH 67 AN9286 | An14g05800 | 56619 ATEG_06 | 5085 AO090026000127 | AFL2G_07114 | ACLA_017270 NFIA_0725 | 510 AFUB_062100 Afu5g14380 | Cluster 260A | - | | | |
| R | alpha glucuronidase | GH 67 | | ATEG_09 | 975 | | | | Cluster 260B | | | | - |
| G | xyloglucan active cellobiohydrolase | GH 74 AN1542 | | | | | NFIA_0960 | 100 AFUB_084270 Afu8g02330 | Cluster 261 | | | - | |
| EG | xyloglucan active endoglucanase | GH 74 AN5061 | An01g01870 | 206333 ATEG_04 | 1708 | | ACLA_044310 NFIA_0949 | 390 AFUB_085120 Afu8g01490 | Cluster 262 | - | | | |
| ≰ | alpha rhamnosidase | GH 78 AN8465 | An07g00240 | 40264 | | AFL2G_05364 | | | Cluster 263 | | | - | |
| ≰ | alpha rhamnosidase | GH 78 | An15g04530 | | | | | | Cluster 264 | | | | - |
| 4 | alpha rhamnosidase | GH 78 AN2631 | An08g09140 | 176718 ATEG_05 | 5089 AO090001000105 | AFL2G_07340 | NFIA_0045 | 560 AFUB_045370 Afu3g02880 | Cluster 265 | - | | | |
| ≰ | alpha rhamnosidase | GH 78 AN11954 | An04g09070 | 51410 | AO090003001016 | AFL2G_02014 | | | Cluster 266A | | - | | |
| ≰ | alpha rhamnosidase | GH 78 AN12368 | | | AO090113000149/ | 4 AFL2G_08627 | NFIA_0579 | 330 AFUB_078020 Afu6g14610 | Cluster 266B | | - | | |
| ≰ | alpha rhamnosidase | GH 78 AN7151 | | ATEG_02 | 2922 AO090010000561 | AFL2G_11713 | NFIA_0579 | 130 AFUB_078020 Afu6g12030 | Cluster 266C | - | | | |
| ≰ | alpha rhamnosidase | GH 78 | | | | AFL2G_10644 | NFIA_0605 | 560 AFUB_000160 | Cluster 266D | | | - | |
| ¥ | alpha rhamnosidase | GH 78 AN10277 | An01g06620 | 170172 | AO090003001291 | AFL2G_01780 | NFIA_0229 | 170 AFUB_002060 Afu1g01660 | Cluster 267 | • | | | |
| ≰ | alpha rhamnosidase | GH 78 | An18g04800 | 42916 ATEG_03 | 8018 AO090009000471 | AFL2G_10227 | NFIA_0186. | 320 AFUB_006490 Afu1g06130 | Cluster 268 | - | | | |
| ¥ | alpha rhamnosidase | GH 78 AN3780 | | | | | | | Cluster 269A | | | | - |
| ¥ | alpha rhamnosidase | GH 78 AN10867 | An10g00290 | 44977 ATEG 04. | 1706 AO090012000058 | AFL2G 02993 | NFIA 0261 | 130 AFUB 090610 Afr/7905040 | Cluster 269B | - | | | |
| 4 | alpha rhamnosidase | GH 78 | | | A0090103000432 | AFL2G 11972 | | | Cluster 270 | | | | |
| ¥ | alpha rhamnosidase | GH 78 AN6929 | An12g05700 | 131668 | AO090005001416 | AFL2G 03939 | | | Cluster 271 | | - | | |
| ж | unsaturated galacturonyl hydrolase | GH 88 | | | AO090005000324 | AFL2G 00324 | NFIA 0262 | 10 AFUB 090670 Afu7g05090 | Cluster 272 | | - | | |
| ж | unsaturated galacturonyl hydrolase | GH 88 AN3991 | An01g01340 | 36414 | AO09013800087 | AFL2G08766 | NFIA_0233 | 140 AFUB 079550 Afu1g01250 | Cluster 273 | - | | | |
| 풍 | unsaturated galacturonyl hydrolase | GH 88 AN11078 | | | | AFL2G 06465 | | | Cluster 274 | | | - | |
| H | unsaturated galacturonyl hydrolase | GH 88 | | | AO090701000907 | | | | Cluster 275 | | | | - |
| ж | unsaturated galacturonyl hydrolase | GH 88 AN4629 | | | | | | | Cluster 276 | | | | - |
| X | exoarabinanase | GH 93 AN2060 | | ATEG_06 | 5045 AO090003001017 | AFL2G_02013 | NFIA_0813. | 120 AFUB_021620 Afu2G04576 | 3 Cluster 277 | - | | | |
| Ň | And and a second se | 0000 | | | A.000044000444 | ACI OC AKONO | | | Chietor 278 | | | | |

Chapter 2

Supplemental Table 4A Cont: Orthology clusters of feruloyl esterase (SF), glycoside hydrolase (GH), carbohydrate esterase and polysaccharide lyase (PL) families.

| ABX | exoarabinanase | GH 93 AN5231 | | 49311 ATE | G_07909_AO0 | 90012000101 | AFL2G_03035 | ACLA_008170 NFW | 027720 AFUE | 091990 Afu7g0643 | 0 Cluster 279 | - | | | | | | |
|------|--|----------------|-----------------|--|-------------|---------------|---------------|------------------|--------------|---------------------|----------------|------|------|---------|-----|-----|------|------|
| ABX | exoarabinanase | GH 93 | | ATE | G_08029 | | | | | | Cluster 280 | | | | | | | - |
| ABX | exoarabinanase | GH 93 | | ATE | G 00891 | | | NFU | 058060 AFUE | 8 078110 Afu6g1212 | 0 Cluster 281 | | | | | - | | |
| AFC | alpha fucosidase | GH 95 AN8149 | An16g02760 | 184037 ATE | G 01560 AO0 | 90005000382 | AFL2G 00373 | | | | Cluster 282 | | | | _ | | | |
| AFC | alpha fucosidase | GH 95 | | | AO0 | 90005000512 | AFL2G_00515 | | | | Cluster 283A | | | | | | - | |
| AFC | alpha fucosidase | GH 95 AN6673 | An16g00540 | 53702 ATE | G 09768 AO0 | / 98000060006 | AFL2G 10565 | ACLA_004070 NFV | 040960 AFUE | 3 049660 Afu5g0119 | 0 Cluster 283B | - | | | | | | |
| AFC | alpha fucosidase | GH 95 AN10376 | | ATE | G 04136 | | | NFI | 064720 AFUE | 3 036590 Afu3g1259 | 0 Cluster 284 | | | | - | | | |
| URH | unsaturated rhamnogalacturonyl hydrolase | GH 105 AN3196 | An14g02920 | 41703 ATE | G_07907_AO0 | 90001000174 | AFL2G_07407 | ACLA_056060 NFI/ | 030670 AFUE | 3_100260 Afu4g0286 | 0 Cluster 285 | - | | | | | | |
| URH | unsaturated mamnogalacturonyl hydrolase | GH 105 | | | A00 | 90003000153 / | AFL2G 02808 | | | | Cluster 286 | | | | | | - | |
| URH | unsaturated rhamnogalacturonyl hydrolase | GH 105 AN9383 | An14g05340 | 41877 | AO0 | 90113000146 | AFL2G 08624 | ACLA 077810 | | | Cluster 287 | | | | - | | | |
| URH | unsaturated rhamnogalacturonyl hydrolase | GH 105 AN10505 | | ATE | G 02892 AO0 | 9000100063 | AFL2G 07308 | ACLA 072870 NFI | 089840 AFUE | 3 030270 Afu2g1463 | 0 Cluster 288 | | - | | | | | |
| URH | unsaturated mamnogalacturonyl hydrolase | GH 105 AN7828 | | | | | | | | | Cluster 289 | | | | | | | - |
| AGU | alpha glucuronidase | GH115 | | ATE | G 04355 AO0 | 90005001415 | AFL2G 01323 | | | | Cluster 290A | | | | | - | | |
| AGU | alpha glucuronidase | GH115 AN9329 | | ATE | G 09974 AO0 | 9001000038 | AFL2G 11304 | ACLA 006360 NFI | 025630 AFUE | 3 090220 Afu7g0468 | 0 Cluster 290B | | - | | | | | |
| AGU | alpha ducuronidase | GH115 | | | AOO | 90001000267 | AFL2G 07498 | | | | Cluster 290C | | | | | | - | |
| AGU | alpha ducuronidase | GH115 | | | AOC | 90113000058 | | | | | Cluster 290D | | | | | | | - |
| ΡLΥ | pectate Nase | PL 1 AN0741 | | ATE | G 08834 AO0 | 90011000673 | AFL2G 05417 | | | | Cluster 291 | | | | - | | | |
| μΥ | pectate lvase | PL 1 AN5333 | | ATE | G 05467 AO0 | 90102000072 | AFL2G 09523 | NEN | 060270 AFUE | 3 000360 Afu6a1440 | D Cluster 292 | | | | | | | |
| ΡLΥ | pectate lvase | PL 1 AN7646 | An10a00870 | 45021 ATE | G 08123 AO0 | 90701000321 | AFL2G 05954 | NFI | 033040 AFUE | 3 017840 Afu2q0076 | 0 Cluster 293 | | - | | | | | |
| ΡLΥ | pectate lvase | PL 1 AN9367/AM | N9368 | | AOO | 90011000030 | AFL2G 04835 | | | | Cluster 294 | | | | | - | | |
| H | pectin lvase | d. | | ATE | G 00950 AO0 | 90003001295 | AFL2G 01776 | | | | Cluster 295A | | | | | - | | |
| H | partin lvase | ā | | | ADD | 90103000463 | AFI 2G 11948 | | | | Cluster 295B | | | | | | - | |
| E | pactin Ivasa | PL 1 AN4882 | An15a07160 | 40837 ATE | G 7577 AO0 | 90012000121 | AFL2G 03052 | NFI | 077100 AFUE | 3 057770 Afu5a1017 | D Cluster 295C | | • | | | | | |
| H | nactin lyase | ā | An19n00270 | 66212 | ADD | 9001000030 | AFI 2G 11297 | NFIL | 026110 AFLIE | 8 090600 Afri700503 | D Chister 296 | | | | | | | |
| H | nactin lyaca | DI 1 4N2331 | An0300190 | 45821 | 4004 | 101000504 | 3FI 2G 11666 | ACI A 012470 NFI | 076850 AFLIE | 057970 A41501038 | Cluster 247 | | Ŧ | | | | | |
| i u | Potent grade | DI 1 AN10147 | An11000030 | 208760 | OUV | 00010000100 | VEI 20 03360 | | | And Roman Andreas | Cluster 208 | | | | • | | | |
| i ii | percent gase | | ACCESSION AND A | 2007 | | 4 NOCODI 2000 | VEI 2/2 08823 | | | | Cluster 200 | | | | | | - | |
| 1 | hereit i Jase | PU 4 410700 | | THE PARTY OF THE P | 200 | +0200000100 | C2000 1100 1 | 1111 01010 1 101 | 1111 | 0000 0 11 0 0000 0 | Cluster 200 | • | | | | | - | |
| Į į | pectin lyase | PL 1 AN2569 | An14gu43/U | 41615 AIE | 1216 AU | 19000001006 | AFLZ0_11352 | HULA U34210 NFM | 033080 AFU | 01/880 Auzguue | Cluster 301 | - | | | | | | • |
| Į | bectin iyase | 1 | | | | | | ILN | 001020 | | Ciuster JUT | | | | | | | - |
| E E | pectin lyase | PL 1 AN9439 | | | | | | | | | Cluster 302 | | | | | | | - |
| ۲Y | pectate lyase | PL 3 AN6748 | | ATE | G_06314_A00 | 90005000472 | AFL2G_00461 | NFL | 027690 AFUE | 091960 Afu7g0640 | 0 Cluster 303A | | | - | | | | |
| ΡLΥ | pectate lyase | PL 3 AN8453 | | ATE | G_06285 | | | | | | Cluster 303B | | | | | | - | |
| ۲Y | pectate lyase | PL 3 AN3337 | | | AOO | 90010000706 | AFL2G_11846 | ACLA_059210 NFW | 038670 AFUE | 3_081630 Afu8g0591 | 0 Cluster 304A | | | - | | | | |
| ΡLΥ | pectate lyase | PL 3 AN2542 | | | | | | | | | Cluster 304B | | | | | | | - |
| ΡLΥ | pectate lyase | PL 3 AN6106 | | ATE | G_08626 AO0 | 90038000502 | AFL2G_07839 | NFL | 023470 AFUE | 3_079680 Afu1g0112 | 0 Cluster 305 | | | F | | | | |
| RGL | rhamnogalacturonan Iyase | PL 4 AN7135 | An14g01130 | 210947 ATE | G_02193 A00 | 90011000349 | AFL2G_05136 | ACLA_054660 NFU | 029620 AFUE | 5_099240 Atu4g0378 | 0 Cluster 306 | - | | | | | | |
| RGL | rhamnogalacturonan lyase | PL 4 AN6395 | An11g00390 | 47780 ATE | G_10327 A00 | 90012000147// | AFL2G 03075 | ACLA_018320 NFI/ | 008140 AFUE | 3_016620 Afu1g1723 | 0 Cluster 307A | - | | | | | | |
| RGL | rhamnogalacturonan lyase | PL 4 AN12097 | | | A00 | 90113000057 | | | | | Cluster 307B | | | | | | - | |
| RGL | rhamnogalacturonan Iyase | PL 4 | | ATE | G 08610 AO0 | 90138000119 / | AFL2G_08794 | NFU | 094270 AFUE | 3_085750 Afu8g0082 | 0 Cluster 308 | | | | - | | | |
| RGL | rhamnogalacturonan lyase | PL 4 AN3950 | | | | | | | | | Cluster 309 | | | | | | | - |
| PLY | pectate lyase | PL 9 AN2537 | | ATE | G_04635_AO0 | 90038000131 | AFL2G_08953 | NFU | 062360 AFUE | 3_034350 Afu3g1489 | 0 Cluster 310A | | | - | | | | |
| ΡLΥ | pectate lyase | PL 9 | | | AOO | 90038000132 | | | | | Cluster 310B | | | | | | | - |
| ΡLΥ | pectate lyase | PL 9 | | ATE | G_03526 | | | | | | Cluster 311 | | | | | | | - |
| RGL | rhamnogalacturonan lyase | PL 11 AN2543 | | | | | | | | | Cluster 312 | | | | | | | - |
| | | | | | | | | | | | | 57 | 44 | 32 26 | 35 | 37 | 61 | 106 |
| | unique genes p | per species 2 | 29 16 | 16 25 | | 7 | 7 | 9 | 7 | 4 | 4 | 14.7 | 11.3 | 8.2 6.7 | 9.0 | 9.5 | 13.1 | 27.2 |
| | strain spe | scific genes | o | 4 | | | | | | 2 | 7 | | | | | | | |

| (AA). |
|----------------|
| activities |
| i auxiliary |
| e. |
| clusters |
| Orthology |
| |
| Table 4B |
| T |
| Supplements |

Colour codes: Yellow = at least one of the orthologs has biochemical support

Green = only present in one species

Orange = present in all species

Blue = present in only one strain of this species

The number of species in which an ortholog is present is listed after the orthologous cluster and a sum of this is given at the bottom.

The number of unique genes per species is given at the bottom of the species column. (Continued on next 2 pages).

010 011A 110 012 012A 012B 016B 017A 017B 007B 017C 04A 05B 07A ő 60 013 015 015 018 80 Cluster A. flows A. clevatus A. flochert A. floringatus A. fumigatus A. fumigatus A. fumigatus A. tumigatus A. Lordon A. Lordon B. Lordon B. A. Lordon B. A. Lordon B. A. Lordon B. A. Lordon B. ACLA_076450 NFIA_092960 AFUB_033230 Afu2g17540 AFUB 071740 Afu4g14490 AFL2G_09420 ACLA_019700 NFIA_009660 AFUB_015200 Afv1g15670 AFL2G 01113 ACLA_093090 NFIA_034090 AFUB_018840 Afu2g01770 AFUB_030480 Afu2g14840 AFL2G 01667 ACLA 078430 NFIA 045070 AFUB 101160 Afu4g00610 VFUB_046830 Afu3g01580 ACLA_072460 NFIA_090260 AFUB_030670 Afv2g16020 AFL2G_09924 ACLA_076440 NFIA_092950 AFUB_033220 AFL2G_08693 NFIA_072100 NFIA_101880 / NFIA_009370 CLA 056040 NFIA 036190 NFIA ACLA AFL2G_10583 AFL2G_11750 1096 AFL2G_01047 AFL2G_03439 12283 AFL2G_07249 VFL2G AFL2G A. niger A. roryzae A. oryzae A. 11927 ATEG_08023 A. 00090102000589 A. 111927 ATEG_08023 A.0039124000358 A. A. 0039124000358 178468 ATEG_03639 A009000900067 189367 ATEG_08458 A0090010000607 A0090102000546 3001409 A0090003001420 00081 A0090103000214 A0090012000422 A009011300007 35672 ATEG_03000 A009001 A009000 ATEG_04852 A009 40254 ATEG 07516 79579 ATEG 08295 TEG_07840 171385 ATEG 05229 ATEG_05264 ATEG_07309 09074 40542 ATEG_07855 53801 ATEG 07905 ATEG 31188 85409 5227 A. niger CBS513.88 An15g05520 An14g05370 An07g00450 An11g05580 An11g03580 5a03100 An18g01120 An01g08960 An01g14740 Nodule A. nidulans AA1_2 AA1_2 AA1_2 AA1_2 AA1_2 AA1_3 AN1597 AA1_3 AN1597 AA1_3 AA1_2 AA1_2 AA1_2 AA1_2 AA1_2 AA1_2 AA1_2 AA1_2 AA1_3 AA3 AA3 AA3 AA3 AA3 AA3 AA3A AA3_2 AN10931 AA3_2 AA3_2 AN10931 AA3_2 AN6445 AN7389 AN10931 AN0901 AN2175 **M8329** M8547 **NN7408** M3206 M1_3 Å1.3 A1_3 441_3 441_3 A13 4A1.2 **W3 2** M3 2 4A3 2 4A3 2 4A3 2 4A3 2 4A3 2 4A3 2 **W3 2** ¥ ¥ ¥ **M3 A**3 ¥ k ₹ ¥ ŝ oxidase nulticopper oxidase oidase e-like n -like accase-like elated to anzyme 8 AAO AAO

AFL2G_01375 ACLA_036030 NFIA_069070 AFUB_041050 Afri3g08070

A0090005001472

37397 ATEG_01660

An02g11560

Supplemental Table 4B Cont: Orthology clusters of auxiliary activities (AA). (Continued on next page)

| Mode: Mode: <th< th=""><th></th><th>Amfig/2010 Yooka ATEG, Orige ActA, Microli (Net, A) (2010) Acta (2011) Acta (2011)</th></th<> <th>MA2 Mady CDB Order ACID Order ACID Order ACID Control CDB Contro CDB Contro C</th> | | Amfig/2010 Yooka ATEG, Orige ActA, Microli (Net, A) (2010) Acta (2011) | MA2 Mady CDB Order ACID Order ACID Order ACID Control CDB Contro CDB Contro C |
|--|--|---|--|
| ACIA, 57700 ACIA, 77700 PA, 91420 | 100000 ALLA, 177000 PAL, 20200 ALLA, 17700 PAL, 20200 PAL, 20200 <th< td=""><td>Marky Sintol 10060 Altra, 00100 Altra, 001000 Altra, 00100</td><td>AAJ Madig_C200 10000 Madig_C200 10000 Madig_C200 10000 Madig_C200 10000 Madig_C200 Madig_</td></th<> | Marky Sintol 10060 Altra, 00100 Altra, 001000 Altra, 00100 | AAJ Madig_C200 10000 Madig_C200 10000 Madig_C200 10000 Madig_C200 10000 Madig_C200 Madig_ |
| A 000001200015 M 123, 1026 A 000001200015 M 123, 1026 A 000001200015 M 123, 1026 A 000001200015 M 123, 1026 A 00000100015 M 123, 1021 A 000001000015 M 123, 1021 A 00001000015 M 123, 1021 A 000010000015 M 123, 1021 A 000001000015 M 123, 1021 A 000001000015 M 123, 1021 A 000001000015 M 123, 1021 A 000000000012 M 123, 1021 A 00000000000000012 M 123, 1021 A 000000000012 M 123, 1021 A 000000000000000000000000000000000000 | 19008 FTC_0145 ATEC_0145 ATE | Antigrizzition 19000 ATEG, 01065 Antigrizzition 44243 ATEG, 01065 Antigrizzition AtEG, 01065 Antigrizzition AtEG, 01065 Antigrizzition AtEG, 01065 AtEG, 010666 AtEG, 01066 AtEG, 0 | AA.2 Moligization 1990al AFEG, 0105 AA.2 Moligization 1990al AFEG, 0105 AA.2 Moligization AA.3 Moligiz |
| | 19043 ATEC 0195 4443 ATEC 0455 4445 ATEC 0455 ATEC 0455 5125 5126 5126 5126 5126 5126 5126 51 | Adiojatication 190668 ATEC 01166 Adiojatication 4443 ATEC 05456 Adiojatication 4443 ATEC 05456 Adiojatication 20238 Adiojatication 20258 Adiojatication 20265 Adiojatication 2025 Adiojatication 2025 Adiojaticatication 2025 Adiojatication 2025 Adiojaticatication 2025 Adiojaticaticaticaticaticaticaticaticaticatic | AMJ Autigradie Pollogicatio Pollogicatio |

| | | | | | | | | - | - | | | | - | | | | | | | - | | | | | | - | - | | 49 | 40.8 |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|----------------|
| | | - | | | | | - | | | | | | | | | | | | | | | - | - | | | | | | 6 | 7.5 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 14 | 11.7 |
| | | | | | - | | | | | | | - | | | | | | | | | | | | | - | | | | 13 | 10.8 |
| | - | | | - | | | | | | | | | | - | | | | | | | | | | - | | | | | 10 | 833 |
| _ | | | | | | | | | | | - | | | | | | | - | | | - | | | | | | | | 5 | 42 |
| | | | | | | | | | | | | | | | | | - | | - | | | | | | | | | | | 8 |
| 60B | 61A | 61B | 62A | 628 | 8 | 15 | 65 | 99 | 67 | 8 | 69 | 70 | 42 | 43A | 43B | 44 | 45 | 46 | 47A | 47B | 48 | 49 | 50 | 51 | 52 | 8 | 54 | 55 | - | 10 |
| Cluster 0 | Cluster 0 | Cluster 0 | Cluster 2 | 0 | 2 |
| | Mu3g13650 | | | | | | | | | Mu2g17620 | Mu2g01180 | | | Mu3g03870 | | Vfu1g12560 | Mu8g06830 | Vfu2g14490 | Vfu2g14540 | | Mu4g07850 | | | Mu6g09540 | | | | | | |
| | B_035540 A | | | | | | | | | B_033310 / | B_018620 / | | | B_044090 / | B_101610 | B_012030 / | B_080950 / | B_030110 / | B_030160 / | | B_064960 / | | | B_075590 / | | | | | 0 | - |
| 45610 | 63710 AFU | | | | | | | | | 93020 AFU | 33480 AFU | | | 06140 AFU | 44390 AFU | 12990 AFU | 99510 AFU | 89670 AFU | 89730 AFU | | 08320 AFU | | | 65220 AFU | | | | | | |
| 90 NFIA_0 | NFIA_0 | 30 | | | | | | | | 10 NFIA_0 | 90 NFIA_0 | | | 90 NFIA_0 | NFIA_0 | 60 NFIA_0 | 90 NFIA_0 | 30 NFIA_0 | 60 NFIA_0 | 20 | 20 NFIA_1 | | | NFIA_0 | | | | | 2 | |
| ACLA_0596 | | ACLA_0548 | | | | | | | | ACLA_0765 | ACLA_0944 | | | ACLA_0608 | | ACLA_0229 | ACLA_0697 | ACLA_0730 | ACLA_0550 | ACLA_0174 | ACLA_0472 | | | | | | | | | |
| L2G_11894 | 1.26_07032 | | | 126_05344 | 126_03990 | | 126_01626 | | | L2G_09511 | L2G_06016 | L2G_08542 | | | 126_03026 | 126_07454 | L2G_00532 | | 1.26_04048 | | 1.2G_04596 | | | 126 12282 | 12G_08699 | | | | 2 | |
| 000757 AF | 000220 AF | | | 000693 AF | 000095 AF | | 001458 AF | | | 000058 AF | AF | 000054 AF | | | 000090 AF | 000221 AF | 000631 AF | 000056 | 000159 AF | | 000787 AF | | | 000087 AF | 000004 AF | | | | e | |
| A0090010 | A0090026 | | | A0090011 | A0090023 | | A0090003 | | | A0090102 | | A0090113 | | | A0090012 | A0090001 | A0090005 | A0090023 | A0090023 | | A0090023 | | | A0090103 | A0090138 | | | | | |
| TEG_10370 | TEG_02996 | TEG_10246 | TEG_08150 | TEG_01526 | TEG_04326 | TEG_08151 | | | | TEG_09993 | TEG_08151 | TEG_00720 | | TEG_04210 | TEG_07920 | TEG_00448 | TEG_07790 | TEG_01035 | TEG_08113 | | TEG_05416 | TEG_06077 | TEG_10194 | TEG_05081 | | TEG_08942 | | TEG_01456 | Ű | |
| 134460 A | 4 | × | < | 173811 A | A | A | | | 39013 | ¥ | 192610 A | 173720 A | | 211595 A | 53797 A | 52688 A | 182430 A | 4 | 43784 A | | × | × | 56338 A | A | 194765 | < | | × | 15 | 0 |
| n14g02900 | | | | n02g06090 | | | | | n11g04100 | | n10g00390 | n02g09270 | | n12g04610 | n14g02670 | n08g05230 | n15g04900 | | n12g02540 | | | | n15g04570 | | n04g08550 | | | | 15 | e |
| 390 V | | | | 0820 A | 2326 | | | 3488 | < | 530 | ∢ | ∢ | 0419 | 4 | 524 A | M1 A | 502 A | 388 | 346 A | | | 128 | × | | 391 A | | 960 | | 13 | |
| AA8 AN81 | AA8 | AA8 | AA8 | AA8 AN11 | AA8 AN1: | AAB | AAB | AA8 AN1 | AA8 | AA3_1 AN7: | AA3_1 | AA3_1 | AA9 AN1 | eve A | AA9 AN9 | ITNA 9AA | AA9 AN1 | AA9 AN2: | AA9 AN3 | AA9 | AA9 | AA9 ANG | AA9 | AA9 | TWA BAA | 6VV | AA9 ANB | 6VV | pecies | genes |
| | | | | | | | | | | A8- | A8- | A8- | | | | | | | | | | | | | | | | | genes per s | train specific |
| | | | | | | | | | | 05 | | e. | se | se | 88 | 68 | 50 | 26 | 56 | se | se | 88 | Se | se | se | 26 | 88 | 05 | unique | 10 |
| se domain | ehydrogena | nase | ehydrogena | ionooxygen8 | ionooxygen: | ionooxygens | ionooxygens | tonooxygent | tonooxygena | ionooxygens | ionooxygens | ionoxygena | onooxygena | ionooxygena | ionooxygen8 | tonooxygenz | ionooxygeni | ionooxygens | tonooxygen: | | |
| iron reducta | cellobiose d | ehydroge | cellobiose d | accharide m | | |
| candidate. | candidate | candidate (| candidate | cellobiose | candidate | lytic polysi | lytic polys. | lytic polysi | lytic polys. | lytic polysi | lytic polys. | lytic polysi | lytic polysi | lytic polys, | lytic polys. | lytic polysi | lytic polysi | lytic polysi | lytic polysi | lytic polys: | lytic polys: | | |
| | | | | | | | | | | CDH | CDH | CDH | LPMO | LPMO | LPM0 | DMO | DMO | LPMO | LPMO | LPMO | LPMO | LPMO | LPMO | PMO | DMO | LPMO | DMO | DMO | | |

Supplemental Table 4B Cont: Orthology clusters of auxiliary activities (AA).

| | Rhamnose | Arabinose | Xylose | Mannose | Galactose | Glucose | Uronic acid | Polysaccharides |
|----------------|----------|-----------|--------|---------|-----------|---------|-------------|-------------------------------|
| Vheat bran | 0 | 17 | 35 | 1 | 2 | 42 | e | cellulose, (arabino)xylan |
| ugar beet pulp | 1 | 28 | 2 | 2 | 7 | 33 | 26 | cellulose, pectin, xyloglucan |

Supplemental Table 6A: Detection of proteins in cultures grown on wheat bran sorted by CAZy family. The colour codes indicate which percentage of the total number of detected peptides was of the specific protein. (Continued on next 4 pages).

| | | | 0-0.1% | 0.1-0.5% | 0.5-1% | 1-2% | 2-5% | 5-10% | >10% | |
|-------------|------------------------------------|-------|-------------|----------|-------------|-----------------|--------------|-------------|--------------|--------------|
| | | | | | | | | | | |
| enzyme code | function | CAZY | A. nidulans | A. niger | A. terreus | A. oryzae | A. flavus | A. clavatus | A. fischeri | A. fumigatus |
| FAE SF7 | ferulovi esterase | faeA | | 51662 | ATEG 08907 | A0090001000207 | AFL2G 07436 | | | |
| FAE SF1 | feruloyl esterase | SF1 | AN1772 | 51478 | ATEG_06663 | A0090001000582 | AFL2G_09228 | ACLA_083360 | NFIA_054700 | Afu6g09040 |
| FAE SF1 | feruloyl esterase | SF1 | | | ATEG_02212 | A0090001000066 | AFL2G_07310 | | NFIA_047590 | Afu6g00450 |
| FAE SF3 | feruloyl esterase | SF3 | 1 | | | A0090102000013 | | | | |
| FAE SF3 | feruloyl esterase | SF3 | 1 | | ATEG_02415 | | | | | |
| FAE SF4 | feruloyl esterase | SF4 | l . | 190471 | | A0090010000573 | AFL2G_11725 | | | |
| FAE SF4 | feruloyl esterase | SF4 | 1 | 43194 | | | | | | |
| AXE | acetyl xylan esterase | CE 1 | AN6093 | 211544 | ATEG_09843 | A0090011000745 | AFL2G_05471 | ACLA_081220 | NFIA_099230 | Afu8g06570 |
| AXE | acetyl xylan esterase | CE 1 | AN8320 | | | | | | | Afu1g17510 |
| AXE | acetyl xylan esterase | CE 1 | | | | | | | NFIA_084920 | |
| FAE SF5 | feruloyl esterase | CE | | | | A0090005000945 | AFL2G_00922 | ACLA_065130 | NFIA_115130 | |
| FAE SF5 | feruloyl esterase | CE | AN5267 | 43785 | ATEG_08112 | A0090023000158 | AFL2G_04047 | ACLA_055050 | NFIA_089720 | Afu2g14530 |
| FAE SF5 | teruloyi esterase | CE | | | ATEG_06644 | A0090701000884 | AFL2G_06446 | ACLA_061520 | | |
| FAE SFS | teruloyi esterase | CE | | | 1750 01011 | | | | | Atu2g09440 |
| FAE SF5 | feruloyi esterase | CE | | | A1EG_01914 | | | ACLA 047490 | | |
| PAE SF5 | Teruloyi esterase | CE | A N9793 | 63346 | ATEO 00420 | 4 0000005000077 | 451.00 00084 | ACLA_017480 | NEIA 077450 | A 6-E=00900 |
| r DME | pactic methyl asterase | CE | ANO/02 | 55515 | ATEG_00430 | A0090003000277 | AFL2G_00281 | ACLA_012070 | NFIA_077450 | Alubgusoou |
| DME | pectin methyl esterase | CER | | 445.95 | | A0090003001314 | AFL20_01070 | ACLA 044240 | NEIA 005020 | A 5-9-01520 |
| DME | nectin methyl esterase | CER | AN4860 | 174365 | ATEG 01704 | A0090012000149 | AFL2G_00016 | ACLA_035610 | NEIA 069500 | Afu3e07650 |
| PME | pectin methyl esterase | CE 6 | AN3390 | 214857 | | A0090102000010 | AFL2G 09467 | | NEIA 099600 | Afu8006880 |
| PME | pectin methyl esterase | CE 8 | AN7966 | 214007 | | | | | | |
| PME | pectin methyl esterase | CE 8 | | | | A0090113000039 | | | NFIA 100100 | Afu8q07250 |
| PME | pectin methyl esterase | CE 8 | ; | | | | | ACLA_059970 | | |
| PME | pectin methyl esterase | CE 8 | 5 | | | | AFL2G_08528 | | | |
| PME | pectin methyl esterase | CE 8 | 5 | | | | _ | ACLA_059980 | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | 2 | | | A0090003001268 | AFL2G_01797 | _ | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | 2 | | | | | | NFIA_099110 | Afu8g06480 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | 2 | 51400 | ATEG_10016 | A0090102000092 | AFL2G_09543 | | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | AN2834 | | | A0090113000155 | AFL2G_08631 | | NFIA_092640 | Afu2g17250 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | AN2528 | 189254 | ATEG_03511 | A0090701000556 | AFL2G_06177 | ALCL_041970 | NFIA_062750 | Afu3g14510 |
| GE | glucuronoyl esterase | CE15 | i | | ATEG_00945 | | | ACLA_087520 | NFIA_060260 | Afu6g14390 |
| BGL | beta glucosidase | GH 1 | | 140573 | | | | | | |
| BGL | beta glucosidase | GH | AN10124 | 213437 | ATEG_00687 | A0090003000497 | AFL2G_02496 | ACLA_020660 | NFIA_010690 | Afu1g14710 |
| BGL | beta glucosidase | GH | | | | | | ACLA_019180 | NFIA_009040 | Atu1g16400 |
| BGL | beta glucosidase | GH | 4110400 | 101717 | 4750 00057 | A0090113000148 | AFL2G_08626 | | NFIA_060550 | Atu6g14600 |
| BOL | beta glucosidase | GH | AN9103 | 131/4/ | ATEG_02057 | A0090120000075 | AFL2G_00111 | | NEIA_064710 | A100g00970 |
| BOL | beta glucosidase | GH | AN10375 | | ATEG_04135 | | | ACLA 064280 | INFIA_004710 | Aluby12600 |
| BGL | beta glucosidase | GH | | | | | | ACLA_004200 | | |
| GUS | beta glucuronidase | GH | AN3200 | 189620 | ATEG 09745 | | AEL2G 05262 | A02A_040420 | | |
| GUS | beta glucuronidase | GH 2 | AN2395 | 52111 | ATEG 01031 | A0090023000053 | AEL2G_03956 | | NEIA 089700 | Afu2n14520 |
| LAC | beta galactosidase | GH 2 | | | ATEG 10255 | | | | | |
| LAC | beta galactosidase | GH 2 | 2 | | ATEG_04784 | | | | | |
| LAC | beta galactosidase | GH 2 | AN2463 | | ATEG_00712 | | | | | |
| GUS | beta glucuronidase | GH 2 | AN5361 | 46827 | | A0090038000009 | AFL2G_08828 | | | |
| LAC | beta galactosidase | GH 2 | AN3201 | | ATEG_10243 | A0090012000389 | AFL2G_03296 | | NFIA_072410 | Afu5g14550 |
| LAC | beta galactosidase | GH 2 | AN6388 | | | | | | | |
| LAC | beta galactosidase | GH 2 | AN1107 | | | | | | NFIA_072890 | Afu5g14090 |
| MND | beta mannosidase | GH 2 | AN1742 | 138876 | ATEG_06636 | A0090001000556 | AFL2G_09201 | ACLA_083570 | NFIA_054490 | Afu6g08840 |
| MND | beta mannosidase | GH 2 | AN11680 | 172587 | ATEG_07339 | A0090003001410 | AFL2G_01666 | ACLA_078510 | NFIA_045250 | Afu4g00390 |
| MND | beta mannosidase | GH 2 | | | | A0090005000740 | AFL2G_00728 | | | |
| MND | beta mannosidase | GH 2 | AN3368 | 212893 | ATEG_08684 | AU090010000208 | AFL2G_11464 | ACLA_066240 | NFIA_114030 | Atu7g01320 |
| mNU ROL | beta aluensidase | GH 2 | | | ATEG_09890 | | | | | |
| BOL | beta glucostase | GH | | 120007 | ATEG_102/4 | | | | | |
| BOL | beta glucosidase | GH | | 139037 | | | | | | |
| BOL | beta glucosidase | OH 2 | | 30643 | | | | | | |
| BGL | beta glucosidase | GH | AN7915 | 38077 | | 40090001000266 | AEL2G 07497 | | | |
| BGL | beta glucosidase | GH 2 | AN10482 | 208871 | ATEG 06617 | A0090001000544 | AFL2G 09187 | ACLA 083710 | NFIA 054350 | Afu6a08700 |
| BGI | beta glucosidase | GH 3 | | | | | | | | |
| BGL | beta glucosidase | GH 3 | | 210981 | ATEG 02806 | A0090003001511 | ALF2G 01582 | | NFIA 095760 | Afu8a02100 |
| BGL | beta glucosidase | GH 3 | 1 | | ATEG 02724 | A0090005000337 | AFL2G 00334 | | - | |
| BGL | beta glucosidase | GH 3 | AN4102 | 56782 | ATEG_03047 | A0090009000356 | AFL2G_10322 | ACLA_028810 | NFIA_018950 | Afu1g05770 |
| BGL | beta glucosidase | GH 3 | AN6652 | 182309 | ATEG_07121 | A0090009000554 | AFL2G_10164 | ACLA_096980 | NFIA_050080 | Afu6g03570 |
| BGL | beta glucosidase | GH 3 | 5 | 44520 | 1 | A009001000034 | AFL2G_11300 | | | |
| BGL | beta glucosidase | GH 3 | 1 | | | A0090012000003 | AFL2G_02949 | | | |
| BGL | beta glucosidase | GH 3 | AN7396 | 179265 | ATEG_10320 | A0090012000135 | AFL2G_03066 | | NFIA_007920 | Afu1g17410 |
| BGL | beta glucosidase | GH 3 | AN1804 | | | A0090026000123 | AFL2G_07119 | | | |
| BGL | beta glucosidase | GH 3 | 5 | | | A0090038000223 | AFL2G_09023 | | | |
| BGL | beta glucosidase | GH 3 | AN5976 | 181816 | ATEG_02713 | A0090038000425 | AFL2G_07763 | | NFIA_100430 | |
| BGL | beta glucosidase | GH 3 | | | | A0090103000127 | AFL2G_12245 | | | |
| BGL | beta glucosidase | GH 3 | | 176601 | 1880 01077 | A0090166000048 | AFL2G_09452 | | NFIA_112660 | |
| BGL | peta glucosidase | GH 3 | AN3903 | 100000 | A (EG_04069 | AU090166000090 | AFL2G_09413 | ACLA_087610 | NFIA_060370 | Atu6g14490 |
| BGL | peta glucosidase | GH 3 | AN2227 | 129891 | ATEG_09329 | A0090701000244 | AFL2G_05886 | ACLA_010450 | NFIA_080070 | Atu5g07190 |
| BGL | beta glucosidase | GH 3 | AN0740 | | ATEG_07931 | | | | | |
| BOL | beta glucosidase | GH 3 | AN0712 | | | | | | | 4.6.7=00240 |
| DOL | Deta giucosidase | GHQ | AN2012 | | | | | | | A10700240 |

Supplemental Table 6A Cont: Detection of proteins in cultures grown on wheat bran sorted by CAZy family. (Continued on next 3 pages).

| BGL | beta glucosidase | GH | 3 AN2828 | | ATEG_07419 | AO090701000841 | AFL2G_06408 | ACLA_007810 | NFIA_027390 | Afu7g06140 |
|--|---|---|--|--|---|--|--|---|---|--|
| BGL | beta glucosidase | GH | 3 AN3949 | | | | | | | |
| BGL | beta glucosidase | GH | 3 AN7865 | | | | | | NFIA_057590 | Afu6g11910 |
| BGL | beta glucosidase | GH | 3 | 129779 | ATEG_00157 | | | | | |
| BGL | beta glucosidase | GH | 3 | | | | | | NFIA 000750 | Afu3q00230 |
| BGL | beta glucosidase | GH | 3 | | | | | | NFIA 098520 | |
| BGL | beta glucosidase | GH | 3 | | | | | | NFIA_057910 | |
| BGL/BXL | beta glucosidase/beta xylosidase | GH | 3 AN0479 | | | | | | | |
| BXL | beta xylosidase | GH | 3 AN2359 | 205670 | ATEG_05106 | A0090005000986 | AFL2G_00957 | | | Afu1g16920 |
| BXL | beta xylosidase | GH | 3 | | ATEG 07383 | A0090011000140 | AFL2G 04928 | | | |
| BXL | beta xylosidase | GH | 3 | | _ | | _ | ACLA_018590 | | |
| BXL/ABF | beta xylosidase/alpha arabinofuranosidas | GH | 3 AN8401 | | ATEG 09052 | A0090103000120 | AFL2G 12252 | ACLA 062400 | NFIA 003180 | Afu3a02090 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosidas | GH | 3 AN2217 | 50997 | ATEG 09314 | A0090701000274 | AFL2G 05912 | ACLA 010340 | NFIA 080180 | Afu5q07080 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosidas | GH | 3 | | ATEG 08027 | | _ | - | - | 1 |
| ? | | GH | 3 AN1416 | 45461 | ATEG 00018 | A0090103000019 | AFL2G 12338 | ACLA 057390 | NFIA 096770 | Afu8a04060 |
| ? | | GH | 3 | | - | | - | - | - | Afu1000540 |
| ? | | GH | 3 AN3360 | 37673 | ATEG 04963 | | | ACLA 052760 | NFIA 102600 | Afu4q13770 |
| ? | | GH | 3 | 120104 | ATEG 04729 | A0090003000741 | AFL2G 02272 | ACLA 039660 | NFIA 065550 | Afu3q11780 |
| ? | | GH | 3 | | _ | | AFL2G 08686 | _ | | |
| ? | | GH | 3 AN2599 | | | | | | | |
| EGL | endoglucanase | GH | 5 AN3013 | 210716 | ATEG 01592 | A0090005001389 | AFL2G 01298 | ACLA 036770 | NFIA 068300 | Afu3q08820 |
| EGL | endoqlucanase | GH | 5 AN1285 | 205580 | ATEG 05002 | | _ | ACLA 085250 | NFIA 057290 | Afu6a11600 |
| EGL | endoglucanase | GH | 5 | | - | | | _ | NFIA 095570 | |
| EGL | endoglucanase | GH | 5 AN8068 | | ATEG 09802 | A0090003001341+/ | AFL2G 01726 | | NFIA 040280 | Afu5q01830 |
| EGL | endoglucanase | GH | 5 AN5214 | 209376 | _ | A0090005001553 | AFL2G 01447 | ACLA 081650 | NFIA 053150 | Afu6q07480 |
| EGL | endoglucanase | GH | 5 | 214608 | ATEG 04390 | A0090011000715 | AFL2G 05447 | ACLA 081310 | NFIA 085010 | Afu2q09520 |
| EGL | endoglucanase | GH | 5 | | ATEG 05003 | | _ | - | - | |
| GLN | exo 1.6 galactanase | GH | 5 AN9166 | 194447 | ATEG 10242 | AO090012000046 | AFL2G 02982 | | NFIA 072400 | Afu5q14560 |
| EXG | exo 1,3 galactanase | GH | 5 | | ATEG_08371 | | | | NFIA_056040 | |
| EXG | exo 1,3 galactanase | GH | 5 AN8947 | 175759 | ATEG_07719 | | | | | |
| EXG | exo 1,3 galactanase | GH | 5 AN7533 | 123981 | ATEG_06686 | A0090001000604 | AFL2G_09249 | ACLA_083150 | NFIA_054930 | Afu6g09250 |
| EXG | exo 1,3 galactanase | GH | 5 AN4052 | 202490 | ATEG_03849 | A0090003000990 | AFL2G_02039 | ACLA_031040 | NFIA_021060 | Afu1g03600 |
| EXG | exo 1.3 galactanase | GH | 5 | | ATEG 06369 | A0090005000423 | AFL2G 00412 | ACLA 007330 | NFIA 026860 | Afu7q05610 |
| EXG | exo 1.3 galactanase | GH | 5 | | ATEG 03062 | AO090009000373 | AFL2G 10308 | - | - | |
| EXG | exo 1,3 galactanase | GH | 5 AN1332 | 52811 | _ | A0090012000917 | AFL2G_03782 | | NFIA_113230 | |
| EXG | beta 1,6 glucanase | GH | 5 AN3777 | | ATEG_09844 | A0090011000757 | AFL2G_05484 | | NFIA_084850 | Afu2g09350 |
| MAN | endomannanase | GH | 5 AN3358 | 50378 | ATEG 08654 | A0090010000122 | AFL2G_11381 | ACLA 066420 | NFIA_113780 | Afu7q01070 |
| MAN | endomannanase | GH | 5 | | ATEG 10292 | A0090012000006 | AFL2G 02951 | | NFIA 041960 | Afu5q00550 |
| MAN | endomannanase | GH | 5 AN7639 | | | A0090038000444 | AFL2G 07781 | ACLA 044470 | NFIA 099770 | Afu8q07030 |
| MAN | endomannanase | GH | 5 | | ATEG_02669 | | | | | |
| MAN | endomannanase | GH | 5 AN2709 | | | | | | | |
| MAN | endomannanase | GH | 5 AN3297 | | | | | | | |
| MAN | endomannanase | GH | 5 AN6427 | | ATEG_09991 | | | | | |
| MAN | endomannanase | GH | 5 AN9276 | | | | | | | |
| MAN | endomannanase | GH | 5 | | ATEG_01374 | | | | | |
| | | GH | 5 | | ATEG_03677 | | | | | |
| CBH | cellobiohydrolase | GH | 6 AN5282 | 54490 | ATEG_07493 | | | ACLA_062560 | NFIA_002990 | Afu3g01910 |
| CBH | cellobiohydrolase | GH | 6 AN1273 | 133986 | ATEG_00193 | AO090038000439 | AFL2G_07776 | ACLA_025560 | NFIA_015680 | |
| CBH | cellobiohydrolase | GH | 7 AN0494 | 51773 | ATEG_05002 | AO090001000348 | AFL2G_07571 | ACLA_085260 | NFIA_057300 | Afu6g11610 |
| CBH | cellobiohydrolase | GH | 7 | | | | | | NFIA_095570 | |
| CBH | cellobiohydrolase | GH | 7 AN5176 | 53159 | ATEG_03727 | A0090012000941 | AFL2G_03805 | ACLA_088870 | NEIA 052720 | Afu6g07070 |
| EGL | endoglucanase | | | | | | | | 1110-002120 | |
| EGL | | GH | 7 AN3418 | | ATEG_08700 | AO090010000314 | ALF2G_11497 | ACLA_066030 | NFIA_114250 | Afu7g01540 |
| | endoglucanase | GH GH | 7 AN3418 7 | | ATEG_08700 ATEG_08705 | A0090010000314 | ALF2G_11497 | ACLA_066030 ACLA_098940 | NFIA_114250 NFIA_047960 | Afu7g01540 Afu6g01800 |
| XLN | endoglucanase endoxylanase | GH GH GH 1 | 7 AN3418 7 0 | | ATEG_08700 ATEG_08705 | A0090010000314 A0090001000208 | ALF2G_11497 AFL2G_07437 | ACLA_066030 ACLA_098940 | NFIA_114250 NFIA_047960 | Afu7g01540 Afu6g01800 |
| XLN XLN | endoglucanase endoxylanase endoxylanase | GH GH GH 1 GH 1 | 7 AN3418 7 0 AN7401 | | ATEG_08700 ATEG_08705 ATEG_03410 | A0090010000314 A0090001000208 A0090103000326 | ALF2G_11497 AFL2G_07437 AFL2G_12071 | ACLA_066030 ACLA_098940 ACLA_086910 | NFIA_047960 NFIA_059570 | Afu7g01540 Afu6g01800 Afu6g13610 |
| XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase | GH GH GH 1 GH 1 GH 1 | 7 AN3418 7 0 0 AN7401 0 AN1818 | 57436 | ATEG_08700 ATEG_08705 ATEG_03410 ATEG_00809 | A0090010000314 A0090001000208 A0090103000326 A0090701000887 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_059570 NFIA_059570 NFIA_06540 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 |
| XLN XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase endoxylanase | GH GH GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN1818 0 | 57436 | ATEG_08700 ATEG_08705 ATEG_03410 ATEG_0809 ATEG_08906 | A0090010000314 A0090001000208 A0090103000326 A0090701000887 A0090103000423 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_114250 NFIA_047960 NFIA_059570 NFIA_106540 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 |
| XLN XLN XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase | GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN7401 0 AN1818 0 0 AN2356 | 57436 | ATEG_08700 ATEG_08705 ATEG_03410 ATEG_00809 ATEG_08906 | A0090010000314 A0090001000208 A0090103000326 A0090701000887 A0090103000423 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_114250 NFIA_047960 NFIA_059570 NFIA_106540 NFIA_057510 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 Afu6g11820 |
| XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase | GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN1818 0 0 AN2356 0 | 57436 50977 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_00809 ATEG_08906 ATEG_07190 | AO090010000314 AO090001000208 AO090103000326 AO090701000887 AO090103000423 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_114250 NFIA_047960 NFIA_047960 NFIA_059570 NFIA_106540 NFIA_057510 NFIA_061880 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09460 Afu6g11820 Afu3g15210 |
| XLN XLN XLN XLN XLN XLN XLN | endoplucanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase | GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN1818 0 0 AN2356 0 1 AN3613 | 57436 50977 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_00809 ATEG_08906 ATEG_07190 | AO090010000314 AO090001000208 AO090103000326 AO090701000887 AO090103000423 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_114250 NFIA_047960 NFIA_047960 NFIA_106540 NFIA_057510 NFIA_061880 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 Afu6g11820 Afu3g15210 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase | GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN1818 0 AN1818 0 AN2356 0 1 AN3613 | 57436 50977 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_00809 ATEG_08906 ATEG_07190 | A0090010000314 A0090001000208 A0090103000326 A0090701000887 A0090103000423 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 | ACLA_066030 ACLA_098940 ACLA_086910 ACLA_048770 | NFIA_114250 NFIA_047960 NFIA_059570 NFIA_106540 NFIA_061880 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 Afu6g11820 Afu3g15210 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase endoxylanase | GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 | 7 AN3418 7 0 AN7401 0 AN1818 0 0 0 AN2356 0 AN2356 1 AN3613 1 | 57436 50977 171269 | ATEG_08700 ATEG_08705 ATEG_03410 ATEG_00809 ATEG_08906 ATEG_07190 ATEG_04943 | A0090010000314 A0090010000268 A0090103000326 A0090701000887 A0090103000423 A0090001000111 | ALF2G_11497 AFL2G_07437 AFL2G_12071 AFL2G_06449 AFL2G_11983 AFL2G_07347 | ACLA_066030 ACLA_098940 ACLA_098940 ACLA_048770 ACLA_048770 | NFIA_059520 NFIA_047960 NFIA_059570 NFIA_106540 NFIA_057510 NFIA_061880 NFIA_058160 | Afu7g01540 Afu6g01800 Afu6g13610 Afu4g09480 Afu6g11820 Afu6g12210 |
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| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase xyloglucan active endoglucanase xyloglucan active endoglucanase yloglucana etive endoglucanase yloglucana etive endoglucanase yloglucana etive endoglucanase | GH GH GH GH GH GH GH GH GH GH GH GH GH G | AN3418 AN3418 Q AN7401 Q Q Q AN1818 Q AN2356 Q AN25613 I | 57436 50977 171269 183088 52011 211053 191511 191511 191511 191511 191511 | ATEG_08700 ATEG_08700 ATEG_08705 ATEG_08066 ATEG_08066 ATEG_07401 ATEG_04943 ATEG_07461 ATEG_07461 ATEG_07466 ATEG_07466 ATEG_07466 ATEG_07466 | A0990010000314 Obeen 10000314 Obeen 100003 A09901000038 A0990701000887 A099010001111 A0990103001423 A0990026000103 A099010300141 A099012000026 A099003000905 A099003000155 A0990701000185 A0990701000185 | ALF20_11497 AFL20_07437 AFL20_07437 AFL20_1071 AFL20_06449 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_08984 AFL20_ | ACLA_066030 ACLA_069340 ACLA_09340 ACLA_09340 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_04370 ACLA_063300 ACLA_060330 ACLA_060330 | NFA_114250 NFA_05750 NFA_055710 NFA_055710 NFA_05510 | Afu7g01540 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g12210 Afu6g12210 Afu6g12210 Afu3g00320 Afu3g00470 Afu3g03610 Afu3g03610 Afu3g01160 Afu3g01540 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase endo | GH GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH | 7 AN3418 7 AN3418 0 AN7401 0 AN1118 0 AN2356 0 AN2356 0 AN2356 1 AN3365 1 AN3365 1 AN3365 1 2 AN3452 | 57438 50977 171289 183088 52011 211053 191511 191511 191511 191511 211162 211162 211162 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_08906 ATEG_08906 ATEG_08906 ATEG_09906 ATEG_09906 ATEG_07401 ATEG_07401 ATEG_07406 ATEG_07406 ATEG_07406 ATEG_07406 ATEG_07406 ATEG_07406 | A0090010000314 Ocean 10000314 Ocean 100003 A00901000038 A0090701000887 A009010000423 A0090020001111 A0090020001101 A009002000102 A0090020001000483 A0090010000483 | ALF20_11497 AFL20_07497 AFL20_07497 AFL20_07497 AFL20_07497 AFL20_07497 AFL20_07387 AFL20_07387 AFL20_07387 AFL20_07387 AFL20_07587 AFL20_07587 AFL20_08984 AFL20 | ACLA_066030 ACLA_069340 ACLA_099340 ACLA_099340 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043710 ACLA_069310 ACLA_060930 ACLA_060930 ACLA_060930 | NFA_114250 NFA_07960 PFA_106540 NFA_05510 NFA_05510 NFA_05510 NFA_05510 NFA_05540 NFA_05540 NFA_05540 NFA_0050 NFA_02020 NFA_020200 NFA_02040 NFA_05810 NFA_05670 NFA_106670 | Afu7g01540 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g12810 Afu6g12210 Afu3g00120 Afu3g00120 Afu3g00120 Afu3g00470 Afu3g03610 Afu3g03610 Afu3g01160 Afu5g10540 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase xyloglucan active endoglucanase xyloglucan active endoglucanase ative endoglucanase yloglucan active endoglucanase alpha glucosidase | GH GH GH GH GH GH GH GH GH GH GH GH GH G | AN3418 AN3418 AN4411 AN7401 AN1413 AN34118 AN256 AN255 AN256 AN256 AN256 AN355 AN355 AN355 AN355 AN355 AN355 AN4552 AN40452 AN40452 AN40452 AN42314 | 57436 50977 171269 183088 52071 52071 211053 191511 191511 191511 191512 211162 221162 221162 50027 52452 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_08806 ATEG_08806 ATEG_07490 ATEG_07491 ATEG_07491 ATEG_07451 ATEG_07456 ATEG_07456 ATEG_07456 ATEG_07456 ATEG_07456 | A0990010000314 Openational A099010000314 Openational A09901000038 A0990701000887 A0990103000423 A099026000103 A0990103000414 A099012000028 A099026000102 A099020000175 A099020000175 A099020000175 A099020000175 A09902000175 A099020000175 A09902000175 A099020000175 A099020000175 A099020000175 A09902000175 A099020000175 A099020000175 A099020000175 A09902000000000000000000000000000000000 | ALF20_11497 AFL20_07437 AFL20_07437 AFL20_1071 AFL20_06449 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_01216 AFL20_08984 AFL20_01216 AFL20_0126 AFL20_ | ACLA_066030 ACLA_069840 ACLA_09840 ACLA_089410 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_045710 ACLA_05140 ACLA_05140 ACLA_060930 ACLA_060930 ACLA_013550 ACLA_055550 | NFA_114250 NFA_051760 NFA_05510 NFA_056510 NFA_056510 NFA_056510 NFA_056570 NFA_056880 | Afu7g01540 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g12210 Afu6g12210 Afu6g12210 Afu3g00320 Afu3g00470 Afu3g03610 Afu3g03610 Afu3g01160 Afu5g10540 Afu3g07380 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylana active endoplucanase xyloglucan active endoplucanase alpha glucoxidase | GH GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH | AN3418 AN3418 0 AN7401 0 AN1118 0 AN2356 0 AN2356 1 AN3613 1 AN9365 1 AN9365 2 AN0452 2 2 2 2 2 2 2 3 3 AN2314 3 AN4843 | 57438 50977 171289 183088 52011 211053 191511 191511 191511 191512 119152 211165 250627 50627 52452 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_08705 ATEG_08906 ATEG_08906 ATEG_08906 ATEG_098906 ATEG_07490 ATEG_07491 ATEG_07481 ATEG_07486 ATEG_07466 ATEG_07468 ATEG_07468 | A0090010000314 O090010000314 O090010000314 A009010300328 A0090701000887 A0090103000423 A009001000100111 A0090103000423 A009002000135 A009002000135 A009002000175 A0090020000175 A0090000000000000000000000000000000000 | ALF20_11497 AFL20_07457 AFL20_07457 AFL20_10649 AFL20_07457 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_068841 AFL20_068841 AFL20_068841 AFL20_08984 AFL20_07477 AFL20_07487 AFL20_074777 AFL20_074777 AFL20_0747777 AFL20_0747777777777777777777777777777777777 | ACLA_066030 ACLA_069340 ACLA_09840 ACLA_09840 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043770 ACLA_043710 ACLA_043710 ACLA_043710 ACLA_060930 ACLA_060930 ACLA_060930 | NFA_114250 NFA_07960 PA_07960 NFA_05540 NFA_05540 NFA_05510 NFA_05540 NFA_05540 NFA_00500 NFA_00100 NFA_00100 NFA_00100 NFA_00300 NFA_005810 NFA_00580 NFA_0 | Afu7g01540 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g11820 Afu6g11820 Afu6g12210 Afu6g12210 Afu6g10220 Afu3g00220 Afu3g00470 Afu3g03810 Afu3g01160 Afu3g07380 Afu3g07380 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase xyloglucan active endoglucanase xyloglucan active endoglucanase alpha glucosidase alpha glucosidase alpha glucosidase | GH GH 1 GH 11 GH 1 | AN3418 AN3418 AN4418 AN7401 AN1418 AN3451 AN2356 AN2356 AN2356 AN2356 AN2356 AN34513 1 1 1 2 2 2 2 2 2 2 2 3 | 57436 50977 171269 183088 52071 211053 191511 191511 191511 57002 21182162 221182 52452 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_08705 ATEG_08906 ATEG_07490 ATEG_07490 ATEG_07490 ATEG_07490 ATEG_07450 ATEG_07456 ATEG_07456 ATEG_07456 ATEG_07456 ATEG_07456 | A0990010000314 Openational A099010000314 Openational A09901000038 A0990701000887 A0990103000423 A099026000103 A0990103000411 A0990103000414 A099026000102 A099026000102 A099026000102 A099026000102 A099026000102 A099026000102 A099026000102 A099026000102 A099026000102 A099026000126 A09902000076 A09902000076 A09902000076 A09902000076 A09902000076 A09902000076 A09902000076 A09902000076 A0990200076 A090200076 A09020 | ALF20_11497 AFL20_07437 AFL20_07437 AFL20_10747 AFL20_10747 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_07138 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_08984 AFL20_01111 AFL20_08984 AFL20_011466 AFL20_01146 AFL20_011466 AFL20_011466 AFL20_01146 AFL20_011466 AFL20_01146 AFL20_0146 | ACLA_066030 ACLA_069840 ACLA_09840 ACLA_09840 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_045770 ACLA_069370 ACLA_069390 ACLA_05550 ACLA_035550 ACLA_035550 | IFFA_114250 IFFA_047960 IFFA_05510 IFFA_05540 IFFA_05510 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_05500 IFFA_055000 IFFA_055000 IFFA_055000 IFFA_055000 IFFA_05000 IFFA_ | Afu7g01540 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g13610 Afu6g12210 Afu6g12210 Afu6g12210 Afu3g0320 Afu3g0320 Afu3g03610 Afu3g03610 Afu3g01160 Afu3g07380 Afu3g07380 |
| XLN XLN XLN XLN XLN XLN XLN XLN XLN XLN | endoglucanase endoxylanase exyloglucan active endoglucanase xyloglucan active endoglucanase xyloglucan active endoglucanase yloglucan extive endoglucanase exyloglucan extive endoglucanase yloglucan extive endoglucanase yloglucanase exits endoglucanase exits endoglucana | GH GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH 1 GH | AN3418 AN3418 AN401 AN101256 AN2556 AN2556 AN3613 AN3653 AN93653 AN48433 AN16420 | 57436 50977 171289 183088 52071 52011 211053 191511 191511 191511 191511 191512 119152 211162 211162 | ATEG_08700 ATEG_08705 ATEG_08705 ATEG_08705 ATEG_08906 ATEG_08906 ATEG_08906 ATEG_08906 ATEG_094943 ATEG_07481 ATEG_07481 ATEG_07481 ATEG_07486 ATEG_07466 ATEG_07468 ATEG_07468 | A0090010000314 Ocean Control | ALF20_11497 AFL20_07457 AFL20_07457 AFL20_07457 AFL20_07457 AFL20_07457 AFL20_07457 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_07458 AFL20_0745 AFL20_07458 AFL20_0745 AFL20_ | ACLA_066030 ACLA_069340 ACLA_099340 ACLA_099340 ACLA_049770 ACLA_049770 ACLA_049770 ACLA_049770 ACLA_049770 ACLA_04970 AC | NFA_114250 NFA_047660 NFA_05540 NFA_05540 NFA_05510 NFA_05510 NFA_05540 NFA_05540 NFA_05540 NFA_00100 NFA_00100 NFA_00100 NFA_002040 NFA_005810 NFA_00590 N | Afu7g01540 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g13810 Afu6g12810 Afu6g12210 Afu6g12210 Afu6g10320 Afu3g00470 Afu3g0470 Afu3g03610 Afu3g03610 Afu3g01160 Afu3g07380 Afu7g06380 Afu8g07070 |

Supplemental Table 6A Cont: Detection of proteins in cultures grown on wheat bran sorted by CAZy family. (Continued on next 2 pages).

| AGD | alpha glucosidase | GH 13 | | | | | AFL2G 08694 | | | |
|---------------------------------|--|---|--|--------------------------|--------------------------|--|---|---------------|----------------------------|--------------------------|
| AGD | alpha glucosidase | GH 13 | | | | | | ACLA 070570 | NFIA 086860 | Afu2q11620 |
| AGD | alpha glucosidase | GH 13 | | | | | | ACLA 070560 | NFIA 086850 | Afu2q11610 |
| AGS | alpha olucan synthase | GH 13 | | 212915 | | | | | | |
| AGS | alpha olucan synthase | GH 13 | | 40878 | | | | | | |
| AGS | alpha olucan synthase | GH 13 | AN3307 | 54378 | ATEG 03622 | A0090003001500 | AFL2G 01593 | ACLA 063450 | NFIA 001720 | Afu3o00910 |
| AGS | alpha olucan synthase | GH 13 | | 55204 | ATEG 10371 | A0090010000106 | AFL2G 11365 | ACLA 042430 | NFIA 009960 | Afu1o15440 |
| AGS | alpha olucan synthase | GH 13 | AN5885 | | ATEG 01449 | A0090026000523 | AFL2G_06761 | ACLA 070240 | NFIA 086550 | Afu2o11270 |
| AMY | alpha amylase | GH 13 | | | | | | rios Coros ro | | / though ture |
| AMY | alpha amylase | GH 13 | | | | | | | | |
| AMY | alpha amylase | GH 13 | | | | 40090120000196 | | ACLA 094070 | NEIA 032970 | Afu2n00710 |
| AMY | alpha amylace | GH 13 | AN3402 | 47011 | ATEG 10103 | A0090023000944 | | ACLA 052920 | | rtidEgeerre |
| AMY | alpha amylase | GH 13 | AN2018 | 45304 | ATEG_08279 | A0090003001210 | AEL2G_01841 | ACLA_032320 | NEIA 105920 | Afu4e10130 |
| AGT | A sinha diucanotraneferaea | GH 13 | AN3308 | 188489 | ATEG_03623 | A0090003001210 | AFL2G_01041 | ACLA_0433330 | NEIA 001710 | Afu3e00900 |
| ANY | alpha amulana | 01113 | A110000 | 100403 | A120_03023 | A00000120000262 | AFL20_01334 | ACCA_003440 | 11104_001110 | Alabyoosoo |
| | alpha amylasc | 01113 | | | | A0030120000203 | Ar 220_00210 | | | |
| AMY | alpha amylase | 00 13 | 014507 | | | | | | | |
| AMIT | alpha amylase | 00113 | AN9307 | | ATEO 00545 | | | | | |
| AMY | alpha amylase | GH 13 | AN3300 | | ATEG_02515 | | | A CL A 070070 | | 44-0-10100 |
| AMY | alpha amylase | GH 13 | AN0324 | 400000 | 4750 00004 | 4.000000004.407 | 451.00 04505 | ACLA_072270 | NFIA_000050 | Atu2g13460 |
| AMY | alpha amylase | GH 13 | | 122069 | ATEG_03624 | A0090003001497 | AFL2G_01595 | | | |
| AMY | alpha amylase | GH 13 | AN10060 | 46621 | ATEG_04879 | A0090005000884 | AFL2G_00860 | ACLA_032290 | NFIA_022510 | Afu1g02140 |
| AMY | alpha amylase | GH 13 | AN3309 | 46290 | ATEG_00838 | A0090005001193 | | ACLA_020060 | NFIA_010270 | Atu1g15150 |
| AMY | alpha amylase | GH 13 | | | ATEG_00724 | | | | | |
| AMY | alpha amylase | GH 13 | | | | | AFL2G_01127 | | | |
| AMY | alpha amylase | GH 13 | | | | | | ACLA_091300 | NFIA_035590 | Afu2g03230 |
| AMY | alpha amylase | GH 13 | | | | | | | NFIA_035580 | |
| GLA | glucoamylase | GH 15 | AN7402 | | | | | ACLA_049360 | NFIA_105910 | |
| GLA | glucoamylase | GH 15 | | | | | | | | Afu4g10140 |
| GLA | glucoamylase | GH 15 | | | ATEG_05980 | A0090003000321 | AFL2G_02658 | ACLA_089470 | | |
| GLA | glucoamylase | GH 15 | AN11143 | 213597 | ATEG_04375 | A0090010000746 | AFL2G_11885 | ACLA_094080 | NFIA_032960 | Afu2g00690 |
| GLA | glucoamylase | GH 15 | | 189911 | | A0090138000105 | AFL2G_08782 | ACLA_044430 | NFIA_094880 | Afu8g01390 |
| GLA | glucoamylase | GH 15 | | | | | | ACLA_082570 | NFIA_053760 | Afu6g08080 |
| GLA | glucoamylase | GH 15 | | | | | | ACLA_078620 | NFIA_001210 | Afu3g00610 |
| MAN | endomannanase | GH 26 | AN3336 | 40875 | | A0090011000055 | AFL2G_04859 | | | |
| MAN | endomannanase | GH 26 | AN3326 | | | | | | | |
| MAN | endomannanase | GH 26 | AN7413 | | | | | | | |
| AGL | alpha galactosidase | GH 27 | | | ATEG 01905 | | | | NFIA 048850 | Afu6q02560 |
| AGL | alpha galactosidase | GH 27 | | 37736 | ATEG 04382 | | | ACLA 016820 | NFIA 073100 | Afu5q13830 |
| AGL | alpha galactosidase | GH 27 | AN0022 | 172232 | ATEG 03427 | A0090005000217 | AFL2G 00225 | - | NFIA 023390 | Afu1001200 |
| AGI | alpha galactosidase | GH 27 | AN7624 | 207264 | ATEG 09830 | A0090003001305 | AEL2G 01765 | ACLA 003130 | NEIA 039990 | Afu5002130 |
| AGI | alpha galactosidase | GH 27 | | 39180 | | | | | | |
| AGI | alpha galactosidase | GH 27 | AN7152 | 41606 | ATEG 02160 | A0090023000151 | AEI 2G 04039 | | NEIA 029860 | Afu4003580 |
| AGI | alpha galactosidase | GH 27 | | | | | | ACLA 097900 | | |
| PGA | endopolygalacturonase | GH 28 | AN8327 | 46255 | | | | | NEIA 008150 | Afu1n17220 |
| PGA | endopolygalacturonase | GH 28 | | 43957 | | | | | 1110-000100 | Andrginzzo |
| POA | andopolygalacturonase | OH 28 | | 192156 | | | | | | |
| POA | endopolygalacturonase | 011 20 | | 214609 | | | | | | |
| POA | endopolygalacturonase | 011 20 | ANGESE | 214350 | | 0000005000195 | AEL 20, 00201 | | NEIA 000410 | |
| POA | endopolygalacturonase | 011 20 | AN0030 | 172044 | ATEC 01601 | A0090005000100 | AFL2G_00201 | ACLA 026670 | NEIA 069440 | A 5-2-09690 |
| POA | endopolygalacturonase | 011 20 | AN4272 | 141677 | ATEC_04001 | A009003001400 | AFL2G_01310 | ACLA_050070 | NEIA 102450 | Afu3g00000 |
| POA | endopolygalacturonase | 011 20 | A114372 | 50040 | ATL0_04331 | A0030023000401 | AFL20_04202 | ACCA_032000 | NEIA 005000 | A104913320 |
| PGA | endopolygalacturonase | GH 20 | | 52219 | | A0090023000161 | AFL2G_04049 | | INFIA_095620 | Aluogo 1970 |
| PGA | endopolygalacturonase | GH 20 | | | | A009013000000 | | | | 45-0-00700 |
| PGA | endopolygalacturonase | GH 20 | | | | | | | | Aluoyuorsu |
| PGA | endopolygalacturonase | GH 28 | | | ATEG_07748 | | 15100.00704 | | | |
| PGA | endopolygalacturonase | GH 28 | | 170170 | | | AFL2G_08764 | | NFIA_023290 | Afu1g01320 |
| PGX | exopolygalacturonase | GH 28 | | 1/81/2 | | | | | | |
| PGX | exopolygalacturonase | GH 28 | AN8891 | 191158 | ATEG_10357 | A0090010000753 | AFL2G_11892 | ACLA_043100 | NFIA_096340 | Afu8g02630 |
| PGX | exopolygalacturonase | GH 28 | AN8761 | 42184 | ATEG_07152 | A0090026000784 | AFL2G_06533 | | NFIA_049320 | Afu6g02980 |
| PGX | exopolygalacturonase | GH 28 | AN9045 | 1000000 | | | 1000000 | | | |
| KGX | exornamnogaiacturonase | GH 28 | | 172236 | | AU090001000133 | AFL2G_07371 | | | |
| RGX | exornamnogalacturonase | GH 28 | 414007 | 42917 | 1750 1005 | A0090009000470 | AFL2G_10228 | | NFIA_018590 | A101g06140 |
| RGX | exornamnogalacturonase | GH 28 | AN10274 | 194461 | ATEG_09025 | A0090102000139 | AFL2G_09582 | | NFIA_027700 | A10/g06410 |
| NGX | exornamnogalacturonase | GH 28 | | | ATEG_06408 | A0090113000199 | AFL2G_08671 | | | |
| KGX | exorhamnogalacturonase | GH 28 | | | | A0090138000066 | AFL2G_03102 | | | |
| KGX | exorhamnogalacturonase | GH 28 | AN11626 | | | | | | | |
| KGX | exorhamnogalacturonase | GH 28 | | | | A0090138000067 | AFL2G_08746 | | | |
| KHG | endorhamnogalacturonase | GH 28 | | 178393 | | | | | | |
| KHG | endorhamnogalacturonase | GH 28 | | 180922 | | A0090003000524 | AFL2G_02475 | | | Afu4g00100 |
| KHG | endorhamnogalacturonase | GH 28 | | | | A0090005000067 | AFL2G_00087 | | | |
| KHG | endorhamnogalacturonase | GH 28 | | 211163 | ATEG_07607 | A0090010000484 | AFL2G_11646 | | | |
| RHG | endorhamnogalacturonase | GH 28 | | 123651 | | A0090026000252 | AFL2G_06999 | | | |
| RHG | endorhamnogalacturonase | GH 28 | AN9134 | 189722 | | A0090038000552 | AFL2G_07883 | | | |
| RHG | endorhamnogalacturonase | GH 28 | | 39337 | | A0090124000009 | AFL2G_08037 | | | |
| RHG | endorhamnogalacturonase | GH 28 | | | | | | | NFIA_076680 | Afu5g10530 |
| XGH | xylogalacturonase | GH 28 | | | | A0090026000120 | AFL2G_07122 | | | |
| XGH | xylogalacturonase | GH 28 | AN3389 | 46065 | | A0090102000011 | AFL2G_09468 | | NFIA_099610 | Afu8g06890 |
| PGX | exopolygalacturonase | GH 28 | | | | | | | NFIA_100120 | Afu8g07265 |
| AFC | alpha fucosidase | GH 29 | | 44822 | ATEG_08111 | | | | | |
| AFC | alpha fucosidase | GH 29 | | | ATEG_05691 | | | | | |
| AGD | alpha glucosidase | GH 31 | AN2017 | 214233 | ATEG_00723 | A0090003001209 | AFL2G_01842 | ACLA_049370 | NFIA_105900 | Afu4g10150 |
| AGD | | 011.01 | AN0280 | 55419 | ATEG_02528 | A0090005000767 | AFL2G 00750 | ACLA_031260 | NFIA 021450 | Afu1g03140 |
| | alpha glucosidase | GH 31 | 1010100 | | | | | | | - |
| AGD | alpha glucosidase alpha glucosidase | GH 31 GH 31 | AN0941 | 119858 | ATEG 05177 | A0090005001084 | AFL2G 01038 | ACLA 019300 | NFIA 009180 | Afu1q16250 |
| AGD AGD | alpha glucosidase alpha glucosidase alpha glucosidase | GH 31 GH 31 GH 31 | AN0941 AN3504 | 119858 49940 | ATEG_05177 ATEG 02966 | A0090005001084 A0090023000288 | AFL2G_01038 AFL2G_04152 | ACLA_019300 | NFIA_009180 NFIA_018130 | Afu1g16250 Afu1g06560 |
| AGD AGD AGD | alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase | GH 31 GH 31 GH 31 GH 31 | AN0941 AN3504 AN10935 | 119858 49940 | ATEG_05177 ATEG_02966 | A0090005001084 A0090023000288 A0090026000111 | AFL2G_01038 AFL2G_04152 AFL2G_07131 | ACLA_019300 | NFIA_009180 NFIA_018130 | Afu1g16250 Afu1g06560 |
| AGD AGD AGD AGD | alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase | GH 31 GH 31 GH 31 GH 31 GH 31 GH 31 | AN0941 AN3504 AN10935 AN8953 | 119858 49940 | ATEG_05177 ATEG_02966 | A0090005001084 A0090023000288 A0090026000111 A0090038000471 | AFL2G_01038 AFL2G_04152 AFL2G_07131 AFL2G_07812 | ACLA_019300 | NFIA_009180 NFIA_018130 | Afu1g16250 Afu1g06560 |
| AGD AGD AGD AGD AGD | alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase alpha glucosidase | GH 31 GH 31 GH 31 GH 31 GH 31 GH 31 GH 31 | AN0941 AN3504 AN10935 AN8953 AN11054 | 119858 49940 50055 | ATEG_05177 ATEG_02966 | A0090005001084 A0090023000288 A0090026000111 A0090038000471 A0090102000559 | AFL2G_01038 AFL2G_04152 AFL2G_07131 AFL2G_07812 AFL2G_09934 | ACLA_019300 | NFIA_009180 NFIA_018130 | Afu1g16250 Afu1g06560 |

Supplemental Table 6A Cont: Detection of proteins in cultures grown on wheat bran sorted by CAZy family. (Continued on next page).

| AGD | alpha glucosidase | GH 31 | AN7120 | | | | | | | |
|---|---|---|---|--|--|--|---|--|---|--|
| AGD | alpha glucosidase | GH 31 | AN7345 | | | | | | | |
| AGD | alpha olucosidase | GH 31 | | | ATEG 08278 | | | | | |
| AGD | alpha glucosidase | OH 21 | | | ATEC 08472 | | | | | |
| AGD | alpha glucosidase | Gh 31 | | | ATEG_00472 | | | | | |
| AGD | alpha glucosidase | GH 31 | | | | | | | NFIA_060440 | |
| AXL | alpha xylosidase | GH 31 | AN7505 | | ATEG_06730 | AO090001000649 | AFL2G_09290 | ACLA_089300 | NFIA_082140 | Afu2g05400 |
| AXL | alpha xylosidase | GH 31 | | 40261 | | A0090701000558 | AFL2G 06180 | | | |
| AXL | alpha xylosidase | GH 31 | | 43342 | ATEG 08390 | A0090701000639 | AFL2G 06239 | | NFIA 032680 | |
| AXI | alpha vylogidaga | GH 31 | | | ATEG 00207 | | | | | |
| 110 | upitu Xylosiduse | 01101 | | | 1750 05400 | | | | | |
| AAL | aipna xyiosidase | Grist | | | ATEG_05169 | | | | | |
| INU | endo inulinase | GH 32 | | 52928 | | | | | | Afu5g00530 |
| INU | endo inulinase | GH 32 | | | | | | | | Afu5g00480 |
| INU | endo inulinase | GH 32 | | | ATEG 07356 | | | | | |
| INX | exo inulinase | GH 32 | | | - | | | | | |
| BIN | exe indinase | 011 02 | 4144770 | 50004 | ATEO 00455 | 4.0000704000400 | 45100 00000 | A CL A | NEW 000540 | 46-0-04040 |
| INA | exo inuinase | GR 32 | AN11770 | 20004 | ATEG_00155 | A0090701000400 | AFL2G_00020 | ACLA_094550 | INFIA_033540 | A102g01240 |
| SUC | invertase/beta fructofuranosidase | GH 32 | AN3837 | | ATEG_05860 | A0090701000038 | AFL2G_05693 | | NFIA_051560 | Afu6g05000 |
| SUC | invertase/beta fructofuranosidase | GH 32 | | 198063 | ATEG_07479 | A0090020000640 | AFL2G_10707 | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | | | A0090103000043 | AFL2G 12317 | | | |
| SUC | invertase/beta fructo/furanosidase | GH 32 | | 176039 | | | | | | |
| SUC | invertexe/hets fructe/uraneoidase | 011 02 | | | ATEC 04006 | | | | | |
| 300 | Inventase/beta inuctorunanosidase | Gh 32 | | | ATEG_04990 | | | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | | ATEG_10253 | | | | | |
| LAC | beta galactosidase | GH 35 | AN0194 | | | | | | | |
| LAC | beta galactosidase | GH 35 | | 180727 | | | | | | |
| LAC | beta galactosidase | QH 35 | | | | 40090001000259 | AEL2G 07489 | | | |
| 140 | hele selecteridese | 04.25 | | 477424 | ATEO 07448 | 4 0 0 0 0 0 0 0 0 0 0 0 4 0 | AEL 20, 02004 | A CL A | NEIA 050040 | 44-0-00000 |
| LAC | beta galactosidase | GH 35 | | 177434 | ATEG_07440 | A0090003000042 | AFL2G_02901 | ACLA_000440 | INFIA_052510 | Aluoguocou |
| LAC | beta galactosidase | GH 35 | AN0756 | 51764 | ATEG_00616 | A0090012000445 | AFL2G_03352 | ACLA_021260 | NFIA_011250 | Atu1g14170 |
| LAC | beta galactosidase | GH 35 | | | | A0090012000746 | | | | |
| LAC | beta galactosidase | GH 35 | AN2804 | | ATEG 08255 | A0090023000076 | AFL2G 03977 | | NFIA 041490 | Afu5q00670 |
| LAC | beta galactosidase | GH 35 | AN0980 | 46429 | ATEG 05131 | A0090120000158 | AFI 2G 08182 | | NEIA 008690 | Afu1016700 |
| 140 | bata salastasidasa | 011.00 | | 44040 | | 1.000012000100 | A EL DO _00040 | | | A 6-0+00000 |
| LAC | beta galactosidase | GH 35 | | 41910 | | | AFL2G_03010 | | INFIA_000910 | Alusguusou |
| LAC | beta galactosidase | GH 35 | | | | A0090701000770 | | | | |
| LAC | beta galactosidase | GH 35 | | | | | | ACLA_018830 | | |
| LAC | beta galactosidase | GH 35 | | | | | AFL2G_06343 | | | |
| LAC | heta galactosidase | GH 35 | | | | | AFI 2G 12387 | | | |
| 401 | alaha galactooidago | 011 00 | AN0120 | 212726 | ATEC 07020 | 4.000001000684 | AEL20_12001 | ACLA 044620 | NEIA 004590 | A 6+2+01120 |
| AGE | alpha galactosidase | 011 30 | ANOTOO | 212730 | ATEG_07323 | A0050010000004 | AFL20_11024 | ACLA_044020 | NFIA_094500 | Aluoyo1130 |
| AGL | alpha galactosidase | GH 36 | AN8392 | | ATEG_07935 | A0090011000063 | AFL2G_04865 | ACLA_044560 | NFIA_094540 | A108001100 |
| AGL | alpha galactosidase | GH 36 | AN3874 | 190816 | ATEG_05379 | A0090023000743 | AFL2G_04555 | ACLA_047600 | NFIA_107920 | Afu4g08250 |
| AGL | alpha galactosidase | GH 36 | AN9035 | | ATEG 02312 | | | | | |
| ABE | alpha arabinofuranosidase | GH 43 | AN8472 | | | A0090012000356 | AFI 2G 03265 | | NEIA 032900 | Afu2000650 |
| ABE | alpha arabinofuranceidaea | GH 43 | AN7781 | | ATEC 08386 | 0000005000065 | AEL2G 00086 | | | |
| 405 | alpha arabinoforanoaldaac | 011 43 | 410500 | | AIL0_00000 | A0030003000003 | ATL20_00000 | | NEW 000750 | 4.6-0-04000 |
| ADF | aipna arabinoturanosidase | GH 43 | AN2533 | | | A0090701000636 | AFL2G_06400 | | NFIA_002750 | Atuagu1660 |
| ABN | endoarabinanase | GH 43 | | 128077 | ATEG_05780 | | | | NFIA_078690 | Afu5g08590 |
| ABN | endoarabinanase | GH 43 | | 182100 | ATEG_07817 | A0090026000804 | AFL2G_06513 | ACLA_072730 | NFIA_089980 | Afu2g14750 |
| ABN | endoarabinanase | GH 43 | AN3044 | 184195 | ATEG 01562 | A0090005001320 | AFL2G 01244 | ACLA 037080 | NFIA 068020 | Afu3q09140 |
| ABN | endoarabinanase | GH 43 | | | _ | | | ACLA 018180 | NEIA 008030 | Afu1o17320 |
| 101 | endourabilitatidad | 011 40 | 410050 | 40.4000 | 1750 04407 | | 451.00.04050 | A0LA_070400 | | A101911020 |
| ABN | endoarabinanase | GH 43 | AN6352 | 134398 | ATEG_01407 | A0090023000165 | AFL2G_04053 | ACLA_073460 | NFIA_089320 | A102g14150 |
| ABN* | endoarabinanase | GH 43 | AN8007 | 197735 | | A0090138000055 | AFL2G_08737 | ACLA_098980 | NFIA_047920 | Afu6g00770 |
| ABN | endoarabinanase | GH 43 | AN2534 | 203143 | ATEG_03520 | A0090701000481 | AFL2G_06106 | ACLA_042100 | NFIA_062660 | Afu3g14620 |
| ABN | endoarabinanase | GH 43 | | | | | | | | |
| ABN | endoarabinanaee | GH 43 | | | | 0.000005000064 | AEL20, 00085 | | | |
| ADN | endourdbindindide | 011 43 | | | ATEO 00000 | A00300000004 | AI 220_00000 | | | |
| ADN | endoarabinanase | GR 43 | | | ATEG_03000 | | | | | |
| ABN | endoarabinanase | GH 43 | | | ATEG_07787 | | | | | |
| BXL | beta xylosidase | GH 43 | AN1870 | 179682 | ATEG_06059 | A0090003000239 | AFL2G_02739 | ACLA_090090 | NFIA_081240 | Afu2g04480 |
| BXL | beta xylosidase | GH 43 | AN6751 | 174379 | ATEG 06306 | A0090005000476 | AFL2G 00456 | | NFIA 027350 | Afu7a06110 |
| BXI | heta yvinsidase | GH 43 | | | ATEG 05083 | 40090103000268 | AFI 2G 12122 | ACLA 087680 | NEIA 060490 | Afu6n14550 |
| DXL | beta xylosidase | 011 43 | 4140400 | | ATEC_03003 | A00000100000200 | ATL20_12122 | ACLA_007000 | NEIA 007400# | A 6-0-04740/047 |
| DAL | beta xylosidase | GR 43 | ANTUT99 | | ATEG_00095 | A0090005000696 | AFL2G_00009/ | UACLA_056100/ | UNFIA_097490/ | 0: Aluog04/10/04/ |
| BXL | beta xylosidase | GH 43 | AN10919 | | ATEG_10072 | A0090010000029 | AFL2G_11296 | | NFIA_093350 | |
| BXL | beta xylosidase | GH 43 | | | ATEG_07784 | A0090010000494 | AFL2G_11656 | | NFIA_023360 | Afu1g01230 |
| BXL | beta xylosidase | GH 43 | AN7313 | 122978 | ATEG 01188 | A0090010000562 | AFL2G 11714 | ACLA 043260 | NFIA 096240 | Afu8a02510 |
| BXI | heta vylosidase | GH 43 | AN2664 | | ATEG 06107 | 40090012000350 | AEL2G_03257 | - | NEIA 072780 | Afu5o14190 |
| BYI | hata vulneidaea | 04.43 | AN8477 | | ATEC 04202 | | AEL20 00074 | ACLA 049400 | | |
| DAL | beta Xylosidase | Gn 43 | ANOTI | | ATEG_01292 | | AFL20_00974 | ACLA_010100 | | |
| BXL | beta xylosidase | GH 43 | | | | AU090113000059 | | | | |
| BXL | beta xylosidase | GH 43 | | | ATEG_10193 | | | | | |
| BXL | beta xylosidase | GH 43 | AN7275 | | ATEG_06643 | A0090701000886 | AFL2G_06448 | ACLA_078600 | NFIA_033220 | Afu2g00930 |
| BXI | beta vylosidase | GH 43 | AN7864 | 47677 | ATEG 01292 | A0090102000331 | AEL2G_09750 | ACLA 072000 | NEIA 088370 | Afu2n13190 |
| BVI | heta vuloridare | OH 43 | | 41011 | ATEC 07647 | | | | | |
| DVI | beta solasidase | 01 43 | AN0000 | | A.CO_01317 | | | | | |
| BXL | Deta xylosidase | GH 43 | AN2633 | | | | | | | |
| BXL | beta xylosidase | GH 43 | AN1043 | | | | | | | |
| BXL | beta xylosidase | GH 43 | | | | | AFL2G_08528 | | | |
| ? | ? | GH 43 | AN1477 | | | | | | | |
| FOI | endoplucanase | OH 45 | AN6786 | | | | | | NEIA 028020 | A fu7c06740 |
| ADE | energial canase | 01 40 | AN4077 | 005.15 | ATEO ADAGE | | 451.00.07767 | A.01.A. 000011 | NEIA 015702 | A 5-4-000000 |
| ABF | aipria arabinoturanosidase | GH 51 | AN12// | 38549 | ATEG_00198 | | AFL2G_07796 | AULA_025610 | NFIA_015730 | Attr: 1009900 |
| ABF | alpha arabinofuranosidase | GH 51 | | 50979 | ATEG_03540 | | | | | |
| ABF | alpha arabinofuranosidase | GH 51 | | | | A0090020000712 | AFL2G_10643 | | | |
| ABF | alpha arabinofuranosidase | GH 51 | AN2541 | 206387 | ATEG 02882 | A0090124000023 | AFL2G 08019 | ACLA 099110 | | |
| ABE | alpha arabinofuranceidaee | CH 64 | AN9439 | 131804 | ATEG 07869 | A0090012000209 | AFI 2G 03217 | ACLA 074120 | NEIA 000440 | Afu2n15160 |
| 0.41 | and a grading to a USIUSSC | 011 51 | | 131091 | | | 11-20-03217 | | | , 102g10100 |
| GAL | and a sale stands of | | | | | | | | | |
| | endogalactanase | Gribb | | 407007 | ATEG_02927 | A0090001000492 | AFL2G 09135 | | NEIA 017780 | 0.511006010 |
| GAL | endogalactanase endogalactanase | GH 53 | AN5727 | 10/22/ | _ | | _ | | | Alurguositu |
| GAL GAL | endogalactanase endogalactanase endogalactanase | GH 53 GH 53 GH 53 | AN5727 | 10/22/ | | | AFL2G_07488 | | | Aldigoosito |
| GAL GAL ABF | endogalactanase endogalactanase endogalactanase aloha arabino furanosidase | GH 53 GH 53 GH 53 GH 54 | AN5727 AN1571 | 200605 | ATEG 07939 | A0090023000001 | AFL2G_07488 AFL2G_03901 | ACLA 066470 | NFIA 060630 | Afu6g14620 |
| GAL GAL ABF | endogalactanase endogalactanase endogalactanase alpha arabinofuranosidase celluidee oxidase | GH 53 GH 53 GH 53 GH 54 | AN5727 AN1571 | 200605 | ATEG_07939 | AO090023000001 | AFL2G_07488 AFL2G_03901 | ACLA_066470 | NFIA_060630 | Afu6g14620 |
| GAL GAL ABF CO | endogalactanase endogalactanase alpha arabinofuranosidase cellulose oxidase | GH 53 GH 53 GH 53 GH 54 GH 61 | AN5727 AN1571 AN10419 | 200605 | ATEG_07939 | AO090023000001 | AFL2G_07488 AFL2G_03901 | ACLA_066470 | NFIA_060630 | Afu6g14620 |
| GAL GAL ABF CO CO | endogalactanase endogalactanase alpha arabino furanosidase cellulose oxidase cellulose oxidase | GH 53 GH 53 GH 53 GH 54 GH 61 GH 61 | AN5727 AN1571 AN10419 | 200605 | ATEG_07939 ATEG_04210 | A0090023000001 | AFL2G_07488 AFL2G_03901 | ACLA_066470 ACLA_060890 | NFIA_060630 | Afu6g14620 Afu3g03870 |
| GAL GAL ABF CO CO CO | endogalactanase endogalactanase alpha arabinofuranosidase cellulose oxidase cellulose oxidase | GH 53 GH 53 GH 53 GH 54 GH 61 GH 61 GH 61 | AN5727 AN1571 AN10419 AN1041 | 200605 211595 52688 | ATEG_07939 ATEG_04210 ATEG_00448 | A0090023000001 | AFL2G_07488 AFL2G_03901 AFL2G_07454 | ACLA_066470 ACLA_060890 ACLA_022980 | NFIA_060630 NFIA_006140 NFIA_012990 | Afu6g14620 Afu3g03870 Afu1g12560 |
| GAL GAL ABF CO CO CO CO | endogalactanase endogalactanase alpha arabinofuranosidase cellulose oxidase cellulose oxidase cellulose oxidase | GH 53 GH 53 GH 53 GH 54 GH 61 GH 61 GH 61 GH 61 GH 61 | AN5727 AN1571 AN10419 AN1041 AN1602 | 200605 211595 52688 182430 | ATEG_07939 ATEG_04210 ATEG_00448 ATEG_07790 | A0090023000001 A0090001000221 A0090005000531 | AFL2G_07488 AFL2G_03901 AFL2G_07454 AFL2G_00532 | ACLA_066470 ACLA_060890 ACLA_022980 ACLA_059790 | NFIA_060630 NFIA_006140 NFIA_012990 NFIA_099510 | Afu6g14620 Afu3g03870 Afu1g12560 Afu8g06830 |
| GAL GAL ABF CO CO CO CO CO | endogalactanase endogalactanase endogalactanase alpha arabinofuranosidase cellulose cxidase cellulose cxidase cellulose cxidase cellulose cxidase cellulose cxidase | GH 53 GH 53 GH 53 GH 54 GH 61 GH 61 GH 61 GH 61 | AN5727 AN1571 AN10419 AN1041 AN1602 AN9524 | 200605 211595 52688 182430 53797 | ATEG_07939 ATEG_04210 ATEG_00448 ATEG_07790 ATEG_07920 | AO090023000001 AO090001000221 AO090005000531 | AFL2G_07488 AFL2G_03901 AFL2G_07454 AFL2G_00532 AFL2G_03026 | ACLA_066470 ACLA_060890 ACLA_022980 ACLA_059790 | NFIA_060630 NFIA_006140 NFIA_012990 NFIA_099510 NFIA_044390 | Afu6g14620 Afu3g03870 Afu1g12560 Afu8g06830 |
| GAL GAL ABF CO CO CO CO CO CO | endogalactanase endogalactanase endogalactanase alpha arabinofuranosidase cellulose oxidase cellulose oxidase cellulose oxidase cellulose oxidase cellulose oxidase | GH 53 GH 53 GH 53 GH 54 GH 61 GH 61 GH 61 GH 61 | AN5727 AN1571 AN10419 AN1041 AN1602 AN9524 AN2388 | 200605 211595 52688 182430 53797 | ATEG_07939 ATEG_04210 ATEG_00448 ATEG_07790 ATEG_07920 | A0090023000001 A0090001000221 A0090005000531 A0090012000090 | AFL2G_07488 AFL2G_03901 AFL2G_07454 AFL2G_00532 AFL2G_03026 | ACLA_066470 ACLA_060890 ACLA_022980 ACLA_059790 | NFIA_060630 NFIA_006140 NFIA_012990 NFIA_099510 NFIA_044390 | Afu6g14620 Afu3g03870 Afu1g12560 Afu8g06830 |

Supplemental Table 6A Cont: Detection of proteins in cultures grown on wheat bran sorted by CAZy family.

| 00 | cellulose oxidase | GH 61 | AN3046 | 43784 | ATEG 08113 | A0090023000159 | AEL2G 04048 | ACLA 055060 | NEIA 089730 | Afi(2n14540 |
|--------|--|---------|---------------|--------|-------------|---|----------------|---------------|---------------|----------------|
| 00 | cellulose oxidase | OH 61 | 74113040 | 40704 | ATEC 05446 | A0000023000133 | AFL20_04040 | ACLA_033000 | NEIA 408220 | A102914040 |
| 00 | celulose oxidase | GH 61 | | | ATEG_05416 | A0090023000787 | AFL2G_04596 | ACLA_047220 | NFIA_108320 | Atu4g07850 |
| CO | cellulose oxidase | GH 61 | AN6428 | | ATEG_06077 | | | | | |
| CO | cellulose oxidase | GH 61 | | 56338 | ATEG 05081 | A0090103000087 | AFL2G 12282 | | NFIA 055220 | Afu6q09540 |
| 00 | cellulose ovidase | GH 61 | | | ATEG 10194 | | - | | | |
| 00 | | 01101 | 417004 | 404705 | AILO_IVIO4 | 4.0000439000004 | A EL 20. 09000 | | | |
| 0 | cellulose oxidase | Gnoi | ANTOST | 194/00 | | A0090136000004 | AFL2G_00099 | | | |
| CO | cellulose oxidase | GH 61 | AN3860 | | | | | | | |
| CO | cellulose oxidase | GH 61 | | | ATEG 08942 | | | | | |
| 00 | cellulose oxidase | GH 61 | | | ATEC 01456 | | | | | |
| 00 | | 01101 | | | AILO_01400 | | | | | |
| co | cellulose oxidase | GH 61 | | | | | | ACLA_01/4/0 | | |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | | | ATEG_00186 | AO090103000088 | AFL2G_12281 | ACLA_071560 | NFIA_087900 | Afu2g12770 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | AN2632 | 55136 | ATEG 10071 | A0090701000885 | AFL2G 06447 | ACLA 061510 | NFIA 033210 | Afu2a00920 |
| AXH | arabinovylan arabinofuranobydrolaga | GH 62 | AN7908 | | | | | | | |
| | arabilioxylari arabilioraralionyarolase | 011 02 | AN1 300 | | 177.0 10070 | | | | | |
| AXH | arabinoxylan arabinoturanonydrolase | GH 62 | | | ATEG_10379 | | | | | |
| AGU | alpha glucuronidase | GH 67 | AN9286 | 56619 | ATEG_06085 | A0090026000127 | AFL2G_07114 | ACLA_017270 | NFIA_072510 | Afu5g14380 |
| AGU | alpha olucuronidase | GH 67 | | | ATEG 09975 | | | | | |
| YO CBH | winduces active cellobiobydrolase | CH 74 | AN1542 | | - | | | | NEIA 096000 | A fu8a02330 |
| XO CON | Xylogidean active cellobionydrolase | 01174 | AN1042 | | | | | | NT 04_000000 | Aldogozoo |
| XG EGL | xyloglucan active endoglucanase | GH 74 | AN5061 | 206333 | ATEG_04708 | | | ACLA_044310 | NFIA_094990 | Afu8g01490 |
| RHA | alpha rhamnosidase | GH 78 | AN8465 | 40264 | | | AFL2G_05364 | | | |
| RHA | alpha rhamnosidase | GH 78 | | | | | | | | |
| DHA | alpha rhampoeidaea | CH 79 | AN2634 | 176718 | ATEC 05089 | 0000001000105 | AEL2G 07340 | | NEIA 004560 | A 6u3a02880 |
| RUA . | alpha malinosidase | 01170 | 7412031 | 170710 | AILO_03003 | A0030001000103 | AT 220_07040 | | 141.042004300 | Alabyo2000 |
| RHA | alpha rhamnosidase | GH 78 | AN11954 | 51410 | | A0090003001016 | AFL2G_02014 | | | |
| RHA | alpha rhamnosidase | GH 78 | AN10277 | 170172 | | A0090003001291 | AFL2G_01780 | | NFIA_022970 | Afu1g01660 |
| RHA | alpha rhamposidase | GH 78 | | 42916 | ATEG 03018 | A0090009000471 | AEL2G 10227 | | NEIA 018620 | Afu1c06130 |
| DHA | alpha rhamposidasa | QH 79 | AN7151 | | ATEC 02022 | 0000010000561 | AEL 20 11712 | | MEIA 057020 | A fu6a12020 |
| DUA | alpha maililiosidae | 0170 | 410700 | | A.LO_02822 | A0000010000001 | ALC20_11/15 | | | A.a0912000 |
| кнА | aipna mamnosidase | GH 78 | AN3780 | | | | | | | |
| RHA | alpha rhamnosidase | GH 78 | | | | A0090103000432 | AFL2G_11972 | | | |
| RHA | alpha rhamnosidase | GH 78 | | | | A0090113000149/4 | AFL2G 08627 | | NFIA 057930 | Afu6g14610 |
| DHA | alpha rhampooidaga | ON 70 | AN6020 | 121600 | | 0.0000005001440 | | | | |
| BUA | aipira mattitiusidase | GH 78 | An0929 | 131068 | | A009000001416 | | | | |
| RHA | alpha rhamnosidase | GH 78 | | | | | | | NFIA_060560 | |
| RHA | alpha rhamnosidase | GH 78 | | | | | AFL2G_10644 | | | |
| RHA | alpha rhamonsidase | GH 78 | AN12368 | | | | | | | |
| DHA | alaba shamaajidaaa | 00.70 | | | | | A EL 20. 42022 | | | |
| кла | aipna mannosidase | GH / C | | | | | AFL2G_03939 | | | |
| RHA | alpha rhamnosidase | GH 78 | AN10867 | 44977 | ATEG_04706 | A0090012000058 | AFL2G_02993 | | NFIA_026130 | Afu7g05040 |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | | | | A0090005000324 | AFL2G_00324 | | NFIA_026210 | Afu7g05090 |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | AN3991 | 36414 | | 40090138000087 | AEL 2008766 | | NEIA 023340 | ∆fu1001250 |
| UOII | ansatarated galactarony nyarolase | 011 00 | ANUSSI | 30414 | | A003013000000 | AT 12000100 | | 14110-020040 | Aldigotzoo |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | AN11078 | | | | AFL2G_06465 | | | |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | | | | A0090701000907 | | | | |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | AN4629 | | | | | | | |
| ABY | experabinance | CH 93 | AN2060 | | ATEC 06045 | 0000003001017 | AEL2G 02013 | | MEIA 081320 | A fu2Q04570 |
| ADA | exualabiliariase | GH 33 | An2000 | | ATEG_00045 | A0050003001017 | AFE20_02013 | | NFIA_001320 | A102004570 |
| ABX | exoarabinanase | GH 93 | | | | A0090011000141 | AFL2G_04929 | | | |
| ABX | exoarabinanase | GH 93 | AN5231 | 49311 | ATEG_07909 | A0090012000101 | AFL2G_03035 | ACLA_008170 | NFIA_027720 | Afu7g06430 |
| ABX | exparabinanase | GH 93 | | | ATEG 08029 | | | | | |
| ARY | eventebienenen | 04.02 | | | ATEC 00804 | | | | | 46-6-10100 |
| ADA | exoarabiliariase | Gn 93 | | | ATEG_00691 | | | | INFIA_050000 | A100912120 |
| AFC | alpha fucosidase | GH 95 | AN8149 | 184037 | ATEG_01560 | A0090005000382 | AFL2G_00373 | | | |
| AFC | alpha fucosidase | GH 95 | | | | A0090005000512 | AFL2G_00515 | | | |
| AFC | alpha fucosidase | GH 95 | AN6673 | 53702 | ATEG 09768 | A009000900086 | AEL2G 10565 | ACLA 004070 | NEIA 040960 | Afu5c01190 |
| 450 | alpha facoaldad | 011 00 | AN40070 | 00102 | ATEO_00100 | A000000000000 | AT 220_10000 | AccA_conterto | NEW 004700 | A100g01100 |
| AFC | alpha tucosidase | GH 95 | AN10376 | | ATEG_04136 | | | | NFIA_064720 | Atu3g12590 |
| URH | unsaturated rhamnogalacturonyl hydrolas | GH 105 | AN3196 | 41703 | ATEG_07907 | A0090001000174 | AFL2G_07407 | ACLA_056060 | NFIA_030670 | Afu4g02860 |
| URH | unsaturated rhamnogalacturonyl hydrolas | GH 105 | | | | A0090003000153 | AFL2G 02808 | | | |
| URH | unsaturated rhamongalacturopyl hydrolas | GH 105 | AN10505 | | | A0090001000063 | AEL2G_07308 | ACLA 072870 | NEIA 089840 | Afu2n14630 |
| UDU | uncaterated shares as a start and budgeles | 011 405 | AN0202 | 44077 | ATEO 00000 | A 00000440000440 | A EL DO _00004 | ACLA 077040 | | , though to be |
| UKN | unsaturated maninogalacturonyi nyorolas | GH 105 | AN9303 | 410// | ATEG_02092 | A0090113000146 | AFL2G_00024 | ACLA_0//810 | | |
| URH | unsaturated rhamnogalacturonyl hydrolas | GH 105 | AN7828 | | | | | | | |
| AGU | alpha glucuronidase | GH115 | | | | A0090005001415 | AFL2G_01323 | | | |
| AGU | alpha glucuronidase | GH115 | AN9329 | | ATEG 09974 | 4009001000038 | AEL2G 11304 | ACLA 006360 | NEIA 025630 | Afu7c04680 |
| 100 | alpha glocaronidado | 011440 | 100020 | | | 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | A EL DO 07400 | 100100000 | In Corcoro | riargolooo |
| AGU | aipina giucuronidase | Gn115 | | | | A0090001000267 | ArL2G_07498 | | | |
| AGU | aipna glucuronidase | GH115 | | | | A0090113000058 | | | | |
| AGU | alpha glucuronidase | GH 115 | | | ATEG_04355 | | | | | |
| PFI | pectin Mase | PI 1 | | | ATEG 00950 | A0090003001295 | AFI 2G 01776 | | | |
| DEI | nectin lyage | DL 4 | | 65040 | | 0000010000000 | AEL20 44207 | | NEIA 000110 | A fu7e05020 |
| T LL | pocar yase | PL 1 | | 00212 | | A0030010000030 | ALC20_11297 | | NI PA_020110 | Alarguouou |
| PEL | pectin lyase | PL 1 | | 45821 | | | | | | |
| PEL | pectin lyase | PL 1 | AN4882 | 40837 | ATEG_7577 | A0090012000121 | AFL2G_03052 | | NFIA_077100 | Afu5g10170 |
| PEL | pectin lyase | PL 1 | | | | A0090012000451 | AFL2G 03359 | | | |
| PEI | pectin lyase | PI 4 | | | | A0090103000463 | AEI 2G 11949 | | | |
| 001 | position by an and a second seco | PL 1 | 4140447 | 209700 | | 40000438000304 | AELOO 088900 | | | |
| FEL | pecul lyase | PL 1 | AN10147 | 208760 | | AU090138000204 | AFL2G_08823 | | | |
| PEL | pectin lyase | PL 1 | | | | | | ACLA_094210 | NFIA_033080 | Afu2g00800 |
| PEL | pectin lyase | PL 1 | AN2331 | 41815 | | A0090010000504 | AFL2G 11666 | ACLA_013470 | NFIA_076850 | Afu5g10380 |
| PEL | pectin Mase | pi 1 | AN2569 | | ATEG 01216 | A009001000087 | AEL2G 11352 | | | - |
| DEL | postin lyana | 01.1 | | | | | 1.1.220_11002 | | NEIA 000400 | |
| -EL | pectal lyase | PL 1 | | | | | | | NFIA_023100 | |
| PEL | pectin lyase | PL 1 | AN9439 | | | | | | | |
| PLY | pectate lyase | PL 1 | AN0741 | | ATEG_08834 | A0090011000673 | AFL2G_05417 | | | |
| PLY | pectate lyase | PI 1 | AN5333 | | ATEG 05467 | A0090102000072 | AFL2G 09523 | | NEIA 060270 | Afu6q14400 |
| DIV | nostate hann | Di A | ANTEAC | 45004 | ATEC 08400 | 00000701000304 | AEL20 05054 | | NEIA 022040 | A 512x00760 |
| FLT. | peciale lyase | PL 1 | MN/040 | 45021 | ATEG_08123 | A0090701000321 | AFL26_05954 | | NFIA_033040 | ~iu2g00760 |
| PLY | pectate lyase | PL 1 | AN9367/AN9368 | 5 | | A0090011000030 | AFL2G_04835 | | | |
| PLY | pectate lyase | PL 3 | AN6748 | | ATEG_06314 | A0090005000472 | AFL2G_00461 | | NFIA_027690 | Afu7g06400 |
| PLY | pectate lyase | PI 9 | AN3337 | | ATEG 06285 | | | | | - |
| | postate lyade | 11L 0 | ANDE40 | | | 40000010000700 | AEL20 44072 | ACLA 050010 | | 46.9-05040 |
| PLY | peciale lyase | PL 3 | ANZ54Z | | | A0090010000706 | AFL2G_11846 | AULA_059210 | INFIA_098670 | A108005910 |
| PLY | pectate lyase | PL 3 | AN6106 | | ATEG_08626 | A0090038000502 | AFL2G_07839 | | NFIA_023470 | Afu1g01120 |
| PLY | pectate lyase | PL 3 | AN8453 | | | | | | | |
| RGI | rhamnonalacturonan lvase | PL / | AN7135 | 210947 | ATEG 02193 | A0090011000349 | AFI 2G 05136 | ACLA 054660 | NFIA 029620 | Afu4003780 |
| DOL | shampagalagturanga huang | PL 4 | ANG205 | 47700 | ATEC 10207 | 40000012000147 | AEL20 02070 | ACLA 019000 | NEIA 002140 | A fu1e17020 |
| RUL | mannogalacturonan lyäse | PL 4 | ww03a2 | 4//80 | ATEG_10327 | AU090012000147/A | AFL2G_030/5 | AULA_018320 | NFIA_008140 | Atung17230 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN12097 | | | A0090113000057 | | | | |
| RGL | rhamnogalacturonan lvase | PL 4 | | | ATEG 08610 | A0090138000119 | AFL2G 08794 | | NFIA 094270 | Afu8q00820 |
| RGI | rhampogalacturonan lyase | pi A | AN3950 | | | | | | | |
| DIX | nannogalactaronan iyaac | PL 4 | AN0537 | | | 4000002000401 | A EL DO | | NEW COORE | A 6-2+1 (200 |
| MLY | pectate lyase | PL 9 | AN2537 | | | A0090038000131 | AFL2G_08953 | | NFIA_062360 | Atu3g14890 |
| PLY | pectate lyase | PL 9 | | | ATEG_03526 | | | | | |
| RGI | rhampogalacturonan lyase | PI 11 | AN2543 | | | | | | | |
| | | | | | | | | | | |

Supplemental Table 6B: Detection of proteins in cultures grown on sugar beet pulp sorted by CAZy family. The colour codes indicate which percentage of the total number of detected peptides was of the specific protein. (Continued on next 4 pages).

| | | | 0-0.1% | 0.1-0.5% | 0.5-1% | 1-2% | 2-5% | 5-10% | >10% | |
|-------------|------------------------------------|--------------|-------------|-----------|------------|-----------------|---------------|----------------|--------------|---|
| | | | | | | _ | _ | | | |
| enzyme code | function | CAZY | A. nidulans | A. niger | A. terreus | A. oryzae | A. flavus | A. clavatus | A. fischeri | A. fumigatus |
| | | | | ATCC 1015 | | | | | | Af293 |
| FAE SF7 | feruloyl esterase | faeA | x | 51662 | ATEG_08907 | A0090001000207 | AFL2G_07436 | | | |
| FAE SF1 | feruloyl esterase | SF1 | AN1772 | 51478 | ATEG_06663 | A0090001000582 | AFL2G_09228 | ACLA_083360 | NFIA_054700 | A fu6g09040 |
| FAE SF1 | teruloyi esterase | SF1 | | | ATEG_02212 | A0090001000066 | AFL2G_07310 | | NFIA_047590 | Atu6g00450 |
| FAE SF3 | feruloyi esterase | 553 | | | ATEC 02445 | A0090102000013 | | | | |
| FAE SEA | ferulayi esterase | SEA | | 100471 | A100_02415 | 40090010000573 | AEL20 11725 | | | |
| FAE SE4 | ferulovi esterase | SEA | | 43194 | | A0050010000373 | Art20_11/25 | | | |
| AXE | acetyl xylan esterase | CE 1 | AN6093 | 211544 | ATEG 09843 | A0090011000745 | AEL2G 05471 | ACLA 081220 | NEIA 099230 | Afu8006570 |
| AXE | acetyl xylan esterase | CE 1 | AN8320 | | | | | | | Afu1o17510 |
| AXE | acetyl xylan esterase | CE 1 | | | | | | | NFIA_084920 | - |
| FAE SF5 | feruloyl esterase | CE 1 | | | | A0090005000945 | AFL2G_00922 | ACLA_065130 | NFIA_115130 | |
| FAE SF5 | feruloyl esterase | CE 1 | AN5267 | 43785 | ATEG_08112 | A0090023000158 | AFL2G_04047 | ACLA_055050 | NFIA_089720 | Afu2G14530 |
| FAE SF5 | feruloyl esterase | CE 1 | | | ATEG_06644 | A0090701000884 | AFL2G_06446 | ACLA_061520 | | |
| FAE SF5 | feruloyl esterase | CE 1 | | | | | | | | Afu2g09440 |
| FAE SF5 | feruloyl esterase | CE 1 | | | ATEG_01914 | | | | | |
| FAE SF5 | feruloyl esterase | CE 1 | | | | | | ACLA_017480 | | |
| ? | a settle mothed and settlements | CE 1 | AN8782 | 53315 | ATEG_06438 | A0090005000277 | AFL2G_00281 | ACLA_012870 | NFIA_077450 | A105g09860 |
| PME | pectin methyl esterase | CEO | | 44505 | | A0090003001514 | AFL2G_01576 | ACLA 044240 | NEIA 005020 | A fu?=01520 |
| DME | pectin methyl esterase | CER | ANAREO | 44303 | ATEC 01704 | A0090012000149 | AFL20_00016 | ACLA_044240 | NEIA 0695020 | Afu2x07650 |
| PME | nectin methyl esterase | CE 8 | AN3390 | 214857 | | A0090102000010 | AEL2G 09467 | A00A_000010 | NEIA 099600 | Afu8e06880 |
| PME | pectin methyl esterase | CE 8 | AN7966 | | | | | | | |
| PME | pectin methyl esterase | CE 8 | 8 | | | A0090113000039 | | | NFIA_100100 | Afu8g07250 |
| PME | pectin methyl esterase | CE 8 | 8 | | | | | ACLA_059970 | - | |
| PME | pectin methyl esterase | CE 8 | 8 | | | | AFL2G_08528 | | | |
| PME | pectin methyl esterase | CE 8 | 3 | | | | | ACLA_059980 | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | 2 | | | A0090003001268 | AFL2G_01797 | | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | | | | | | | NFIA_099110 | Afu8g06480 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | | 51400 | ATEG_10016 | A0090102000092 | AFL2G_09543 | | | |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | AN2834 | 100051 | | A0090113000155 | AFL2G_08631 | | NFIA_092640 | Afu2g17250 |
| RGAE | mamnogalacturonan acetyl esterase | CE 12 | AN2528 | 189254 | ATEG_03511 | A0090701000556 | AFL2G_06177 | ALCL_041970 | NFIA_062750 | Afu3g14510 |
| BOI | beta ducosidase | CH 1 | | 140573 | A120_00845 | | | ACCA_007320 | NT IM_000200 | Aldog14550 |
| BGL | beta glucosidase | GH 1 | AN10124 | 213437 | ATEG 00687 | A0090003000497 | AFI 2G 02496 | ACLA 020660 | NEIA 010690 | Afu1o14710 |
| BGL | beta glucosidase | GH 1 | | 210101 | / | , | / | ACLA 019180 | NFIA 009040 | Afu1o16400 |
| BGL | beta glucosidase | GH 1 | | | | A0090113000148 | AFL2G 08626 | | NFIA 060550 | A fu6q14600 |
| BGL | beta glucosidase | GH 1 | AN9183 | 131747 | ATEG_02657 | A0090120000075 | AFL2G_08111 | | NFIA_099670 | Afu8g06970 |
| BGL | beta glucosidase | GH 1 | AN10375 | | ATEG_04135 | | | | NFIA_064710 | Afu3g12600 |
| BGL | beta glucosidase | GH 1 | | | | | | ACLA_064280 | | |
| BGL | beta glucosidase | GH 1 | | | | | | ACLA_040420 | | |
| GUS | beta glucuronidase | GH 2 | 2 AN3200 | 189620 | ATEG_09745 | | AFL2G_05262 | | | |
| GUS | beta glucuronidase | GH 2 | AN2395 | 52111 | ATEG_01031 | A0090023000053 | AFL2G_03956 | | NFIA_089700 | Afu2g14520 |
| LAC | beta galactosidase | GH 2 | | | ATEG_10255 | | | | | |
| LAC | beta galactosidase | GH 2 | AND462 | | ATEG_04784 | | | | | |
| GUS | beta glucuronidase | GH 2 | AN5361 | 46827 | AIL0_00/12 | 0000038000000 | AEL 20, 08828 | | | |
| LAC | beta galactosidase | GH 2 | AN3201 | 10021 | ATEG 10243 | A0090012000389 | AFL2G_03296 | | NEIA 072410 | Afu5o14550 |
| LAC | beta galactosidase | GH 2 | AN6388 | | | | | | | , |
| LAC | beta galactosidase | GH 2 | AN1107 | | | | | | NFIA 072890 | Afu5q14090 |
| MND | beta mannosidase | GH 2 | AN1742 | 138876 | ATEG_06636 | A0090001000556 | AFL2G_09201 | ACLA_083570 | NFIA_054490 | Afu6g08840 |
| MND | beta mannosidase | GH 2 | AN11680 | 172587 | ATEG_07339 | A0090003001410 | AFL2G_01666 | ACLA_078510 | NFIA_045250 | Afu4g00390 |
| MND | beta mannosidase | GH 2 | 2 | | | A0090005000740 | AFL2G_00728 | | | |
| MND | beta mannosidase | GH 2 | 2 AN3368 | 212893 | ATEG_08684 | A0090010000208 | AFL2G_11464 | ACLA_066240 | NFIA_114030 | Afu7g01320 |
| MND | beta mannosidase | GH 2 | 2 | | ATEG_09890 | | | | | |
| BGL | beta giucosidase | GH 3 | 5 | 400007 | AIEG_10274 | | | | | |
| BOL | beta glucosidase | GH 3 | | 139037 | | | | | | |
| BGL | beta glucosidase | GH 3 OF 2 | | 20642 | | | | | | |
| BGL | beta glucosidase | GH 3 | AN7915 | 38077 | | A0090001000266 | AFI 2G 07497 | | | |
| BGI | beta glucosidase | GH 3 | AN10482 | 208871 | ATEG 06617 | A0090001000544 | AFL2G_01437 | ACI A 083710 | NEIA 054350 | Afu6n08700 |
| BGL | beta glucosidase | GH 3 | | 200011 | | | 10220_00101 | 1000 (2000) 10 | in Conner | ridogeeree |
| BGL | beta glucosidase | GH 3 | 1 | 210981 | ATEG 02806 | A0090003001511 | ALF2G 01582 | | NFIA 095760 | Afu8q02100 |
| BGL | beta glucosidase | GH 3 | 8 | | ATEG_02724 | A0090005000337 | AFL2G_00334 | | | - |
| BGL | beta glucosidase | GH 3 | AN4102 | 56782 | ATEG_03047 | A0090009000356 | AFL2G_10322 | ACLA_028810 | NFIA_018950 | Afu1g05770 |
| BGL | beta glucosidase | GH 3 | AN6652 | 182309 | ATEG_07121 | A0090009000554 | AFL2G_10164 | ACLA_096980 | NFIA_050080 | A fu6g03570 |
| BGL | beta glucosidase | GH 3 | 1 | 44520 | | A0090010000034 | AFL2G_11300 | | | |
| BGL | beta glucosidase | GH 3 | | | | A0090012000003 | AFL2G_02949 | | | |
| BGL | peta glucosidase | GH 3 | AN7396 | 179265 | ATEG_10320 | A0090012000135 | AFL2G_03066 | | NFIA_007920 | Afu1g17410 |
| BGL | beta glucosidase | GH 3 | AN1804 | | | A0090026000123 | AFL2G_07119 | | | |
| BOL | beta glucosidase | GH 3 | ANE076 | 404040 | ATEC 00740 | A0090038000223 | AFL2G_09023 | | NEIA 100/00 | |
| BOL | beta glucosidase | GH 3 CH 3 | AN0070 | 101016 | AIE0_02/13 | A0090030000425 | AFL20_07763 | | NTIM_100430 | |
| BGI | beta glucosidase | OH 3 | | 176604 | | A00901660000127 | AFL20_12240 | | NEIA 112660 | |
| BGL | beta glucosidase | GH 3 | AN3903 | 110001 | ATEG 04069 | A0090166000040 | AFL2G 09413 | ACLA 087610 | NEIA 060370 | Afu6q14490 |
| BGL | beta glucosidase | GH 3 | AN2227 | 129891 | ATEG 09329 | A0090701000244 | AFL2G 05886 | ACLA 010450 | NFIA 080070 | Afu5q07190 |
| BGL | beta glucosidase | GH 3 | 5 | | ATEG_07931 | | | | | |
| BGL | beta glucosidase | GH 3 | AN0712 | | | | | | | |
| BGL | beta glucosidase | GH 3 | AN2612 | | | | | | | Afu7g00240 |

Supplemental Table 6B Cont: Detection of proteins in cultures grown on sugar beet pulp sorted by CAZy family. (Continued on next 3 pages).

| BOI | hata ducceidaea | CH 3 | AN2828 | | ATEC 07419 | 00000701000841 | AEL2G 06408 | ACLA 007810 | NEIA 027300 | A fu7o06140 |
|---------|--|-------|----------|--------|-------------|---|--------------|--------------|--------------|--------------|
| BOL | beta glucosidase | OH 3 | AN2020 | | A1EG_07415 | A0050701000041 | AFL20_00400 | ACLA_007810 | NFIA_027380 | Alurgooliko |
| BOL | beta glucosidase | 01 3 | AN3045 | | | | | | | 44-0-44040 |
| DGL | beta glucosidase | GH 3 | ANTODO | 100770 | 1750 00457 | | | | INFIA_057590 | Aluogitisto |
| BGL | beta giucosidase | GH 3 | 5 | 129779 | ATEG_00157 | | | | 1000750 | 4.6-0-00000 |
| BGL | beta glucosidase | GH 3 | | | | | | | NFIA_000750 | Atu3g00230 |
| BGL | beta glucosidase | GH 3 | 5 | | | | | | NFIA_098520 | |
| BGL | beta giucosidase | GH 3 | 5 | | | | | | NFIA_057910 | |
| BGL/BXL | beta glucosidase/beta xylosidase | GH 3 | 3 AN0479 | | | | | | | |
| BXL | beta xylosidase | GH 3 | AN2359 | 205670 | ATEG_05106 | A0090005000986 | AFL2G_00957 | | | Afu1g16920 |
| BXL | beta xylosidase | GH 3 | 3 | | ATEG_07383 | A0090011000140 | AFL2G_04928 | | | |
| BXL | beta xylosidase | GH 3 | 3 | | | | | ACLA_018590 | | |
| BXL/ABF | beta xylosidase/alpha arabinofuranosidas | GH 3 | 3 AN8401 | | ATEG_09052 | AO090103000120 | AFL2G_12252 | ACLA_062400 | NFIA_003180 | Afu3g02090 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosida: | GH 3 | AN2217 | 50997 | ATEG_09314 | A0090701000274 | AFL2G_05912 | ACLA_010340 | NFIA_080180 | Afu5g07080 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosidas | GH 3 | 3 | | ATEG_08027 | | | | | |
| ? | | GH 3 | 3 AN1416 | 45461 | ATEG_00018 | A0090103000019 | AFL2G_12338 | ACLA_057390 | NFIA_096770 | Afu8g04060 |
| ? | | GH 3 | 3 | | | | | | | Afu1g00540 |
| ? | | GH 3 | AN3360 | 37673 | ATEG_04963 | | | ACLA_052760 | NFIA_102600 | Afu4g13770 |
| ? | | GH 3 | 3 | 120104 | ATEG_04729 | A0090003000741 | AFL2G_02272 | ACLA_039660 | NFIA_065550 | Afu3g11780 |
| ? | | GH 3 | 3 | | | | AFL2G_08686 | | | |
| ? | | GH 3 | 3 AN2599 | | | | | | | |
| EGL | endoglucanase | GH 5 | 5 AN3013 | 210716 | ATEG_01592 | AO090005001389 | AFL2G_01298 | ACLA_036770 | NFIA_068300 | Afu3g08820 |
| EGL | endoglucanase | GH 5 | AN1285 | 205580 | ATEG_05002 | | | ACLA_085250 | NFIA_057290 | Afu6g11600 |
| EGL | endoglucanase | GH 5 | 5 | | | | | | NFIA_095570 | |
| EGL | endoglucanase | GH 5 | AN8068 | | ATEG_09802 | AO090003001341+ | AFL2G_01726 | | NFIA_040280 | Afu5g01830 |
| EGL | endoglucanase | GH 5 | 5 AN5214 | 209376 | | AO090005001553 | AFL2G_01447 | ACLA_081650 | NFIA_053150 | Afu6g07480 |
| EGL | endoglucanase | GH 5 | 5 | 214608 | ATEG_04390 | AO090011000715 | AFL2G_05447 | ACLA_081310 | NFIA_085010 | Afu2g09520 |
| EGL | endoglucanase | GH 5 | 5 | | ATEG 05003 | | | | | |
| GLN | exo 1,6 galactanase | GH 5 | 5 AN9166 | 194447 | ATEG_10242 | A0090012000046 | AFL2G_02982 | | NFIA_072400 | Afu5g14560 |
| EXG | exo 1,3 galactanase | GH 5 | 5 | | ATEG_08371 | | | | NFIA_056040 | |
| EXG | exo 1,3 galactanase | GH 5 | AN8947 | 175759 | ATEG_07719 | | | | | |
| EXG | exo 1,3 galactanase | GH 5 | AN7533 | 123981 | ATEG 06686 | AO090001000604 | AFL2G 09249 | ACLA 083150 | NFIA 054930 | Afu6q09250 |
| EXG | exo 1,3 galactanase | GH 5 | AN4052 | 202490 | ATEG_03849 | A0090003000990 | AFL2G 02039 | ACLA_031040 | NFIA_021060 | Afu1g03600 |
| EXG | exo 1.3 galactanase | GH 5 | 5 | | ATEG 06369 | A0090005000423 | AFL2G 00412 | ACLA 007330 | NFIA 026860 | Afu7q05610 |
| EXG | exo 1.3 galactanase | GH 5 | 5 | | ATEG 03062 | A0090009000373 | AFL2G 10308 | | | |
| EXG | exo 1.3 galactanase | GH 5 | AN1332 | 52811 | _ | A0090012000917 | AEL2G_03782 | | NEIA 113230 | |
| FXG | beta 1.6 olucanase | GH 5 | AN3777 | | ATEG 09844 | A0090011000757 | AFI 2G 05484 | | NEIA 084850 | Afu2009350 |
| MAN | endomannanase | GH 5 | AN3358 | 50378 | ATEG 08654 | A0090010000122 | AFL2G 11381 | ACLA 066420 | NFIA 113780 | Afu7q01070 |
| MAN | endomannanase | GH 5 | 1 10000 | 00010 | ATEG 10292 | A0090012000006 | AEL2G_02951 | 100 2000 120 | NEIA 041960 | A fu5q00550 |
| MAN | endomannanase | GH 5 | AN7639 | | | A0090038000444 | AEL2G_07781 | ACLA 044470 | NEIA 099770 | Afu8a07030 |
| MAN | endomannanase | GH 5 | 1 | | ATEG 02669 | | 20100 | | 1110000110 | Alladgerebe |
| MAN | endomannanase | GH 5 | AN2709 | | ///20_02000 | | | | | |
| MAN | endomenananase | 0110 | AN2207 | | | | | | | |
| MAN | endomannandase | 0110 | ANE427 | | ATEC 00001 | | | | | |
| MAN | endomannanase | CH 5 | AN07276 | | A120_00001 | | | | | |
| MAN | endomannanase | On 5 | AN3270 | | ATEC 01274 | | | | | |
| MAIN | endomannanase | 010 | | | ATEG_01374 | | | | | |
| CDU | a a lla his hu das las a | GRO | ANC202 | 54400 | ATEG_030/7 | | | A CL A | NELA 002000 | 44-2-04040 |
| CDH | cellobiohydrolase | Grid | AN5262 | 54490 | ATEG_07493 | 4.0.00000000000000000000000000000000000 | 451.00 07770 | ACLA_002500 | NFIA_002990 | Alusgolialo |
| CDH | cellobionydrolase | Gnic | AN1273 | 535500 | ATEG_00195 | A0090038000439 | AFL2G_07776 | ACLA_025560 | NFIA_013000 | 44-0-44040 |
| CDN | cellobiohydrolase | GH / | ANU494 | 51//3 | ATEG_05002 | A0090001000346 | AFL2G_07571 | AGLA_005260 | NFIA_057300 | Atuogiitoitu |
| CON | cellobiohydrolase | GH / | | 50450 | | | | | NFIA_095570 | |
| CBH | cellobionydrolase | GH / | AN5176 | 53159 | ATEG_03727 | A0090012000941 | AFL2G_03805 | ACLA_088870 | NFIA_052720 | A106g07070 |
| EGL | endogiucanase | GH / | AN3418 | | ATEG_08700 | A0090010000314 | ALF2G_11497 | ACLA_066030 | NFIA_114250 | Atu7g01540 |
| EGL | endoglucanase | GH / | | | ATEG_08705 | | | ACLA_098940 | NFIA_047960 | Afu6g01800 |
| XLN | endoxylanase | GH 10 | | | | A0090001000208 | AFL2G_07437 | | | |
| XLN | endoxylanase | GH 10 | AN7401 | | ATEG_03410 | A0090103000326 | AFL2G_12071 | ACLA_086910 | NFIA_059570 | Afu6g13610 |
| XLN | endoxylanase | GH 10 | ANT818 | 57436 | ATEG_00809 | A0090701000887 | AFL2G_06449 | ACLA_048770 | NEIA_106540 | A fu4g09480 |
| ALN | endoxylanase | GH 10 | | | ATEG_08906 | A0090103000423 | AFL2G_11983 | | | |
| XLN | endoxylanase | GH 10 | AN2356 | 50977 | | | | | NFIA_057510 | Afu6g11820 |
| XLN | endoxyianase | GH 10 | | - | ATEG_07190 | | | | NFIA_061880 | Afu3g15210 |
| XLN | encoxylanase | GH 11 | AN3613 | | | | | | | |
| ALN | endoxylanase | GH 11 | | 101000 | 1.000 0.00 | | - | | | |
| ALN | endoxylanase | GH 11 | | 171269 | AIEG_04943 | A0090001000111 | AFL2G_07347 | ACLA_085410 | NFIA_058160 | A106012210 |
| XLN | endoxyianase | GH 11 | | 183088 | | AU090026000103 | AFL2G_07138 | | NFIA_055240 | |
| XLN | encoxylanase | GH 11 | | | | A0090103000141 | AFL2G_12233 | | | |
| ALN | endoxylanase | GH 11 | AN9365 | 52071 | ATEG_07461 | A0090120000026 | AFL2G_08066 | ACLA_063140 | NEIA_000850 | A103g00320 |
| XLN | endoxylanase | GH 11 | | | | | | | NFIA_001010 | Atu3g00470 |
| XLN | endoxylanase | GH 11 | | | | | | ACLA_064270 | | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | AN0452 | 52011 | ATEG_03755 | A0090003000905 | AFL2G_02120 | ACLA_029940 | NFIA_020020 | Atu1g04730 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | 211053 | ATEG_07420 | A0090026000102 | AFL2G_07140 | ACLA_007820 | NFIA_027400 | Afu7g06150 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | ATEG_05519 | | | | | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | ATEG_09894 | | | | | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | | A0090038000175 | AFL2G_08984 | | NFIA_002040 | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | | AO090701000185 | AFL2G_05831 | ACLA_060930 | NFIA_005810 | Afu3g03610 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | 191511 | | | | | NFIA_100330 | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | | | AFL2G_04893 | | | |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | | | | | | Afu3g01160 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | ATEG_07466 | | | | | |
| AGT | 4 alpha glucanotransferase | GH 13 | 3 | 57002 | | | | | | |
| ? | | GH 13 | 3 | 182162 | | | | | | |
| GBA | glycogen debranching enzyme | GH 13 | 8 AN2314 | 211162 | ATEG_07608 | A0090010000483 | AFL2G_11646 | ACLA_013550 | NFIA_076670 | A fu5g10540 |
| AGD | alpha glucosidase | GH 13 | 3 | 50927 | | | | | | |
| AGD | alpha glucosidase | GH 13 | AN4843 | 52452 | ATEG_01729 | A0090020000176 | AFL2G_11111 | ACLA_035550 | NFIA_069880 | Afu3g07380 |
| AGD | alpha glucosidase | GH 13 | 3 | | | A0090026000034 | AFL2G_07207 | | | |
| AGD | alpha glucosidase | GH 13 | 3 | | | A0090038000234 | AFL2G_09036 | ACLA_008120 | NFIA_027660 | Afu7g06380 |
| AGD | alpha glucosidase | GH 13 | AN10420 | | ATEG_01029 | A0090103000129 | AFL2G_12243 | ACLA_059930 | NFIA_099830 | Afu8g07070 |
| AGD | alpha glucosidase | GH 13 | 3 | | _ | AO090103000378 | AFL2G_12021 | _ | | |
| | | | | | | | | | | |

Supplemental Table 6B Cont: Detection of proteins in cultures grown on sugar beet pulp sorted by CAZy family. (Continued on next 2 pages).

| AGD | alpha olucosidase | GH 13 | | | | | AFI 2G 08694 | | | |
|-----|----------------------------|-------|---------|--------|-------------|------------------|---------------|-------------|--------------|--------------|
| AGD | alpha glucosidase | GH 13 | | | | | | ACLA 070570 | NFIA 086860 | Afu2q11620 |
| AGD | alpha glucosidase | GH 13 | | | | | | ACLA 070560 | NEIA 086850 | Afu2011610 |
| AGS | alpha ducan synthase | GH 13 | | 212915 | | | | ACCA_010300 | 1110-000000 | Allazyriolo |
| AGS | alpha glucan synthase | GH 13 | | 40979 | | | | | | |
| A03 | alpha glucan synthaso | GH 13 | AN2207 | 64070 | ATEC 02622 | 4.0000002001500 | AEL 20, 04602 | ACLA 062450 | NEIA 001720 | A \$12x00010 |
| A05 | alpha glucan synthase | OH 13 | AN3307 | 54370 | ATEC 10271 | A0090003001300 | AFL20_01395 | ACLA_003430 | NEIA_000060 | A103g00510 |
| A05 | alpha glucan synthase | 01113 | ANCOOL | 33204 | ATEG_10371 | A0050010000100 | AFL20_11303 | ACLA_042430 | NEIA_0005500 | Alu 1913440 |
| AGS | alpha glucan synthase | GH 13 | ANDOOD | | ATEG_01449 | A0090026000525 | AFL2G_00701 | ACLA_070240 | INFIA_000000 | Aluzgitizio |
| AMY | alpha amylase | GH 13 | | | | | | | | |
| AMY | alpha amylase | GH 13 | | | | | | | | |
| AMY | alpha amylase | GH 13 | | | | A0090120000196 | | ACLA_094070 | NFIA_032970 | Afu2g00710 |
| AMY | alpha amylase | GH 13 | AN3402 | 47911 | ATEG_10103 | A0090023000944 | | ACLA_052920 | | |
| AMY | alpha amylase | GH 13 | AN2018 | 45304 | ATEG_08279 | AO090003001210 | AFL2G_01841 | ACLA_049350 | NFIA_105920 | Afu4g10130 |
| AGT | 4 alpha glucanotransferase | GH 13 | AN3308 | 188489 | ATEG_03623 | AO090003001498 | AFL2G_01594 | ACLA_063440 | NFIA_001710 | Afu3g00900 |
| AMY | alpha amylase | GH 13 | | | | A0090120000263 | AFL2G_08276 | | | |
| AMY | alpha amylase | GH 13 | | | | | | | | |
| AMY | alpha amylase | GH 13 | AN4507 | | | | | | | |
| AMY | alpha amylase | GH 13 | AN3388 | | ATEG 02515 | | | | | |
| AMY | alpha amylase | GH 13 | AN6324 | | | | | ACLA 072270 | NEIA 088650 | Afu2n13460 |
| AMY | alpha amylaco | GH 13 | /110021 | 122069 | ATEG 03624 | 0000003001497 | AEL2G 01595 | ACCA_CTEETO | | ring for too |
| AMY | sinha amylaco | GH 13 | AN10060 | 46621 | ATEG 04879 | A0090005000884 | AFL2G_00860 | ACLA 032290 | NEIA 022510 | A fu1e02140 |
| AMY | alpha amylase | 01113 | AN2200 | 46200 | ATEC 00929 | A00000000000000 | Ar 220_00000 | ACLA_032250 | NEIA 010270 | Afu1g02140 |
| ANT | alpha amylase | GH 13 | AN3309 | 40290 | ATEG_00030 | A0090005001195 | | ACLA_020060 | NFIA_010270 | Alurgisisu |
| AMY | aipna amyiase | GH 13 | | | ATEG_00724 | | | | | |
| AMY | alpha amylase | GH 13 | | | | | AFL2G_01127 | | | |
| AMY | alpha amylase | GH 13 | | | | | | ACLA_091300 | NFIA_035590 | Afu2g03230 |
| AMY | alpha amylase | GH 13 | | | | | | | NFIA_035580 | |
| GLA | glucoamylase | GH 15 | AN7402 | | | | | ACLA_049360 | NFIA_105910 | |
| GLA | glucoamylase | GH 15 | | | | | | | | Afu4g10140 |
| GLA | glucoamylase | GH 15 | | | ATEG_05980 | A0090003000321 | AFL2G_02658 | ACLA_089470 | | |
| GLA | glucoamylase | GH 15 | AN11143 | 213597 | ATEG_04375 | A0090010000746 | AFL2G_11885 | ACLA_094080 | NFIA_032960 | Afu2g00690 |
| GLA | glucoamylase | GH 15 | | 189911 | - | A0090138000105 | AFL2G 08782 | ACLA 044430 | NFIA 094880 | Afu8g01390 |
| GLA | olucoamviase | GH 15 | | | | | | ACLA 082570 | NEIA 053760 | Afu6q08080 |
| GLA | olucoamylase | GH 15 | | | | | | ACLA 078620 | NEIA 001210 | Afu3e00610 |
| MAN | endomennansee | GH 26 | AN3336 | 40875 | | 0000011000055 | AEL2G 04859 | | | Anabyouoro |
| MAN | endomannanase | 01120 | AN2226 | 40013 | | A0030011000033 | AI 220_04033 | | | |
| MAN | endomannanase | 01/20 | AN7442 | | | | | | | |
| MAN | endomannanase | GH 26 | AN/413 | | | | | | | |
| AGL | alpha galactosidase | GH 27 | | | ATEG_01905 | | | | NFIA_048850 | Afu6g02560 |
| AGL | alpha galactosidase | GH 27 | | 37736 | ATEG_04382 | | | ACLA_016820 | NFIA_073100 | Afu5g13830 |
| AGL | alpha galactosidase | GH 27 | AN0022 | 172232 | ATEG_03427 | A0090005000217 | AFL2G_00225 | | NFIA_023390 | Afu1g01200 |
| AGL | alpha galactosidase | GH 27 | AN7624 | 207264 | ATEG_09830 | AO090003001305 | AFL2G_01765 | ACLA_003130 | NFIA_039990 | Afu5g02130 |
| AGL | alpha galactosidase | GH 27 | | 39180 | | | | | | |
| AGL | alpha galactosidase | GH 27 | AN7152 | 41606 | ATEG_02160 | A0090023000151 | AFL2G_04039 | | NFIA_029860 | Afu4g03580 |
| AGL | alpha galactosidase | GH 27 | | | | | | ACLA_097900 | | |
| PGA | endopolygalacturonase | GH 28 | AN8327 | 46255 | | | | | NFIA_008150 | Afu1g17220 |
| PGA | endopolygalacturonase | GH 28 | | 43957 | | | | | | |
| PGA | endopolygalacturonase | GH 28 | | 182156 | | | | | | |
| PGA | endopolygalacturonase | GH 28 | | 214598 | | | | | | |
| PGA | endonolygalacturgnase | GH 28 | AN6656 | 50161 | | 40090005000186 | AEL2G 00201 | | NEIA 099410 | |
| PGA | endonolygalacturonase | GH 28 | / | 172944 | ATEG 01601 | A0090005001400 | AFL2G_01310 | ACLA 036670 | NEIA 068440 | A fu3r08680 |
| PCA | endopolygalacturonase | OH 28 | AN/272 | 141677 | ATEC 04991 | A0090022000404 | AFL20_01010 | ACLA_052860 | NEIA 102450 | A fu4a12920 |
| POA | endepolygalacturonase | 01 20 | MN4372 | 62240 | AILO_04881 | A0000023000401 | AFL20_04232 | ACCA_032000 | NEIA_005630 | Afu9g13320 |
| POA | endopolygalacturonase | 01/20 | | 32219 | | A0090023000161 | AFL20_04049 | | NFIA_093020 | Aluoyu1970 |
| PGA | endopolygalacturonase | GH 20 | | | | A009013600006 | | | | |
| PGA | endopolygalacturonase | GH 28 | | | | | | | | A108006730 |
| PGA | endopolygalacturonase | GH 28 | | | ATEG_07748 | | | | | |
| PGA | endopolygalacturonase | GH 28 | | | | | AFL2G_08764 | | NFIA_023290 | Afu1g01320 |
| PGX | exopolygalacturonase | GH 28 | | 178172 | | | | | | |
| PGX | exopolygalacturonase | GH 28 | AN8891 | 191158 | ATEG_10357 | AO090010000753 | AFL2G_11892 | ACLA_043100 | NFIA_096340 | Afu8g02630 |
| PGX | exopolygalacturonase | GH 28 | AN8761 | 42184 | ATEG_07152 | AO090026000784 | AFL2G_06533 | | NFIA_049320 | A fu6g02980 |
| PGX | exopolygalacturonase | GH 28 | AN9045 | | | | | | | |
| RGX | exorhamnogalacturonase | GH 28 | | 172236 | | A0090001000133 | AFL2G_07371 | | | |
| RGX | exorhamnogalacturonase | GH 28 | | 42917 | | A0090009000470 | AFL2G_10228 | | NFIA_018590 | Afu1g06140 |
| RGX | exorhamnogalacturonase | GH 28 | AN10274 | 194461 | ATEG_09025 | A0090102000139 | AFL2G_09582 | | NFIA_027700 | Afu7g06410 |
| RGX | exorhamnogalacturonase | GH 28 | | | ATEG_06408 | A0090113000199 | AFL2G_08671 | | _ | |
| RGX | exorhamnogalacturonase | GH 28 | | | | A0090138000066 | AFL2G 03102 | | | |
| RGX | exorhamnogalacturonase | GH 28 | AN11626 | | | | | | | |
| RGX | exorhampogalacturonase | GH 28 | | | | 40090138000067 | AEL2G 08746 | | | |
| PHG | andorhamnonalacturonasa | GH 20 | | 178202 | | | | | | |
| DHO | endormannogalactaronase | 01120 | | 480000 | | 4.00000000000504 | 451.00 00475 | | | 44-4-00400 |
| PHO | andorhamnogalacturonase | GH 20 | | 100822 | | A0090005000524 | AEL20_02475 | | | A langed foo |
| DHO | anderhamnenslacturonase | GH 20 | | 244402 | ATEO ATOST | A0000010000000 | AFL20_00067 | | | |
| RIG | engomamnogalacturonase | GH 28 | | 211163 | ATEG_07607 | A0090010000484 | AFL20_11646 | | | |
| RHG | endornamnogalacturonase | GH 28 | | 123651 | | AU090026000252 | AFL2G_06999 | | | |
| RHG | endorhamnogalacturonase | GH 28 | AN9134 | 189722 | | AU090038000552 | AFL2G_07883 | | | |
| KHG | endorhamnogalacturonase | GH 28 | | 39337 | | A0090124000009 | AFL2G_08037 | | | |
| RHG | endorhamnogalacturonase | GH 28 | | | | | | | NFIA_076680 | Afu5g10530 |
| XGH | xylogalacturonase | GH 28 | | | | A0090026000120 | AFL2G_07122 | | | |
| XGH | xylogalacturonase | GH 28 | AN3389 | 46065 | | A0090102000011 | AFL2G_09468 | | NFIA_099610 | Afu8g06890 |
| PGX | exopolygalacturonase | GH 28 | | | | | | | NFIA_100120 | A fu8g07265 |
| AFC | alpha fucosidase | GH 29 | | 44822 | ATEG_08111 | | | | | |
| AFC | alpha fucosidase | GH 29 | | | ATEG, 05691 | | | | | |
| AGD | alpha glucosidase | GH 31 | AN2017 | 214233 | ATEG 00723 | A0090003001209 | AFL2G 01842 | ACLA 049370 | NFIA 105900 | Afu4q10150 |
| AGD | alpha olucosidase | GH 31 | AN0280 | 55410 | ATEG 02528 | A0090005000767 | AEL2G_00750 | ACLA 031260 | NEIA 021450 | Afu1003140 |
| AGD | alpha glucosidase | GH 34 | AN0941 | 119259 | ATEG 05177 | A0090005001084 | AFL2G_01039 | ACLA 019300 | NEIA 009190 | Afu1o16250 |
| AGD | alpha glucoeidaea | GH 24 | AN3504 | 40040 | ATEG_02000 | A0090023000299 | AEL20_01030 | ACCA_010300 | NEIA 018120 | A fu1006560 |
| 400 | alpha glucosidase | 01131 | AN10005 | +5340 | A1EG_02800 | A0000023000200 | AFL20_04152 | | NI PA_010130 | Analyuusuu |
| AGD | aipna giucosidase | GH 31 | AN10935 | | | A0090026000111 | AFL2G_07131 | | | |
| AGD | aipna giucosidase | GH 31 | AN8953 | | | A0090038000471 | AFL2G_07812 | | | |
| AGD | alpha glucosidase | GH 31 | AN11054 | 50055 | A1EG_08065 | AU090102000559 | AFL2G_09934 | ACLA_001670 | NEIA_038610 | Atu5g03500 |

Supplemental Table 6B Cont: Detection of proteins in cultures grown on sugar beet pulp sorted by CAZy family. (Continued on next page).

| 1.00 | status strandidana | 011.01 | 4117400 | | | | | | | |
|-------------|-----------------------------------|--------|---------|---------|-------------|---------------------------|-----------------|--------------|---------------|------------------|
| AGD | alpha glucosidase | GH 31 | AN7120 | | | | | | | |
| AGD | aipna glucosidase | GH 31 | AN7345 | | | | | | | |
| AGD | alpha glucosidase | GH 31 | | | ATEG_08278 | | | | | |
| AGD | alpha glucosidase | GH 31 | | | ATEG_08472 | | | | | |
| AGD | alpha glucosidase | GH 31 | | | | | | | NFIA_060440 | |
| AXL | alpha xylosidase | GH 31 | AN7505 | | ATEG_06730 | AO090001000649 | AFL2G_09290 | ACLA_089300 | NFIA_082140 | Afu2g05400 |
| AXL | alpha xylosidase | GH 31 | | 40261 | | A0090701000558 | AFL2G_06180 | | | |
| AXL | alpha xylosidase | GH 31 | | 43342 | ATEG 08390 | AO090701000639 | AFL2G 06239 | | NFIA 032680 | |
| AXL | alpha xylosidase | GH 31 | | | ATEG 00207 | | - | | - | |
| AXI | alpha xylosidase | GH 31 | | | ATEG 05169 | | | | | |
| NU | endo inulinase | GH 32 | | 52928 | | | | | | ∆fu5a00530 |
| MU | anda inulinase | 011 32 | | 32320 | | | | | | A fuEc00420 |
| MU | ando inulinase | 011 32 | | | ATEC 07256 | | | | | Alabyooroo |
| NV NV | endo indinase | 011 32 | | | AILG_07330 | | | | | |
| INA BUV | exo inuinase | GH 32 | 4144770 | 50004 | ATEO 00455 | 4 0 0 0 0 7 0 4 0 0 4 0 0 | A 51 000 000000 | A CL A | NELA 000540 | 44-0-04040 |
| INA OUIO | exo inuinase | GH 32 | AN11776 | 20004 | ATEG_06155 | A0090701000400 | AFL2G_06026 | ACLA_094550 | NFIA_033540 | A102g01240 |
| SUC | invertase/beta fructofuranosidase | GH 32 | AN3837 | | ATEG_05860 | A0090701000038 | AFL2G_05693 | | NFIA_051560 | A106g05000 |
| SUC | invertase/beta fructofuranosidase | GH 32 | | 198063 | ATEG_07479 | A0090020000640 | AFL2G_10707 | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | | | AO090103000043 | AFL2G_12317 | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | 176039 | | | | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | | ATEG_04996 | | | | | |
| SUC | invertase/beta fructofuranosidase | GH 32 | | | ATEG_10253 | | | | | |
| LAC | beta galactosidase | GH 35 | AN0194 | | | | | | | |
| LAC | beta galactosidase | GH 35 | | 180727 | | | | | | |
| LAC | beta galactosidase | GH 35 | | | | A0090001000259 | AFI 2G 07489 | | | |
| 140 | heta galactosidase | GH 35 | | 177434 | ATEG 07446 | A0090003000042 | AEL2G 02901 | ACLA 088440 | NEIA 052310 | A fu6a06660 |
| LAC | beta galactosidase | GH 34 | AN0756 | 51764 | ATEG 00616 | A0090012000445 | AFL2G_03352 | ACLA 021260 | NEIA 011250 | Afu1q14170 |
| LAC | beta galactosidase | CH 20 | | 01104 | | A0090012000746 | | | | |
| LAC | heta galactosidase | GH 25 | AN2804 | | ATEG 08265 | A00900230000740 | AFI 2G 03977 | | NEIA 041400 | Afu5a00670 |
| LAC | hata galactoridara | GH 35 | AN0000 | 40400 | ATEG_06255 | A0090023000076 | AFL20_03977 | | NELA_041490 | Afu1016700 |
| LAC | beta galdClosidase | GH 35 | MM0300 | 40429 | A166_05131 | A0090120000158 | AFL2G_00182 | | NELA_000090 | All 1910/00 |
| LAC | ueta galactosidase | GH 35 | | 41910 | | | AFL2G_03616 | | INFIA_000910 | A103000380 |
| LAC | beta galactosidase | GH 35 | | | | A0090701000770 | | | | |
| LAC | beta galactosidase | GH 35 | | | | | | ACLA_018830 | | |
| LAC | beta galactosidase | GH 35 | | | | | AFL2G_06343 | | | |
| LAC | beta galactosidase | GH 35 | | | | | AFL2G_12387 | | | |
| AGL | alpha galactosidase | GH 36 | AN8138 | 212736 | ATEG_07929 | A0090010000684 | AFL2G_11824 | ACLA_044620 | NFIA_094580 | Afu8g01130 |
| AGL | alpha galactosidase | GH 36 | AN8392 | | ATEG_07935 | A0090011000063 | AFL2G_04865 | ACLA_044560 | NFIA_094540 | Afu8g01100 |
| AGL | alpha galactosidase | GH 36 | AN3874 | 190816 | ATEG_05379 | A0090023000743 | AFL2G_04555 | ACLA_047600 | NFIA_107920 | Afu4g08250 |
| AGL | alpha galactosidase | GH 36 | AN9035 | | ATEG_02312 | | | | | |
| ABF | alpha arabinofuranosidase | GH 43 | AN8472 | | - | A0090012000356 | AFL2G 03265 | | NFIA 032900 | Afu2q00650 |
| ABF | alpha arabinofuranosidase | GH 43 | AN7781 | | ATEG 08386 | A0090005000065 | AFL2G 00086 | | - | |
| ABE | alpha arabinofuranosidase | GH 43 | AN2533 | | | A0090701000838 | AEL2G_06400 | | NEIA 002750 | A fu3c01660 |
| ABN | endoarabinanase | GH 43 | | 128077 | ATEG 05780 | | | | NEIA 078690 | A fu5c08590 |
| ADN | andearabinanase | 01143 | | 120077 | ATEC 07917 | 4.0000026000804 | AEL 20, 06512 | ACLA 072720 | NEIA 020020 | Afu3g00330 |
| APN | andoarabinanaso | 01143 | AN2044 | 194106 | ATEC 01662 | A00000020000004 | AFL20_00313 | ACLA_0727090 | NEIA 068030 | A fu2e00140 |
| ADN | endoarabirariase | 01143 | ANJ044 | 104133 | A1L0_01302 | A0050003001320 | AI L20_01244 | ACLA_037000 | NEIA_000020 | A103g03140 |
| ABN | endoarabinanase | GH 43 | 410050 | 10,1000 | 1750 04407 | 4.000000000000000 | 451.00.04050 | ACLA_018180 | NFIA_008030 | Afu1g17320 |
| ABN | endoarabinanase | GH 43 | AN6352 | 134398 | ATEG_01407 | A0090023000165 | AFL2G_04053 | ACLA_073460 | NFIA_089320 | Afu2g14150 |
| ABN* | endoarabinanase | GH 43 | AN8007 | 197735 | | A0090138000055 | AFL2G_08737 | ACLA_098980 | NFIA_047920 | ATU6000770 |
| ABN | endoarabinanase | GH 43 | AN2534 | 203143 | ATEG_03520 | A0090701000481 | AFL2G_06106 | ACLA_042100 | NFIA_062660 | Afu3g14620 |
| ABN | endoarabinanase | GH 43 | | | | | | | | |
| ABN | endoarabinanase | GH 43 | | | | AO090005000064 | AFL2G_00085 | | | |
| ABN | endoarabinanase | GH 43 | | | ATEG_03688 | | | | | |
| ABN | endoarabinanase | GH 43 | | | ATEG_07787 | | | | | |
| BXL | beta xylosidase | GH 43 | AN1870 | 179682 | ATEG_06059 | A0090003000239 | AFL2G_02739 | ACLA_090090 | NFIA_081240 | Afu2g04480 |
| BXL | beta xylosidase | GH 43 | AN6751 | 174379 | ATEG_06306 | AO090005000476 | AFL2G_00456 | | NFIA_027350 | Afu7g06110 |
| BXL | beta xylosidase | GH 43 | | | ATEG 05083 | A0090103000268 | AFL2G 12122 | ACLA 087680 | NFIA 060490 | Afu6q14550 |
| BXL | beta xylosidase | GH 43 | AN10199 | | ATEG 00093 | A0090005000698 | AFL2G 00689/ | ACLA 058100/ | NFIA 097490/0 | Afu8a04710/04720 |
| BXL | beta xylosidase | GH 43 | AN10919 | | ATEG 10072 | A0090010000029 | AFL2G 11296 | - | NFIA 093350 | - |
| BXL | beta xylosidase | GH 43 | | | ATEG 07784 | A0090010000494 | AFL2G 11656 | | NFIA 023360 | Afu1q01230 |
| BXL | beta xylosidase | GH 43 | AN7313 | 122978 | ATEG 01188 | A0090010000562 | AFL2G 11714 | ACLA 043260 | NFIA 096240 | Afu8q02510 |
| BXI | beta xylosidase | GH 43 | AN2664 | | ATEG 06107 | A0090012000350 | AFI 2G 03257 | | NEIA 072780 | Afu5q14190 |
| BXI | heta vylosidase | GH 43 | AN8477 | | ATEG 01292 | | AFI 2G 06974 | ACLA 018160 | | |
| BXI | heta xylosidase | GH 43 | | | | 40090113000059 | | | | |
| BXI | hets vulneidese | 00 43 | | | ATEG 10103 | | | | | |
| BVI | hata vulneidaea | OH 43 | AN7275 | | ATEC 06642 | 0000701000000 | AEL20, 06449 | ACLA 078600 | NEIA 033330 | A 6/2×00930 |
| DAL | bota xyuSidase | GH 43 | AN7924 | 47077 | ATEC 010043 | A0000101000886 | AFL20_00448 | ACLA_070600 | NEIA 099270 | Afu2g00930 |
| DAL | beta xylUSIO8Se | GH 43 | AN1/004 | 4/6// | ATEG_01292 | A0090102000331 | ArL2G_09750 | ACLA_072000 | INFIA_000370 | A102013190 |
| DXL | Deta Xylosidase | GH 43 | 4100000 | | ATEG_07517 | | | | | |
| BXL | beta xylosidase | GH 43 | AN2633 | | | | | | | |
| RXL | beta xylosidase | GH 43 | AN1043 | | | | | | | |
| BXL | beta xylosidase | GH 43 | | | | | AFL2G_08528 | | | |
| ? | ? | GH 43 | AN1477 | | | | | | | |
| EGL | endoglucanase | GH 45 | AN6786 | | | | | | NFIA_028020 | Afu7g06740 |
| ABF | alpha arabinofuranosidase | GH 51 | AN1277 | 38549 | ATEG_00198 | | AFL2G_07796 | ACLA_025610 | NFIA_015730 | Afu1g09900 |
| ABF | alpha arabinofuranosidase | GH 51 | | 50979 | ATEG_03540 | | | | | |
| ABF | alpha arabinofuranosidase | GH 51 | | | | A0090020000712 | AFL2G_10643 | | | |
| ABF | alpha arabinofuranosidase | GH 51 | AN2541 | 206387 | ATEG 02882 | A0090124000023 | AFL2G 08019 | ACLA 099110 | | |
| ABF | alpha arabinofuranosidase | GH 51 | AN9439 | 131891 | ATEG 07868 | A0090012000298 | AFL2G 03217 | ACLA 074120 | NFIA 090410 | Afu2q15160 |
| GAI | endonalactanase | GH 53 | | | | | | | | |
| GAL | endogalactanase | GH 53 | AN5727 | 187227 | ATEG 02927 | 40090001000492 | AEL2G 09135 | | NEIA 017790 | Afu1c06910 |
| GAL | andonalactanaea | CH 53 | 10121 | 10/22/ | A120_02021 | A00000000492 | AEL20_07499 | | | |
| ARE | alaba arabiaafuranasidana | GH 53 | AN1571 | 200000 | ATEC 07020 | 4.0000022000004 | AFL20_07400 | ACLA 066470 | NELA DEDEDA | A 6+6+14620 |
| AUF CO | aipira arabitoturatiosidase | GH 54 | AN1071 | 200605 | HIEG_01938 | A0090025000001 | APL26_03901 | ACLA_000470 | NI 14_000030 | A100014020 |
| 00 | celulose oxidase | GH 61 | AN10419 | | 1750 04515 | | | | 10004-00 | 4.6-0-00070 |
| 00 | cellulose oxidase | GH 61 | | 211595 | AIEG_04210 | | | ACLA_060890 | NEIA_006140 | A103g03870 |
| CO | cellulose oxidase | GH 61 | AN1041 | 52688 | ATEG_00448 | A0090001000221 | AFL2G_07454 | ACLA_022980 | NFIA_012990 | Afu1g12560 |
| CO | cellulose oxidase | GH 61 | AN1602 | 182430 | ATEG_07790 | A0090005000531 | AFL2G_00532 | ACLA_059790 | NFIA_099510 | Afu8g06830 |
| CO | cellulose oxidase | GH 61 | AN9524 | 53797 | ATEG_07920 | A0090012000090 | AFL2G_03026 | | NFIA_044390 | |
| CO | cellulose oxidase | GH 61 | AN2388 | | ATEG_01035 | A0090023000056 | | ACLA_073030 | NFIA_089670 | Afu2g14490 |
| | | | | | | | | | | |

Supplemental Table 6B Cont: Detection of proteins in cultures grown on sugar beet pulp sorted by CAZy family.

| CO | cellulose ovidase | CH 61 | AN3046 | 43784 | ATEC 08113 | A0000023000150 | AEL2G 04048 | ACLA 055060 | NEIA 089730 | A fu2o14540 |
|--------------|---|---------|-------------|--------|-------------|------------------|---------------|-------------|--------------|----------------|
| 00 | collulose exidade | CH 61 | /10010 | 10701 | ATEC 05 416 | A00000220000787 | AEL20_04506 | ACLA 047220 | NEIA 109220 | A fu 4 n0 7950 |
| 0 | cellulose oxidase | GH 61 | | | ATEG_05416 | A0090023000767 | AFL2G_04596 | ACLA_047220 | NFIA_106520 | A104g07650 |
| co | cellulose oxidase | GH 61 | AN6428 | | ATEG_06077 | | | | | |
| CO | cellulose oxidase | GH 61 | | 56338 | ATEG_05081 | A0090103000087 | AFL2G_12282 | | NFIA_055220 | Afu6g09540 |
| 00 | cellulose ovidase | GH 61 | | | ATEG 10194 | | - | | - | |
| 00 | | 011.01 | 4117004 | 404705 | 10100 | 4.000040000004 | 4.51.00.00000 | | | |
| 0 | celulose oxidase | GH 61 | AN7091 | 194/03 | | A0090136000004 | AFL2G_00099 | | | |
| CO | cellulose oxidase | GH 61 | AN3860 | | | | | | | |
| CO | cellulose oxidase | GH 61 | | | ATEG 08942 | | | | | |
| 00 | cellulone ovidane | CH 61 | | | ATEC 01456 | | | | | |
| 00 | Celulose oxidase | GITUI | | | MILO_01450 | | | | | |
| CO | cellulose oxidase | GH 61 | | | | | | ACLA_017470 | | |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | | | ATEG_00186 | A0090103000088 | AFL2G_12281 | ACLA_071560 | NFIA_087900 | Afu2g12770 |
| AVH | arabinovylan arabinofuranobydrolasa | CH 62 | AN2632 | 55136 | ATEC 10071 | A0000701000885 | AEL2G_06447 | ACLA 061510 | NEIA 033210 | A fu 2000920 |
| 4301 | arabinoxyian arabinoraranonyarolase | 011 02 | 4112002 | 00100 | AILO_IOUTI | A0030101000003 | AI 220_00441 | ACEA_001010 | NI 14_000210 | Allazgooszo |
| АХН | arabinoxylan arabinoturanonydrolase | GH 62 | AN/908 | | | | | | | |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | | | ATEG_10379 | | | | | |
| AGU | alpha glucuronidase | GH 67 | AN9286 | 56619 | ATEG 06085 | A0090026000127 | AFL2G 07114 | ACLA 017270 | NFIA 072510 | Afu5q14380 |
| AGU | alpha olucuropidase | GH 67 | | | ATEC 00075 | | - | - | - | 3 |
| AGU | aipita giucuroniuase | Giror | | | AILG_03575 | | | | | |
| XG CBH | xyloglucan active cellobiohydrolase | GH 74 | AN1542 | | | | | | NFIA_096000 | Afu8g02330 |
| XG EGL | xyloglucan active endoglucanase | GH 74 | AN5061 | 206333 | ATEG 04708 | | | ACLA 044310 | NFIA 094990 | Afu8q01490 |
| RHA | alpha rhamoosidase | GH 78 | AN8465 | 40264 | - | | AEL2G 05364 | | | |
| BULL | | 01170 | 7410100 | 102.01 | | | | | | |
| RNA | aipna mamnosidase | GH / C | • | | | | | | | |
| RHA | alpha rhamnosidase | GH 78 | AN2631 | 176718 | ATEG_05089 | A0090001000105 | AFL2G_07340 | | NFIA_004560 | Afu3g02880 |
| RHA | alpha rhamposidase | GH 78 | AN11954 | 51410 | | A0090003001016 | AEL2G_02014 | | | |
| DHA | alpha shamaasidaaa | 01.70 | AN40277 | 470472 | | 4.00000000004004 | AEL 20 04790 | | NEIA 022070 | 46-4-04660 |
| RHA | aipna rnamnosidase | GH 78 | AN10277 | 1/01/2 | | A0090003001291 | AFL2G_01780 | | NFIA_022970 | Aturguteeu |
| RHA | alpha rhamnosidase | GH 78 | 5 | 42916 | ATEG_03018 | A0090009000471 | AFL2G_10227 | | NFIA_018620 | Afu1g06130 |
| RHA | alpha rhamnosidase | GH 78 | AN7151 | | ATEG 02922 | A0090010000561 | AFL2G 11713 | | NFIA 057930 | Afu6a12030 |
| DHA | alaba shamaqaidaga | 04.79 | AN2790 | | | | | | | |
| RITA | alpha mannosluase | GITTO | ANJTOU | | | | | | | |
| RHA | alpha rhamnosidase | GH 78 | | | | A0090103000432 | AFL2G_119/2 | | | |
| RHA | alpha rhamnosidase | GH 78 | 1 | | | A0090113000149/A | AFL2G 08627 | | NFIA 057930 | Afu6q14610 |
| RHA | alpha rhamposidase | GH 78 | AN6929 | 131668 | | 40090005001416 | | | | |
| NIIA DULA | apria mannosidase | 01170 | AN0323 | 131000 | | A0030003001410 | | | | |
| кĦА | aipna rnamnosidase | GH 78 | • | | | | | | INFIA_060560 | |
| RHA | alpha rhamnosidase | GH 78 | | | | | AFL2G_10644 | | | |
| RHA | alpha rhamposidase | GH 78 | AN12368 | | | | | | | |
| DUA | alpha mamrooldage | 01170 | | | | | 4.51.00.00000 | | | |
| RNA | aipna rnamnosidase | GH /c | • | | | | AFL2G_03939 | | | |
| RHA | alpha rhamnosidase | GH 78 | AN10867 | 44977 | ATEG_04706 | A0090012000058 | AFL2G_02993 | | NFIA_026130 | Afu7g05040 |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | | | | A0090005000324 | AFL2G 00324 | | NFIA 026210 | Afu7o05090 |
| IIOH | uppaturated galacturopy/ hydrolago | 04.99 | AN2004 | 26414 | | 0000122000027 | A EL 2009766 | | NEIA 022240 | A fu1e01250 |
| UGH | unsaturated galacturonyi nydrolase | GH oc | ANJAAT | 30414 | | A0090136000067 | AFL2G00700 | | INFIA_025540 | Alu Igu 1250 |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | AN11078 | | | | AFL2G_06465 | | | |
| UGH | unsaturated galacturonyl hydrolase | GH 88 | 1 | | | A0090701000907 | | | | |
| UGH | unesturated galacturopyl hydrolaea | GH 88 | AN/629 | | | | | | | |
| 1001 | unsaturated galactaronymydrolase | 01100 | ANHO23 | | 1750 00015 | | 15100.00040 | | | |
| ABX | exoarabinanase | GH 93 | AN2060 | | ATEG_06045 | A0090003001017 | AFL2G_02013 | | NFIA_081320 | Afu2G04570 |
| ABX | exoarabinanase | GH 93 | 1 | | | A0090011000141 | AFL2G_04929 | | | |
| ABX | exoarabinanase | GH 93 | AN5231 | 49311 | ATEG 07909 | A0090012000101 | AEL2G_03035 | ACLA 008170 | NEIA 027720 | Afu7o06430 |
| 4.01/ | and the second se | 011.02 | | | ATEO 00000 | | | | | |
| ADA | exoarabinanase | GH 93 | | | ATEG_06029 | | | | | |
| ABX | exoarabinanase | GH 93 | | | ATEG_00891 | | | | NFIA_058060 | Afu6g12120 |
| AFC | alpha fucosidase | GH 95 | AN8149 | 184037 | ATEG 01560 | A0090005000382 | AFL2G 00373 | | | |
| AEC | alpha fucosidasa | CH 05 | | | - | 40000005000512 | AEL 20, 00515 | | | |
| Arc | alpha lucosidase | 011 30 | | | | A0030003000312 | ATE26_00010 | | | |
| AFC | alpha fucosidase | GH 95 | AN6673 | 53702 | ATEG_09768 | A0090009000086 | AFL2G_10565 | ACLA_004070 | NFIA_040960 | Afu5g01190 |
| AFC | alpha fucosidase | GH 95 | AN10376 | | ATEG_04136 | | | | NFIA_064720 | Afu3g12590 |
| IIDH | unceturated rhamponalacturopyl hydrolas | GH 105 | AN3196 | 41703 | ATEC 07907 | 0000001000174 | AEL2G 07407 | ACLA 056060 | NEIA 030670 | A fu4c02860 |
| UDU | unsaturated mannogulactoronymydrolas | 011100 | Anotoo | 41103 | AILO_01001 | 100000000000000 | ATL20_01401 | ACCA_000000 | 1110-000010 | A104902000 |
| URH | unsaturated mamnogalacturonyl hydrolas | GH 105 | | | | A0090003000153 | AFL2G_02808 | | | |
| URH | unsaturated rhamnogalacturonyl hydrolas | GH 105 | AN10505 | | | A0090001000063 | AFL2G_07308 | ACLA_072870 | NFIA_089840 | Afu2g14630 |
| URH | unsaturated rhamnogalacturonvl hydrolas | GH 105 | AN9383 | 41877 | ATEG 02892 | A0090113000146 | AFL2G 08624 | ACLA 077810 | | |
| UDH | upportunated champopologituropul budgolog | OH 105 | AN7939 | | - | | - | - | | |
| URI | unsaturateu mannogalacturonymyurolas | Gir ius | ANTO20 | | | | | | | |
| AGU | alpha glucuronidase | GH115 | | | | A0090005001415 | AFL2G_01323 | | | |
| AGU | alpha glucuronidase | GH115 | AN9329 | | ATEG_09974 | A0090010000038 | AFL2G_11304 | ACLA_006360 | NFIA_025630 | Afu7g04680 |
| AGU | alpha glucuronidase | GH115 | | | | A0090001000267 | AEL2G 07498 | | | |
| 1.00 | apra glacaromadoo | 011110 | | | | 10000001000201 | A 220_01400 | | | |
| AGU | aipna giucuronidase | Gn115 | | | | A0090113000058 | | | | |
| AGU | alpha glucuronidase | GH 115 | | | ATEG_04355 | | | | | |
| PEL | nectin lyase | PL 1 | | | ATEG 00950 | A0090003001295 | AEL2G 01776 | | | |
| DEI | pactin lyana | DI 4 | | 66040 | | 0000010000030 | AEL 20 11207 | | NEIA 026140 | A fu7a05030 |
| PLL . | poun yase | PL 1 | | 55212 | | A0090010000030 | ALC20_11297 | | NI PA_020110 | Miarguouou |
| PEL | pectin lyase | PL 1 | | 45821 | | | | | | |
| PEL | pectin lyase | PL 1 | AN4882 | 40837 | ATEG_7577 | A0090012000121 | AFL2G_03052 | | NFIA_077100 | Afu5g10170 |
| PEI | nectin lyase | DI 4 | | | | A0090012000454 | AEL2G 03350 | | | - |
| - total | possil gudo | | | | | 10000012000431 | | | | |
| PEL | pectin iyase | PL 1 | | | | A0090103000463 | AFL2G_11948 | | | |
| PEL | pectin lyase | PL 1 | AN10147 | 208760 | | AO090138000204 | AFL2G_08823 | | | |
| PEL | pectin lyase | PI 1 | | | | | _ | ACLA 094210 | NEIA 033080 | Afu2000800 |
| DEL | postin hano | | AN0224 | 440.00 | | 40000010000501 | AEL 20 44000 | ACLA 040470 | NEIA 070000 | A fuEa10200 |
| PEL | pecun iyase | PL 1 | ANZ331 | 41015 | | A0090010000504 | AFL2G_11000 | ACLA_013470 | INFIA_070000 | Alubg10560 |
| PEL | pectin lyase | PL 1 | AN2569 | | ATEG_01216 | A0090010000087 | AFL2G_11352 | | | |
| PEL | pectin lyase | PL 1 | | | | | | | NFIA 023100 | |
| DEI | nectin brase | DI 4 | AN0430 | | | | | | | |
| PLL | poun yase | PL 1 | A113433 | | 1700 1111 | | 1000 | | | |
| PLY | pectate lyase | PL 1 | AN0741 | | ATEG_08834 | A0090011000673 | AFL2G_05417 | | | |
| PLY | pectate lyase | PL 1 | AN5333 | | ATEG 05467 | A0090102000072 | AFL2G 09523 | | NFIA 060270 | Afu6q14400 |
| PLY | pectate lvase | DI 4 | AN7646 | 45024 | ATEG 08122 | A0090701000321 | AFI 20, 05954 | | NEIA 033040 | A fu2000760 |
| PLACE I | poolato iyase | PL 1 | 410007/4 | +5021 | A100_00125 | 4.000004400021 | AFL00_03034 | | 11 M_000040 | Alazyourou |
| PLY | pectate lyase | PL 1 | AN9367/AN93 | 306 | | A0090011000030 | AFL2G_04835 | | | |
| PLY | pectate lyase | PL 3 | AN6748 | | ATEG_06314 | A0090005000472 | AFL2G_00461 | | NFIA_027690 | Afu7g06400 |
| PLY | pectate lyase | PL 3 | AN3337 | | ATEG 06285 | | _ | | _ | |
| DLV. | pactate lyane | DI C | AN2542 | | | 40000010000700 | AEL20 44942 | ACLA 0500/0 | NEIA 000070 | A fu9a05040 |
| PLY | pectate lyase | PL 3 | ANZ54Z | | | A0090010000706 | AFL2G_11846 | ACLA_059210 | INFIA_098670 | A106005910 |
| PLY | pectate lyase | PL 3 | AN6106 | | ATEG_08626 | AO090038000502 | AFL2G_07839 | | NFIA_023470 | Afu1g01120 |
| PLY | pectate lvase | PL 3 | AN8453 | | | | | | | |
| POL | rhamnonalacturonac hisso | DI 4 | AN7125 | 210047 | ATEC 02400 | A 0000014000270 | AEL20 05400 | ACLA 054000 | NEIA 020620 | A fu4o03790 |
| NOL | manifiogalacturonari iyase | PL 4 | -441135 | 210947 | A100_02193 | ~0000011000049 | AFE20_05136 | ACLA_034060 | WTH_029020 | A104903700 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN6395 | 47780 | ATEG_10327 | A0090012000147/A | AFL2G_03075 | ACLA_018320 | NFIA_008140 | Atu1g17230 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN12097 | | | A0090113000057 | | | | |
| RGI | rhampogalacturopan lyase | pi 4 | | | ATEG 08610 | A0090138000449 | AEL2G 08794 | | NEIA 094270 | A fu8e00820 |
| ROL | manifogalactaronari iyase | PL 4 | | | A100_00010 | 20000130000119 | AT L26_00/94 | | 10 04_094210 | A100900020 |
| KGL | mamnogalacturonan lyase | PL 4 | AN3950 | | | | | | | |
| PLY | pectate lyase | PL 9 | AN2537 | | | A0090038000131 | AFL2G_08953 | | NFIA_062360 | Afu3g14890 |
| PLY | pectate lyase | PI C | | | ATEG 03526 | | _ | | _ | |
| 1.0.00 | boorgro Ange | riu a | | | | | | | | |
| 001 | all a second a second | | 0.01110.011 | | | | | | | |

Chapter 2

Supplemental Table 6C: Detected proteins in cultures grown on wheat bran sorted by number of species that contain an orthologue. The colour codes indicate which percentage of the total number of detected peptides was of the specific protein. (Continued on next page; Please see pdf file for enlargened text).

| | | | 0-0.1% | 0.1-0.5% | 0.5-1% | 1-2% | 2-5% | 5-10% | >10% | | | | |
|----------------|--|----------------|--------------|----------|-------------|-----------------|---------------|-----------------|--------------|-----------------|-------------|----------------|---------------------|
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | no. species | no. of species | percentage detected |
| enzyme code | function | CAZY | A. nidulans | A. niger | A. terreus | A. oryzae | A. flavus | A. clavatus | A. fischeri | A. fumigatus | protein | gene | orthologs |
| BGL | beta glucosidase | GH 3 | AN4102 | 56782 | ATEG_03047 | A0090009000356 | AFL2G_10322 | ACLA_028810 | NFIA_018950 | Afu1g05770 | 8 | 8 | 100 |
| CBH | cellobiohydrolase | GH 7 | AN0494 | 51773 | ATEG_05002 | A0090001000348 | AFL2G_07571 | ACLA_085260 | NFIA_057300 | Afu6g11610 | 8 | 8 | 100 |
| LAC | beta galactosidase | GH 35 | 5 AN0756 | 51764 | ATEG_00616 | A0090012000445 | AFL2G_03352 | ACLA_021260 | NFIA_011250 | Afu1g14170 | 8 | 8 | 100 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosida: | GH 3 | 3 AN2217 | 50997 | ATEG_09314 | A0090701000274 | AFL2G_05912 | ACLA_010340 | NFIA_080180 | Afu5g07080 | 7 | 8 | 88 |
| XLN | endoxylanase | GH 10 | AN1818 | 212507 | ATEC_00809 | A0090701000887 | AFL2G_06449 | ACLA_048770 | NFIA_106540 | A104009480 | 1 | 8 | 88 |
| AGD | aloba olucosidase | GH 31 | AN11143 | 21009/ | ATEG_04375 | A0090005001084 | AFL2G_11005 | ACLA_094080 | NEIA 009180 | Afu102000090 | 7 | 0 | 00 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | AN2632 | 55136 | ATEG 10071 | A0090701000885 | AFL2G_01030 | ACLA_013500 | NEIA 033210 | Afu2000920 | 7 | 8 | 88 |
| CBH | cellobiohydrolase | GH 6 | AN5282 | 54490 | ATEG 07493 | | | ACLA 062560 | NFIA 002990 | Afu3q01910 | 6 | 6 | 100 |
| ABX | exoarabinanase | GH 93 | AN2060 | | ATEG_06045 | A0090003001017 | AFL2G_02013 | | NFIA_081320 | Afu2G04570 | 6 | 6 | 100 |
| BXL/ABF | beta xylosidase/alpha arabinofuranosida: | GH 3 | 3 AN8401 | | ATEG_09052 | A0090103000120 | AFL2G_12252 | ACLA_062400 | NFIA_003180 | Afu3g02090 | 6 | 7 | 86 |
| AGL | alpha galactosidase | GH 27 | AN7152 | 41606 | ATEG_02160 | A0090023000151 | AFL2G_04039 | | NFIA_029860 | Afu4g03580 | 6 | 7 | 86 |
| ABN* | endoarabinanase | GH 43 | 3 AN8007 | 197735 | | A0090138000055 | AFL2G_08737 | ACLA_096960 | NFIA_047920 | Afu6g00770 | 6 | 7 | 86 |
| XLN | endoxylanase | GH 11 | 1 AN9365 | 52071 | ATEG_07461 | A0090120000026 | AFL2G_08066 | ACLA_063140 | NFIA_000850 | Afu3g00320 | 6 | 8 | 75 |
| ABF | alpha arabinoturanosidase | GH 54 | AN1571 | 200605 | ATEG_07939 | A0090023000001 | AFL2G_03901 | ACLA_066470 | NFIA_060630 | Afu6g14620 | 8 | 8 | /5 |
| EGL | endoglucanase | GH S | AN1285 | 205580 | ATEG_05002 | 4.000004000405 | 451.00.00000 | ACLA_085250 | NFIA_057290 | Atubg11600 | 5 | 6 | 83 |
| VIN | endovulanana | OH 10 | AN7 390 | 1/9205 | ATEG_10320 | A0090012000135 | AFL2G_03066 | ACLA 025010 | NFIA_007920 | Alu1917410 | 5 | 7 | 71 |
| XG FOI | vyloglucan active endoplucanase | GH 12 | | 211053 | ATEG 07420 | A0090026000102 | AFL20_07140 | ACLA 007820 | NEIA 027400 | A fu7c06150 | 5 | 7 | 71 |
| PGX | exopolygalacturonase | GH 28 | AN8761 | 42184 | ATEG 07152 | A0090026000784 | AFL2G 06533 | | NFIA 049320 | Afu6q02980 | 5 | 7 | 71 |
| LAC | beta galactosidase | GH 35 | 5 AN0980 | 46429 | ATEG 05131 | A0090120000158 | AFL2G 08182 | | NFIA 008690 | Afu1g16700 | 5 | 7 | 71 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | AN2528 | 189254 | ATEG_03511 | A0090701000556 | AFL2G_06177 | ALCL_041970 | NFIA_062750 | Afu3g14510 | 5 | 8 | 63 |
| CBH | cellobiohydrolase | GH 7 | AN5176 | 53159 | ATEG_03727 | A0090012000941 | AFL2G_03805 | ACLA_088870 | NFIA_052720 | Afu6g07070 | 5 | 8 | 63 |
| PGX | exopolygalacturonase | GH 28 | 3 AN8891 | 191158 | ATEG_10357 | A0090010000753 | AFL2G_11892 | ACLA_043100 | NFIA_096340 | Afu8g02630 | 5 | 8 | 63 |
| NX | exo inulinase | GH 32 | 2 AN11778 | 56684 | ATEG_08155 | A0090701000400 | AFL2G_06028 | ACLA_094550 | NFIA_033540 | Afu2g01240 | 5 | 8 | 63 |
| ABF | alpha arabinofuranosidase | GH 51 | AN9439 | 131891 | ATEG_07868 | A0090012000298 | AFL2G_03217 | ACLA_074120 | NFIA_090410 | Afu2g15160 | 5 | 8 | 63 |
| PEL | pectin lyase | PL 1 | AN2569 | 17011 | ATEG_01216 | A0090010000087 | AFL2G_11352 | A CL A . 050000 | | | 4 | 4 | 100 |
| ARE | alpha arabiaofuranosidana | OH 13 | AN0402 | 4/511 | ATEG_10103 | A0090023000944 | AEL 20 02265 | ACEA_052920 | NEIA 022000 | 0.002000650 | | 5 | 00 |
| PLY | nectate base | PI C | AN2537 | | | A0090038000131 | AFL2G_03203 | | NEIA 062360 | Afu3o14890 | 4 | 5 | 80 |
| PME | pectin methyl esterase | CE 8 | 3 AN3390 | 214857 | | A0090102000010 | AFL2G 09467 | | NFIA 099600 | Afu8g06880 | 4 | 6 | 67 |
| XGH | xylogalacturonase | GH 28 | 3 AN3389 | 46065 | | A0090102000011 | AFL2G 09468 | | NFIA 099610 | Afu8q06890 | 4 | 6 | 67 |
| GUS | beta glucuronidase | GH 2 | 2 AN2395 | 52111 | ATEG_01031 | A0090023000053 | AFL2G_03956 | | NFIA_089700 | Afu2g14520 | 4 | 7 | 57 |
| BGL | beta glucosidase | GH 3 | AN2828 | | ATEG_07419 | A0090701000841 | AFL2G_06408 | ACLA_007810 | NFIA_027390 | Afu7g06140 | 4 | 7 | 57 |
| CBH | cellobiohydrolase | GH 6 | AN1273 | 133986 | ATEG_00193 | A0090038000439 | AFL2G_07776 | ACLA_025560 | NFIA_015680 | | 4 | 7 | 57 |
| ABN | endoarabinanase | GH 43 | 3 | 182100 | ATEG_07817 | A0090026000804 | AFL2G_06513 | ACLA_072730 | NFIA_089980 | Afu2g14750 | 4 | 7 | 57 |
| GAL | endogalactanase | GH 53 | 3 AN5727 | 187227 | ATEG_02927 | A0090001000492 | AFL2G_09135 | | NFIA_017780 | Afu1g06910 | 4 | 7 | 57 |
| PLY EAE CEE | pectate lyase feruleul esterane | PL 1 | AN/646 | 45021 | ATEC_08123 | A0090701000321 | AFL2G_05954 | ACLA 055050 | NFIA_033040 | Afu2g00760 | 4 | / | 5/ |
| PAE SFS | nectio method esterano | CE I | AN3207 | 43/03 | ATEG_00112 | A0090023000156 | AFL2G_04047 | ACLA_035050 | NEIA_069720 | A102014530 | | | 50 |
| MAN | endomannanase | CL C | 5 AN3358 | 50378 | ATEG 08654 | A0090010000122 | AFL2G_10310 | ACLA_055010 | NEIA 113780 | Afu7o01070 | 4 | 8 | 50 |
| AGL | alpha galactosidase | GH 27 | 7 AN7624 | 207264 | ATEG 09830 | A0090003001305 | AFL2G 01765 | ACLA 003130 | NFIA 039990 | Afu5q02130 | 4 | 8 | 50 |
| AGD | alpha glucosidase | GH 31 | AN2017 | 214233 | ATEG_00723 | A0090003001209 | AFL2G_01842 | ACLA_049370 | NFIA_105900 | Afu4g10150 | 4 | 8 | 50 |
| AGU | alpha glucuronidase | GH 67 | 7 AN9286 | 56619 | ATEG_06085 | A0090026000127 | AFL2G_07114 | ACLA_017270 | NFIA_072510 | Afu5g14380 | 4 | 8 | 50 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN7135 | 210947 | ATEG_02193 | A0090011000349 | AFL2G_05136 | ACLA_054660 | NFIA_029620 | Afu4g03780 | 4 | 8 | 50 |
| XLN | endoxylanase | GH 10 |) | | ATEG_07190 | | | | NFIA_061880 | Afu3g15210 | 3 | 3 | 100 |
| AGL | alpha galactosidase | GH 27 | | | ATEG_01905 | | | | NFIA_048850 | Afu6g02560 | 3 | 3 | 100 |
| AGD | alpha glucosidase | GH 31 | AN8953 | | ATEC 09705 | A0090038000471 | AFL2G_07812 | ACLA 002040 | NEIA 047060 | 45-6-01200 | 3 | 3 | 100 |
| AMY | alpha amviase | GH 13 | 1 | | ATEG_00703 | A0090120000196 | | ACLA_094070 | NEIA 032970 | Atu2000710 | 3 | | 75 |
| GLA | olucoamylase | GH 15 | 5 | | ATEG 05980 | A0090003000321 | AFL2G 02658 | ACLA 089470 | 110000010 | And Lycon to | 3 | 4 | 75 |
| SUC | invertase/beta fructofuranosidase | GH 32 | 2 | 198063 | ATEG 07479 | A0090020000640 | AFL2G 10707 | | | | 3 | 4 | 75 |
| ABF | alpha arabinofuranosidase | GH 43 | 3 AN7781 | | ATEG_08386 | A0090005000065 | AFL2G_00086 | | | | 3 | 4 | 75 |
| BXL | beta xylosidase | GH 43 | AN8477 | | ATEG_01292 | | AFL2G_06974 | ACLA_018160 | | | 3 | 4 | 75 |
| BXL | beta xylosidase | GH 3 | AN2359 | 205670 | ATEG_05106 | A0090005000986 | AFL2G_00957 | | | Afu1g16920 | 3 | 5 | 60 |
| ABF | alpha arabinofuranosidase | GH 43 | AN2533 | - | 1750 10077 | AU090701000838 | AFL2G_06400 | | NFIA_002750 | Atu3g01660 | 3 | 5 | 60 |
| DAL EOL | peta xylośidase | GH 43 | AN10919 | | A1EG_10072 | A0090010000029 | AFL2G_11296 | | NFIA_093350 | 46-5-01920 | 3 | 5 | 60 |
| EGL | endogucanase | GRO | ANOUDO | | ATEG_09002 | A00900030013414 | AFL2G_01726 | ACLA 044470 | NFIA_040280 | A105g01630 | 3 | 0 | 50 |
| BXI | heta vulneidase | GH 43 | AN10199 | | ATEG. 00093 | A0090005000698 | AFL2G_07781 | ACLA_058100 | NEIA 0974904 | A fu8o04710/047 | 3 | 6 | 50 |
| PLY | pectate lyase | PL 3 | AN2542 | | | A0090010000706 | AFL2G 11846 | ACLA 059210 | NEIA 098670 | Afu8005910 | 3 | 6 | 50 |
| RGX | exorhamnogalacturonase | GH 28 | AN10274 | 194461 | ATEG 09025 | A0090102000139 | AFL2G 09582 | | NFIA 027700 | Afu7q06410 | 3 | 7 | 43 |
| PEL | pectin lyase | PL 1 | AN2331 | 41815 | _ | A0090010000504 | AFL2G_11666 | ACLA_013470 | NFIA_076850 | Afu5g10380 | 3 | 7 | 43 |
| AXE | acetyl xylan esterase | CE 1 | 1 AN6093 | 211544 | ATEG_09843 | A0090011000745 | AFL2G_05471 | ACLA_081220 | NFIA_099230 | Afu8g06570 | 3 | 8 | 38 |
| BGL | beta glucosidase | GH 3 | 3 AN10482 | 208871 | ATEG_06617 | A0090001000544 | AFL2G_09187 | ACLA_083710 | NFIA_054350 | Afu6g08700 | 3 | 8 | 38 |
| AGD | alpha glucosidase | GH 31 | AN0280 | 55419 | ATEG_02528 | A0090005000767 | AFL2G_00750 | ACLA_031260 | NFIA_021450 | Afu1g03140 | 3 | 8 | 38 |
| BGL | beta glucosidase | GH 3 | 1 10 10 10 7 | | | A0090038000223 | AFL2G_09023 | | | | 2 | 2 | 100 |
| MAN | encomannañase | GH 5 | AN6427 | | A1EG_09991 | 4.0000001000000 | AEL 20. 07407 | - | | | 2 | 2 | 100 |
| ABN | endosrabinanase | GH 10 GH 45 | | | | A009000500094 | AFL2G_07437 | | | | 2 | 2 | 100 |
| ABX | exparabinanase | GH 91 | 1 | | | A0090011000141 | AFI 2G 04929 | | | | 2 | 2 | 100 |
| BXL | beta xylosidase | GH 3 | 3 | | ATEG 07383 | A0090011000140 | AFL2G 04928 | | | | 2 | 3 | 67 |
| XLN | endoxylanase | GH 10 |) | | ATEG_08906 | A0090103000423 | AFL2G_11983 | | | | 2 | 3 | 67 |
| ABX | exoarabinanase | GH 93 | 3 | | ATEG_00891 | | | | NFIA_058060 | Afu6g12120 | 2 | 3 | 67 |
| PEL | pectin lyase | PL 1 | | | | | | ACLA_094210 | NFIA_033080 | Afu2g00800 | 2 | 3 | 67 |
| FAE SF5 | feruloyi esterase | CE 1 | | | ATEG_06644 | A0090701000884 | AFL2G_06446 | ACLA_061520 | | | 2 | 4 | 50 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | 2 | 51400 | ATEG_10016 | AU090102000092 | AFL2G_09543 | | | | 2 | 4 | 50 |
| PME | pectin methyl esterase | CE 8 | 5 | 44585 | | AU090012000749 | AFL2G_03618 | ACLA_044240 | NFIA_095020 | Atu8g01520 | 2 | 5 | 40 |

Supplemental Table 6C Cont: Detected proteins in cultures grown on wheat bran sorted by number of species that contain an orthologue. (Please see pdf file for enlargened text).

| RGAE | rhamnogalacturonan acetvi esterase | CE 12 | 2 AN2834 | | | A0090113000155 | AFL2G 08631 | | NFIA 092640 | Afu2o17250 | 2 | 5 | | 40 |
|--|--|--|--|--|---|---|---|--|---|---|---|---|---|--|
| 401 | alpha calactosidase | 0 4 23 | | 27726 | ATEC 04292 | | | ACLA 016920 | NEIA 072100 | Atu6a12920 | 2 | | | 40 |
| AGE | aipita galactosidase | 01121 | | 37730 | ATEG_04302 | | | HCD4_010020 | 100_013100 | Aluogroood | 4 | | | 40 |
| RGL | rhamnogalacturonan lyase | PL 4 | 4 | | ATEG_08610 | A0090138000119 | AFL2G_08794 | | NFIA_094270 | Afu8g00820 | 2 | 5 | | 40 |
| EXG | exo 1,3 galactanase | GH 5 | 5 | | ATEG_06369 | A0090005000423 | AFL2G_00412 | ACLA_007330 | NFIA_026860 | Afu7g05610 | 2 | 6 | | 33 |
| 00 | cellulose oxidase | GH 61 | | | ATEG 05416 | A0090023000787 | AEL2G 04596 | ACLA 047220 | NEIA 108320 | Afu4e07850 | 2 | 6 | | 33 |
| AVH | anthing of an anthing for an abudantan | OH 61 | | | ATEC 00498 | A 0000102000099 | AEL 00 40084 | ACLA 074520 | NEIA 097000 | 44-2=42770 | 2 | | | 22 |
| AVU | arabitoxyiati arabitoturationyuroiase | 0102 | 6 | | ATEG_00100 | A0050103000088 | AFL20_12201 | ACEA_071560 | NFIA_007500 | Aluzgiziio | 6 | 0 | | 33 |
| PLY | pectate lyase | PL 3 | 3 AN6106 | | ATEG_08626 | A0090038000502 | AFL2G_07839 | | NFIA_023470 | Afu1g01120 | 2 | 6 | | 33 |
| RHA | alpha rhamnosidase | GH 78 | 3 | 42916 | ATEG 03018 | A0090009000471 | AFL2G 10227 | | NFIA 018620 | Afu1o06130 | 2 | 6 | | 33 |
| EOI | andanhannan | CH C | ANEDIA | 200276 | | 4.0000005004552 | AEL 00 01447 | ACLA 094950 | NEIA 052450 | 46-0-07490 | 2 | 7 | | 20 |
| LOL | enuogiucanase | On c | J ANUZ IN | 203370 | | A0050005001555 | Art226_01447 | ACEA_001030 | NFD4_000100 | Allogorado | 4 | | | 20 |
| GLN | exo 1,6 galactanase | GH 5 | 5 AN9166 | 194447 | ATEG_10242 | A0090012000046 | AFL2G_02982 | | NFIA_072400 | Afu5g14560 | 2 | 7 | | 29 |
| EGL | endoqlucanase | GH 7 | AN3418 | | ATEG 08700 | A0090010000314 | ALF2G 11497 | ACLA 066030 | NFIA 114250 | Afu7o01540 | 2 | 7 | | 29 |
| 401 | alaba galactosidana | 0 4 22 | AN0022 | 170000 | ATEC 02427 | A0000005000217 | AEL 20, 00225 | | NEIA 022200 | A fulle01200 | | | | 20 |
| AGL | aipna galactosidase | GH 27 | ANUUZZ | 172232 | ATEG_03427 | A0090005000217 | AFL2G_00225 | | NFIA_025590 | Alu 1gu 1200 | 2 | / | | 29 |
| AXL | alpha xylosidase | GH 31 | AN7505 | | ATEG_06730 | A0090001000649 | AFL2G_09290 | ACLA_089300 | NFIA_082140 | Afu2g05400 | 2 | 7 | | 29 |
| LAC | beta galactosidase | GH 35 | 5 | 177434 | ATEG 07446 | A0090003000042 | AEL2G 02901 | ACLA 088440 | NEIA 052310 | Afu6o06660 | 2 | 7 | | 29 |
| 81/1 | hate underlidence | 011.45 | AN7076 | | ATEO OFFICE | 4.0000704000886 | AELOO 00440 | ACLA 070000 | NELA 022220 | 44-2-00020 | - | | | 20 |
| DAL | beta xylosidase | on +a | AN1215 | | ATEG_00045 | A0030701000666 | AFL20_00440 | ACLA_070000 | NFIA_033220 | A10200930 | 4 | | | 23 |
| AGU | alpha glucuronidase | GH115 | 5 AN9329 | | ATEG_09974 | A0090010000038 | AFL2G_11304 | ACLA_006360 | NFIA_025630 | Afu7g04680 | 2 | 7 | | 29 |
| MND | beta mannosidase | GH 2 | 2 AN1742 | 138876 | ATEG 06636 | A0090001000556 | AFL2G 09201 | ACLA 083570 | NFIA 054490 | Afu6q08840 | 2 | 8 | | 25 |
| BYI | hata voloridara | OH 43 | AN1870 | 179682 | ATEC 08059 | A0090003000239 | AEL 2G 02739 | ACLA 090090 | NEIA 081240 | A fu2e04480 | 2 | | | 25 |
| | bela kylosiduse | 01144 | | 110002 | A120_00000 | A0050005000255 | ALLEO_OLIOS | ACCA_030030 | 11104_001240 | Anazgottoo | | | | |
| BXL | beta xylosidase | GH 43 | 3 AN7313 | 122978 | ATEG_01188 | A0090010000582 | AFL2G_11/14 | ACLA_043260 | NFIA_096240 | Afu8g02510 | 2 | 8 | | 25 |
| BXL | beta xylosidase | GH 43 | 3 AN7864 | 47677 | ATEG_01292 | A0090102000331 | AFL2G_09750 | ACLA_072000 | NFIA_088370 | Afu2g13190 | 2 | 8 | | 25 |
| 00 | cellulase ovidese | GH 61 | AN1041 | 52688 | ATEG 00448 | A0090001000221 | AEL2G 07454 | ACLA 022980 | NEIA 012000 | Afu1012580 | 2 | 8 | | 25 |
| | | 0 | | 02000 | 200,00000 | ADDDDDDDTDDDZET | AT 220_01404 | HOLH_OLLOOD | 1012000 | Andigizooo | | | | |
| CO | cellulose oxidase | GH 61 | AN1602 | 182430 | ATEG_07790 | A0090005000531 | AFL2G_00532 | ACLA_059790 | NFIA_099510 | A108006830 | 2 | 8 | | 25 |
| EXG | exo 1,3 galactanase | GH 5 | 5 AN4052 | 202490 | ATEG_03849 | A0090003000990 | AFL2G_02039 | ACLA_031040 | NFIA_021060 | Afu1g03600 | 2 | 8 | | 25 |
| EAE SES | ferulovi esterase | CE 1 | | | ATEG 01914 | | | | | | 1 | 1 | 1 | 00 |
| POI . | beta elucosidase | 04 1 | | | ATEC 07021 | | | | | | 4 | 4 | 4 | 00 |
| DOL . | nera Ancosinase | Gna | | | ATEG_07931 | | | | | | | | | ~ |
| EGL | endoglucanase | GH S | 5 | | ATEG_05003 | | | | | | 1 | 1 | 1 | 00 |
| MAN | endomannanase | GH 5 | 5 AN3297 | | | | | | | | 1 | 1 | 1 | 00 |
| MAN | endomannanase | GH 6 | AN9276 | | | | | | | | 4 | 4 | 4 | 00 |
| 201 81 | and a damage | on c | 100010 | | | | | | | | - | | | |
| ALN | enooxyianase | GH 11 | AN3613 | | | | | | | | 1 | 1 | 1 | 00 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | 2 | | ATEG_09894 | | | | | | 1 | 1 | 1 | 00 |
| AGD | alpha olucosidasa | GH 13 | 1 | | | | AEL2G 08694 | | | | 4 | 1 | 1 | 00 |
| AGD | aipita giucosiuase | Girls | | | | | AT L20_00034 | | | | - | | | 00 |
| мыА | encoporygalacturonase | GH 28 | > | 182156 | | | | | | | 1 | 1 | 1 | 00 |
| PGX | exopolygalacturonase | GH 28 | 3 | 178172 | | | | | | | 1 | 1 | 1 | 00 |
| AGD | alpha ducosidase | GH 31 | | | ATEG 08278 | | | | | | 1 | 1 | 1 | 00 |
| | alpha glocosloado | 0.1.01 | | | | | | | | | - | | | |
| 500 | invertase/beta tructoturanosidase | GH 32 | | | ATEG_10253 | | | | | | 1 | 1 | 1 | 00 |
| ABN | endoarabinanase | GH 43 | 3 | | ATEG_03688 | | | | | | 1 | 1 | 1 | 00 |
| BXI | beta vylosidase | GH 41 | 1 | | ATEG 10193 | | | | | | 1 | 1 | 1 | 00 |
| 2 | 2 | 011 13 | AN4477 | | | | | | | | | | | 00 |
| 1 | 1 | On +a | 2 AN1477 | | | | | | | | | | | 00 |
| CO | cellulose oxidase | GH 61 | | | ATEG_10194 | | | | | | 1 | 1 | 1 | 00 |
| 00 | cellulose oxidase | GH 61 | AN3860 | | | | | | | | 1 | 1 | 1 | 00 |
| 43/01 | and have deep and have deep and have a | 011.02 | 4417000 | | | | | | | | - | | | |
| AXN | arabinoxyian arabinoturanonydroiase | GH 64 | ANTSUD | | | | | | | | 1 | 1 | 1 | 00 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | 2 | | ATEG_10379 | | | | | | 1 | 1 | 1 | 00 |
| URH | unsaturated rhampogalacturonyl hydrolas | GH 105 | 5 AN7828 | | | | | | | | 1 | 1 | 1 | 00 |
| 400 | alaha aluguraaidaga | CH 115 | | | ATEC 04255 | | | | | | - | | | 00 |
| AGU | aipna giucuronidase | Gnitte | 2 | | ATEG_04355 | | | | | | 1 | 1 | 1 | 00 |
| PLY | pectate lyase | PL 3 | 3 AN8453 | | | | | | | | 1 | 1 | 1 | 00 |
| RGAE | rhamoogalacturonan acetyl esterase | CE 12 | 2 | | | A0090003001268 | AEL2G 01797 | | | | 1 | 2 | | 50 |
| A107 | alaka ang daga | 011.45 | 4112200 | | ATEO 00040 | | | | | | | - | | 50 |
| AMT | alpha amylase | On 13 | 5 MN3300 | | ATEG_02515 | | | | | | | 4 | | 50 |
| AGL | alpha galactosidase | GH 36 | 5 AN9035 | | ATEG_02312 | | | | | | 1 | 2 | | 50 |
| ABF | alpha arabinofuranosidase | GH 51 | | 50979 | ATEG 03540 | | | | | | 1 | 2 | | 50 |
| AGU | alpha glucuropidana | GH115 | | | | 0.0000001000267 | AEL2G 07498 | | | | 4 | 2 | | 50 |
| AGO | aipita giucuronidase | GHTT | 2 | | | A0050001000207 | AFL20_07450 | | | | | 4 | | 50 |
| CO | cellulose ovidase | | 1 AN6428 | | ATEG 08077 | | | | | | 1 | | | 50 |
| | CCHARGE CARGUSC | GH 61 | | | ALCO_00011 | | | | | | | 2 | | 00 |
| BGL | beta olucosidase | GH 61 GH 3 | 3 AN1804 | | A120_00011 | A0090026000123 | AFL2G 07119 | | | | 1 | 2 | | 331 |
| BGL | beta glucosidase | GH 61 GH 3 CH 3 | 3 AN1804 | | ATEG 02724 | A0090026000123 | AFL2G_07119 | | | | 1 | 3 | | 33 |
| BGL | beta glucosidase beta glucosidase | GH 61 GH 3 GH 3 | 3 AN1804 | | ATEG_02724 | A0090026000123 A0090005000337 | AFL2G_07119 AFL2G_00334 | | | | 1 | 3 | | 33 |
| BGL GLA | beta glucosidase beta glucosidase glucoamylase | GH 61 GH 3 GH 3 GH 15 | AN1804 AN7402 | | ATEG_02724 | A0090026000123 A0090005000337 | AFL2G_07119 AFL2G_00334 | ACLA_049360 | NFIA_105910 | | 1 | 3 | | 33 33 33 |
| BGL GLA FAE SF7 | beta glucosidase beta glucosidase glucoamylase feruloyi esterase | GH 61 GH 3 GH 3 GH 15 faeA | 3 AN1804 3 AN7402 | 51662 | ATEG_02724 ATEG_08907 | A0090026000123 A0090005000337 A0090001000207 | AFL2G_07119 AFL2G_00334 AFL2G_07436 | ACLA_049360 | NFIA_105910 | | 1 | 2 3 3 3 4 | | 33 33 33 25 |
| BGL GLA FAE SF7 GF | beta glucosidase beta glucosidase glucoamylase feruloyi esterase ducuronovi esterase | GH 61 GH 3 GH 3 GH 15 faeA CF15 | 3 AN1804 3 AN7402 | 51662 | ATEG_02724 ATEG_08907 ATEG_08907 | A0090026000123 A0090005000337 A0090001000207 | AFL2G_07119 AFL2G_00334 AFL2G_07436 | ACLA_049360 | NFIA_105910 | Afu6014390 | 1 | 2 3 3 3 4 4 | | 33 33 33 25 25 |
| BGL BGL GLA FAE SF7 GE | beta glucosidase beta glucosidase glucoamylase glucoamylase glucuronoyl esterase glucuronoyl esterase | GH 61 GH 3 GH 3 GH 15 faeA CE15 | 3 AN1804 3 AN7402 | 51662 | ATEG_02724 ATEG_08907 ATEG_00945 | A0090026000123 A0090005000337 A0090001000207 | AFL2G_07119 AFL2G_00334 AFL2G_07436 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 | Afu6g14390 | 1 1 1 1 | 23333344 | | 33 33 25 25 |
| BGL BGL GLA FAE SF7 GE PLY | beta glucosidase beta glucosidase glucoamylase feruloyi esterase glucuronoyi esterase pectate lyase | GH 61 GH 3 GH 15 GH 15 faeA CE15 PL 1 | AN1804 AN7402 AN7402 AN0741 | 51662 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 | Afu6g14390 | 1 1 1 1 1 1 | 2 3 3 3 4 4 4 | | 33 33 25 25 25 25 |
| BGL BGL GLA FAE SF7 GE PLY PGA | beta glucosidase beta glucosidase glucoanylase foruloyi esterase glucuronoyi esterase pectate lyase endopolygalacturonase | GH 61 GH 3 GH 15 faeA CE15 PL 1 GH 26 | AN1804 AN7402 AN7402 AN0741 | 51662 | ATEG_02724 ATEG_08907 ATEG_08905 ATEG_08834 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_04049 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 NFIA_095620 | Afu6g14390 Afu8g01970 | 1 1 1 1 1 1 | 2 3 3 4 4 4 5 | | 33 33 25 25 25 25 20 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX | Losso ondaz beta glucosidase beta glucosidase glucosingas feruloyi esterase glucuronyi esterase poctate lyas endopolyaliacturonase exorhamnoalacturonase | GH 61 GH 3 GH 3 GH 15 faeA CE15 PL 1 GH 28 GH 28 GH 28 | AN1804 AN7402 AN7402 | 51662 52219 42917 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 A009000900470 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_05417 AFL2G_04049 AFL2G_10228 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 | Afu6g14390 Afu8g01970 Afu1g06140 | 1 1 1 1 1 1 1 1 | 2 3 3 4 4 4 5 5 | | 33 33 25 25 25 20 20 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXI AXI | bela glucosidase beta glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase glucosidase excitamogalacturonase excitamogalacturonase | GH 61 GH 3 GH 15 GH 15 faeA CE15 PL 1 GH 26 GH 26 GH 26 | AN1804 AN7402 AN7402 AN0741 | 51662 52219 42917 43142 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 A0090009000470 A0090701000679 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_05417 AFL2G_10228 AFL2G_10228 AFL2G_06229 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_012890 | Afu6g14390 Afu8g01970 Afu1g06140 | 1 1 1 1 1 1 1 1 1 | 2 3 3 4 4 4 4 5 5 5 | | 33 33 25 25 25 20 20 20 |
| BGL BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL AXL VO EQU | Losso ordaz beta glucosidase beta glucosidase glucosingas feruloyi esterase glucuronyi esterase poctate lyas endopolyaliacturonase exorhamrogalacturonase alpha xylosidase | GH 61 GH 3 GH 3 GH 15 faeA CE15 PL 1 GH 25 GH 21 GH 21 GH 21 GH 21 | AN1804 AN7402 AN7402 AN0741 AN0741 | 51662 52219 42917 43342 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08390 | A0090026000123 A0090005000337 A009001000207 A0090011000673 A0090023000161 A0090009000470 A0090701000639 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_05417 AFL2G_04049 AFL2G_10228 AFL2G_06239 | ACLA_049360 ACLA_087520 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_010590 NFIA_032680 | Afu6g14390 Afu8g01970 Afu1g06140 | 1 | 233344444455555555555555555555555555555 | | 33 33 25 25 25 20 20 20 20 |
| BGL GLA FAE SF7 GE PLY PGA R0X AXL XG EGL | best glucoadase beta glucoadase glucoanylase feruloy testrase glucoranyl setrase pectate yase endopolygaleurunnase aipha sylvaidase aipha sylvaidase aipha sylvaidase | GH 61 GH 3 GH 3 GH 3 CE15 PL 1 GH 28 GH 28 GH 28 GH 31 GH 74 | AN1804 AN7402 AN7402 AN0741 AN0741 AN0741 | 51662 52219 42917 43342 206333 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08390 ATEG_08390 ATEG_04708 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 A0090009000470 A0090701000639 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_05417 AFL2G_04049 AFL2G_10228 AFL2G_06239 | ACLA_049360 ACLA_087520 ACLA_044310 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_034990 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 | 1 1 1 1 1 1 1 1 1 1 1 1 1 | 2 3 3 4 4 4 5 5 5 5 5 5 5 | | 33 33 25 25 25 20 20 20 20 20 |
| BGL BGL BGL GLA FAE SF7 GE PLY PGA ROX AXL XG EGL AFC | beta glucosadas beta glucosadase glucosanjase faruioyi setarase glucosanjase glucoranoyi setarase glucoranoyi setarase andopolygalautunonase exorhamogalautunonase exorhamogalautunonase aujha xylosidase alpha sulosidase | GH 61 GH 3 GH 3 GH 15 faeA CE15 PL 1 GH 26 GH 26 GH 27 GH 31 GH 74 GH 95 | AN1804 AN7402 AN7402 AN0741 AN14 | 51662 52219 42917 43342 206333 184037 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08834 ATEG_08390 ATEG_04708 ATEG_01560 | A0090026000123 A0090005000337 A009001000207 A0090011000673 A009002000161 A009002000161 A009002000161 A009005000382 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_05417 AFL2G_04049 AFL2G_04049 AFL2G_06239 AFL2G_00373 | ACLA_049360 ACLA_087520 ACLA_044310 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_094990 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 | | 2 3 3 4 4 4 5 5 5 5 5 5 5 5 5 5 5 | | 33 33 25 25 25 20 20 20 20 20 20 20 |
| BGL BGL BGL GE FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? P | beta glucoaddase beta glucoaddase glucoarryllase fruitory esterase glucarroyl esterase polatie tyse endopolyailacturonase endopolyailacturonase alpha sylvostase alpha sylvostase alpha fucosidase | GH 61 GH 3 GH 3 GH 3 GH 3 GH 26 GH 26 GH 26 GH 26 GH 31 GH 74 GH 95 GH 95 GH 95 GH 95 GH 95 GH 95 | AN1804 AN7402 AN7402 AN0741 AN0741 AN0741 AN5061 AN8149 AN380 | 51662 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08390 ATEG_08390 ATEG_04708 ATEG_01560 ATEG_04963 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A009002300167 A0090009000470 A0090005000382 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_00373 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_094990 NFIA_102600 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu4g13770 | | 2 3 3 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | | 33 33 25 25 25 20 20 20 20 20 20 20 |
| BGL BGL BGL GLA FAE SF7 GE PDLY POA RGX AXL XG EGL AFC ? FVG | heta glucostdase beta glucostdase glucoamydase fruibyr asterase glucurnoyi esterase endopolyais duronase exchramogalacturonase abha sylvaidase abha sylvaidase abha to endoglucanase abha to forunase | GH 61 GH 3 GH 15 GH 16 7 CE15 PL 1 OH 26 GH 26 GH 31 GH 74 GH 95 GH 95 G | AN1804 AN7402 AN7402 AN7402 AN0741 AN0741 AN5061 | 51662 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_04963 ATEG_04963 ATEG_04963 | A0090026000123 A0090005000337 A009001000207 A0090011000673 A009002000161 A009002000161 A009002000639 A009005000382 | AFL2G_07119 AFL2G_07436 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_10228 AFL2G_06239 AFL2G_06239 AFL2G_06239 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_0132680 NFIA_032680 NFIA_094990 NFIA_102600 NFIA_048850 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu8g01490 | | 2 3 3 4 4 4 5 5 5 5 5 5 5 5 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 20 17 |
| BGL BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? EXG | beta glucostalase beta glucostalase glucoamyisae feruloyi esterase glucurnoyi esterase pectate yase endopolyaia duronase endopolyaia duronase apha syloxiaase xyloglucan active endoglucanase ajha fucostase beta 1.8 glucanase | GH 61 GH 3 GH 3 GH 12 GH 26 GH 26 GH 26 GH 26 GH 26 GH 30 GH 90 GH | AN1804 AN7402 AN7402 AN0741 AN0741 AN0741 AN0741 AN0741 AN0749 AN0749 AN0349 AN0360 AN0377 | 51662 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08834 ATEG_04708 ATEG_04708 ATEG_01580 ATEG_09844 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 A0090023000161 A00900701000639 A009005000382 A0090011000757 | AFL2G_07119 AFL2G_07034 AFL2G_07436 AFL2G_07436 AFL2G_0449 AFL2G_04049 AFL2G_04239 AFL2G_06239 AFL2G_00373 AFL2G_05484 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_094990 NFIA_102600 NFIA_084850 | Atu6g14390 Atu8g01970 Atu1g06140 Atu8g01490 Atu8g01490 Atu4g13770 Atu2g09350 | | 2 3 3 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | | 33 33 25 25 25 20 20 20 20 20 20 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PQA RQX AXL XG EGL AFC ? EXG BXL | bela gluzostáse bela gluzostáse plucompisée ficulty esterase glucurony esterase glucurony esterase endopolygalacturonase endopolygalacturonase ajba zyskásse zyhoglucan active endoglucanase ajbh fucostáse bela zyskáse | GH 61 GH 3 GH 15 GH 15 TaeA CE15 OH 26 GH 26 GH 26 GH 27 GH 31 GH 74 GH 31 GH 74 GH 33 GH 43 GH 43 | 3 AN1804 3 AN7402 4 AN5061 5 AN5061 5 AN8149 3 AN3360 5 AN3777 | 51662 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08834 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_08844 ATEG_08844 ATEG_08844 | A0090026000123 A0090005000337 A0090001000207 A0090011000673 A0090023000161 A00900000470 A009005000382 A009005000382 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_00373 AFL2G_05484 AFL2G_12122 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 ACLA_052760 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018500 NFIA_0132680 NFIA_094990 NFIA_102600 NFIA_060490 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu4g13770 Afu2g09350 Afu6g14550 | | 2 3 3 3 3 4 4 4 5 5 5 5 5 5 5 6 6 6 6 6 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 17 17 17 |
| BGL BGL GLA FAE SF7 GE PP PGA RGX XG EGL AFC ? EXG BXL BXL | bela glucosidase bela glucosidase glucoamystes ficulto festesse glucumory esterase endopolygalacturonase abha systelase ajbha fucosidase ajbha fucosidase bela systelase bela systelase | GH 61 GH 3 GH 15 GH 15 FleeA CE15 PL 1 GH 28 GH 28 GH 31 GH 73 GH 95 GH 43 GH 45 GH 45 GH 45 | 3 AN1804 3 AN7402 4 AN7402 4 AN741 3 AN0741 3 AN0741 4 AN5061 5 AN8149 5 AN3300 5 AN3300 5 AN3777 3 AN2564 | 51662 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08804 ATEG_08834 ATEG_08834 ATEG_01580 ATEG_01580 ATEG_01580 ATEG_01580 ATEG_05083 ATEG_05083 | A099022600123 A099005000337 A099001000207 A099001000207 A09902100673 A099023000161 A099009000470 A0990701000639 A0990005000382 A099001000757 A099011000757 A099010300288 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_05417 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_05484 AFL2G_05484 AFL2G_05484 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 ACLA_087680 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_032680 NFIA_034990 NFIA_102600 NFIA_084850 NFIA_060490 NFIA_072780 | Atu6g14390 Atu8g01970 Afu1g06140 Afu8g01490 Afu8g01490 Afu2g09350 Afu6g14550 Atu6g14590 | | 2 3 3 4 4 4 4 5 5 5 5 5 5 6 8 8 6 6 6 6 | | 33 33 25 25 20 20 20 20 20 20 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PQA RGX AXL XG EGL AFC ? EXG BXL BXL ABF | bela glucosidase bela glucosidase glucoampiase ficultor testorase glucoumpor seterase endopolyapathotutorase endopolyapathotutorase abha vytostace abha vytostace bela 1.6 gluconase bela 1.6 gluconase bela 1.6 gluconase | GH 6H 6 GH 3 GH 15 GH 15 FleA CE15 PL 1 GH 22 GH 32 GH 31 GH 74 GH 74 GH 74 GH 43 GH 43 GH 43 GH 45 GH 45 | 3 AN1804 3 AN7402 4 AN741 3 AN741 4 AN5061 5 AN8149 AN360 5 AN8149 3 AN8664 AN377 3 AN2664 AN2541 | 51862 52219 42917 43342 206333 184037 37673 | ATEG_02724 ATEG_08907 ATEG_00945 ATEG_08834 ATEG_08834 ATEG_04803 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_05083 ATEG_05083 | A099022600123 A099005000337 A099001000207 A099001000207 A099001000673 A0990023000161 A0990701000639 A099000470 A09901000500382 A099011000757 A099012000350 A099012000350 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_062484 AFL2G_03257 AFL2G_03257 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 ACLA_052760 ACLA_087680 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_032680 NFIA_032680 NFIA_034990 NFIA_102600 NFIA_060490 NFIA_060490 NFIA_072780 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu4g13770 Afu2g9350 Afu2g9350 Afu8g1450 Afu5g14190 | | 2 3 3 4 4 4 5 5 5 5 5 5 5 6 8 8 8 8 8 8 8 8 8 8 8 8 | | 33 33 25 25 20 20 20 20 20 20 20 20 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? EXG BXL ABF PO | bela glucosidase bela glucosidase glucoamyske (ruculor statense glucurotor) statense exortamropalschuronase exortamropalschuronase alpha furcosidase jaba furcosidase bela sylosidase bela sylosidase bela sylosidase | GH 61 GH 2 GH 12 GH 12 GH 12 CE15 Pt 1 GH 26 GH | AN1804 AN7402 AN7402 AN0741 AN | 51662 52219 42917 4342 206333 184037 37673 206387 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_0890834 ATEG_08390 ATEG_04708 ATEG_04708 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_0406107 ATEG_028824 ATEG_02802 ATEG_04107 | A099022600123 A099005000337 A099001000207 A009001000207 A0090023000161 A00900900470 A0090701000639 A009005000382 A099011000757 A099011000757 A099011000288 A099011200028 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_0733 AFL2G_03257 AFL2G_03257 AFL2G_03257 | ACLA_049360 ACLA_087520 ACLA_044310 ACLA_052760 ACLA_087680 ACLA_099110 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_018590 NFIA_014990 NFIA_094990 NFIA_064850 NFIA_060490 NFIA_060490 NFIA_072780 | Atu6g14390 Atu8g01970 Atu1g06140 Atu8g01490 Atu8g01490 Atu8g13770 Atu8g14550 Atu8g14190 | | 2 2 3 3 3 4 4 4 4 5 5 5 5 5 5 5 6 8 8 8 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 20 20 20 20 20 20 20 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? EXG BXL BXL CO | beta glucostase beta glucostase glucoamyses forciuly astases pectate hysis endopolyaticuroase exortamogaticuroase exortamogaticuroase abeta r.6 glucostase beta 1.6 glucostase beta 1.6 glucostase beta 1.6 glucostase coltas systelidas pelas hysindase | GH 61 GH 12 GH 12 GH 12 GH 12 CE15 GH 22 GH 22 GH 22 GH 24 GH 24 GH 24 GH 24 GH 24 GH 24 GH 24 GH 24 GH 24 GH 26 GH 26 G | AN1804 AN1804 AN1402 AN0741 AN0741 AN0741 AN0741 AN38061 AN38061 AN3380 AN3380 AN3380 AN3380 AN3380 AN3380 AN3864 AN2541 AN2541 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08804 ATEG_08834 ATEG_04808 ATEG_04963 ATEG_04963 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_07920 | A099022600123 A099005000337 A099001000207 A099001000207 A099001000207 A099000000011 000500 A0990005000382 A09900100075 A0990100028 A09901200028 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_00239 AFL2G_00239 AFL2G_00239 AFL2G_05484 AFL2G_05484 AFL2G_05484 AFL2G_0325 | ACLA_049360 ACLA_067520 ACLA_044310 ACLA_052760 ACLA_087680 ACLA_099110 | NFIA_105910 NFIA_060260 NFIA_018590 NFIA_018590 NFIA_018590 NFIA_02680 NFIA_024990 NFIA_102600 NFIA_060490 NFIA_060490 NFIA_072780 NFIA_044390 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu4g13770 Afu2g9350 Afu2g9350 Afu6g1450 Afu5g14190 | | 2 2 3 3 3 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6 8 6 8 6 8 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 20 17 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? EXG BXL BXL ABF CO CO | bela glucosidase bela glucosidase glucoampiasea ficuloy testorase glucoumpor setorase endopologia drumose endopologia drumose endopologia drumose endopologia drumose endopologia drumose bela si of glucoanse bela si of g | GH 61 GH 2 GH 12 GH 12 GH 12 FL 1 GH 22 GH 22 GH 22 GH 24 GH 31 GH 74 GH 97 GH | S AN1804 S AN7402 S AN7402 S AN7402 S AN3741 S AN0741 S AN0741 S AN0741 S AN0741 S AN0741 S AN0741 S AN0741 S AN0741 S AN1804 S A | 51662 52219 42917 43342 206333 184037 37673 206387 53797 | ATEG_02724 ATEG_08907 ATEG_00905 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_04963 ATEG_05083 ATEG_0884 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_07840 ATEG | A099022600123 A09900500337 A099001000207 A099011000273 A099011000573 A099071000639 A0990701000639 A099070100059 A09901200050 A09901200050 A09901200050 | AFL26_07119 AFL26_00334 AFL26_07436 AFL26_07436 AFL26_04049 AFL26_04049 AFL26_04028 AFL26_04239 AFL26_04239 AFL26_05484 AFL26_05484 AFL26_03257 AFL26_03056 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_052760 ACLA_087680 ACLA_087680 ACLA_099110 ACLA_073030 | NFIA_105910 NFIA_060260 NFIA_018590 NFIA_018590 NFIA_012500 NFIA_024990 NFIA_084850 NFIA_084850 NFIA_080490 NFIA_060490 NFIA_060490 NFIA_089670 | Atu8g14390 Atu8g01970 Atu1g06140 Atu8g01490 Atu8g01490 Atu8g13770 Atu2g09350 Atu8g14550 Atu5g14190 Atu2g14490 | | 2 2 3 3 3 4 4 4 4 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 17 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL AFC ? EXG BXL BXL ABF CO URH | bela glucosiase bela glucosiane glucoamyske ficulor jestirase glucoamoti jestirase exontamogalistirumase audodovjajalicumase audodovjasi curonase audodovjasi curonase audodovjasi patha fucosiase bela sylvalase bela sylvalase bela sylvalase bela sylvalase bela sylvalase bela sylvalase bela sylvalase bela sylvalase | GH 61 GH 2 GH 12 GH 15 TakeA GH 15 F CE15 PL 1 GH 25 GH 25 GH 25 GH 26 GH 26 G | AN1804 AN1804 AN1402 AN0741 AN0741 AN0741 AN38061 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08304 ATEG_08304 ATEG_08304 ATEG_04903 ATEG_04903 ATEG_04903 ATEG_05083 ATEG_05083 ATEG_05083 ATEG_05083 ATEG_07920 ATEG_07920 ATEG_07920 | A099022600123 A099005000337 A099001000207 A099001000673 A099001000673 A099003000470 A0990701000639 A099005000382 A099011000757 A099011000757 A099011000757 A099011000757 A099012000500 A099012000500 A099012000500 A099012000500 A09902300056 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_00239 AFL2G_00239 AFL2G_00239 AFL2G_00373 AFL2G_00373 AFL2G_0326484 AFL2G_12122 AFL2G_03026 AFL2G_03026 AFL2G_03026 | ACLA_049360 ACLA_067520 ACLA_067520 ACLA_044310 ACLA_052760 ACLA_057680 ACLA_099110 ACLA_072030 ACLA_072870 | NFIA_105910 NFIA_060260 NFIA_013060 NFIA_013060 NFIA_013060 NFIA_024990 NFIA_084850 NFIA_084850 NFIA_084850 NFIA_084430 NFIA_089670 | Atu6g14390 Atu8g01970 Atu1g06140 Atu8g01490 Atu8g01490 Atu8g14370 Atu5g1450 Atu5g14190 Atu2g14490 Atu2g14490 | | 2 3 3 3 4 4 4 4 4 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 20 17 17 17 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL SGE EXG BXL BXL BXL BXL CO CO URH | beta glucostase beta glucostase glucoamystes forculyr saferase podato hysia exchamogalacturonase exchamogalacturonase exchamogalacturonase aba korstase beta 15 gluconase beta 15 gluconase beta 15 gluconase beta 15 gluconase beta 15 gluconase celluses exclase celluses exclase | GH 61 GH 2 GH 12 GH 12 GH 14 PL 1 GH 26 GH 20 GH | S AN1804 S AN7402 S AN7402 AN0741 S AN0741 S AN0 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08945 ATEG_0834 ATEG_0834 ATEG_0834 ATEG_0834 ATEG_09834 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_05083 ATEG_09844 ATEG_02882 ATEG_07920 ATEG_07920 ATEG_07920 | A099022600123 A099001000207 A099001000207 A099001000207 A09900100050 A09900100050 A09900100050 A09900100050 A0990100050 A09901200050 A09901200050 A09901200050 A09901200050 A09901200050 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_06239 AFL2G_06239 AFL2G_06239 AFL2G_05484 AFL2G_03257 AFL2G_08019 AFL2G_08019 AFL2G_08019 AFL2G_08019 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_087680 ACLA_052760 ACLA_052760 ACLA_072870 ACLA_072870 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_015620 NFIA_032680 NFIA_034990 NFIA_034990 NFIA_060490 NFIA_060490 NFIA_072780 NFIA_072780 NFIA_072780 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu4g13770 Afu2g09350 Afu6g14550 Afu5g14190 Afu2g14490 Afu2g14490 | | 2 3 3 3 4 4 4 4 4 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| BGL BGL GLA FAE SF7 GGE PLY PGA RGX AXL XX G EGL AFC ? EXG BXL BXL ABF CO URH | bela glucosisse bela glucosisse glucoamystes ficulty esterase glucourty esterase endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones endopolysacturiones bela 1,6 glucoanse bela 2,9 stolates bela 1,6 glucoanse bela 2,9 stolates bela 3,9 stolates bela 4,9 stolates bela 4,9 stolates bela 4,9 stolates bela 4,9 stolates bela 4,9 stolates bela 4,9 stol | GH 61 GH 2 GH 12 GH 12 GH 12 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH | 3 AN1804 3 AN1804 3 AN7402 4 AN5061 5 AN8149 3 AN3360 5 AN3777 3 AN3564 4 AN5264 4 AN5264 4 AN5265 5 AN3777 3 AN3564 5 AN3777 3 AN3565 5 AN3777 3 AN3565 5 AN3555 5 AN35 | 51862 52219 42917 43342 206333 184037 37673 206387 53797 41877 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08934 ATEG_0834 ATEG_0834 ATEG_0834 ATEG_0834 ATEG_0834 ATEG_0845 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0844 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845 ATEG_0845ATEG_0845ATEG_0845 ATEG_0845ATEG_0845ATEG_0845ATEG_0845ATEG_0845ATEG_0845AT | AC99902500123 AC9900500537 AC99001000207 AC99001000207 AC99001000207 AC9900100050 AC9900200050 AC9900100059 AC99001200050 AC99001200050 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 AC99001200055 | AFL2G_07119 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_04049 AFL2G_04028 AFL2G_04028 AFL2G_04028 AFL2G_04028 AFL2G_04028 AFL2G_03026 AFL2G_03026 AFL2G_03026 AFL2G_04624 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_052760 ACLA_087680 ACLA_087680 ACLA_073030 ACLA_072870 ACLA_077810 | NFIA_105910 NFIA_060260 NFIA_018590 NFIA_018590 NFIA_018590 NFIA_018450 NFIA_084850 NFIA_080490 NFIA_080490 NFIA_0727880 NFIA_080490 NFIA_089670 NFIA_089840 | Atu5g14390 Atu3g01970 Atu1g06140 Atu1g06140 Atu2g01490 Atu2g1490 Atu2g14490 Atu2g14490 | | 2 3 3 3 4 4 4 4 4 5 5 5 5 5 5 6 8 8 8 8 8 8 8 8 8 8 8 8 8 | | 33 33 25 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XG EGL XFC ? EXG BXL BXL ABF CO CO URH URH PLY PLY | bela glucosiase bela glucosiase glucoamyske (returby elatrase glucuropic testrase porto) yeak contantopic testrase contantopic turonase angla sylostican sylogican active endopicanase alba farostase bela sylosticase bela sylosti | GH 61 GH 2 GH 2 GH 15 TakeA CE15 PL 1 GH 25 GH 25 GH 25 GH 25 GH 25 GH 26 GH 2 | AN1804 AN7402 AN7402 AN0741 AN5061 AN5061 AN5061 AN3360 AN3360 AN3360 AN3360 AN3360 AN3360 AN3360 AN3564 AN9524 AN9524 AN9524 AN9524 AN9524 AN9524 AN9524 AN9524 AN9525 AN9533 AN6748 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 41877 | ATEG_02724 ATEG_02724 ATEG_00945 ATEG_00945 ATEG_0834 ATEG_04708 ATEG_04708 ATEG_04708 ATEG_04983 ATEG_04983 ATEG_04983 ATEG_05083 ATEG_06107 ATEG_05083 ATEG_07920 ATEG_07920 ATEG_07920 ATEG_07920 | AC990025000123 AC99005000337 AC99001000207 AC99001000207 AC99001000050 AC9900100050 AC9900100050 AC9900100053 AC99011000757 AC99011000053 AC9901200050 AC99021200050 AC99021200050 AC99021200050 AC990112000163 AC99011000063 AC99011000063 AC99011000063 | AFL2G_00334 AFL2G_00334 AFL2G_07436 AFL2G_07436 AFL2G_04049 AFL2G_04049 AFL2G_04049 AFL2G_04049 AFL2G_04049 AFL2G_04033 AFL2G_0584 AFL2G_0326 AFL2G_03026 AFL2G_03026 AFL2G_03026 AFL2G_0461 | ACLA_049360 ACLA_087520 ACLA_052760 ACLA_052760 ACLA_05760 ACLA_099110 ACLA_073030 ACLA_073030 ACLA_077810 | NFIA_105910 NFIA_060260 NFIA_060260 NFIA_05620 NFIA_032800 NFIA_032800 NFIA_04390 NFIA_064390 NFIA_072780 NFIA_08940 NFIA_089840 NFIA_089840 NFIA_089840 NFIA_089840 | Afu8g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu8g01490 Afu8g01490 Afu8g01490 Afu8g01490 Afu8g1450 Afu8g1450 Afu8g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 | | 2 3 3 3 4 4 4 4 5 5 5 5 5 5 5 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 | | 33 33 25 25 20 20 20 20 20 20 17 17 17 17 17 17 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE FAE SF7 GE PDA RGX AXL XG EGL AFC ? EXG BXL BXL BXL CO CO URH URH PLY EGL EGL | beta glucosisse beta glucosisse glucoamyske ficulty starses plucital yske dodobygatal curonase exortamogatichuronase abha vykoticas abha vykoticas beta size dodoburanase abha tucosisse beta size dodoburanase beta size abha valitase beta size abha valitase celutese oxitase unashurated mamogated uroyi hydroles exotoburase abha | GH 61 GH 2 GH 2 GH 15 GH 15 FatA PL 1 PL 1 PL 1 PL 1 OH 22 GH 22 GH 22 GH 32 GH 32 GH 32 GH 42 GH 42 GH 41 GH 41 GH 41 GH 10 GH 10 G | 3 AN1804 3 AN7402 AN740 AN74 | 51862 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08384 ATEG_08834 ATEG_08834 ATEG_04833 ATEG_04833 ATEG_04833 ATEG_04833 ATEG_05833 ATEG_05833 ATEG_07035 ATEG_02892 ATEG_06314 ATEG_06314 | ACG96025000123 ACG96005000337 ACG96001000207 ACG96001000207 ACG96001000673 ACG960023000161 ACG9600900470 ACG96005000470 ACG9600100065 ACG9601200056 ACG960120056 ACG960120056 ACG960120056 ACG960050050 ACG960120056 ACG960050 ACG960120056 ACG960050 ACG960120056 ACG960050 ACG96050 ACG960050 | AFL26,07119 AFL26,0034 AFL26,0034 AFL26,07436 AFL26,05417 AFL20,0494 AFL20,1028 AFL20,1028 AFL20,0128 AFL20,0128 AFL20,0128 AFL20,0128 AFL20,0128 AFL20,00257 AFL20,00257 AFL20,00257 AFL20,00257 AFL20,00257 AFL20,00257 AFL20,00257 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_044310 ACLA_052760 ACLA_052760 ACLA_07810 ACLA_07810 ACLA_07810 ACLA_07810 ACLA_07810 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_032680 NFIA_032680 NFIA_0326490 NFIA_026490 NFIA_026490 NFIA_027780 NFIA_089670 NFIA_089640 NFIA_059640 NFIA_059640 | Afu6g14390 Afu8g01970 Afu8g01970 Afu8g01490 Afu4g13770 Afu2g09350 Afu5g14550 Afu5g14190 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 | | 2 3 3 3 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 | | 33 33 25 25 20 20 20 20 20 20 20 17 17 17 17 17 17 17 17 17 17 17 17 |
| BGL BGL GLA FAE SF7 GE FAE SF7 GE RGX XXD EGL AFC ? ENG BNL BNL BNL ABF CO CO URH PLY PLY PLY | beta glucositase beta glucositase glucoamytes (molyo startase sea pectato kysas endopolyatalcuronase exontamogitachuronase exontamogitachuronase ajaha frusositase ajaha frusositase beta ryskatase da ryskatase da syskatase celluse oxidas celluse oxidas celluse oxidas exotamogitachuronj kydrolas pectato kysas endopularita | GH 61 GH 2 GH 2 GH 12 GH 12 FateA CE12 FL 1 GH 22 GH 22 GH 22 GH 22 GH 22 GH 22 GH 24 GH 24 GH 26 GH 2 | 3 AN1804 3 AN17402 AN7402 AN0741 3 AN0741 4 AN5061 4 AN5061 4 AN5061 4 AN390 4 AN390 4 AN3924 4 AN2341 4 AN2341 4 AN2341 4 AN2341 4 AN2342 4 AN23854 4 AN19524 5 AN19525 5 AN19526 5 AN19556 5 AN19556 | 51862 52219 42917 43342 206333 184037 37673 206387 53797 41877 214663 17000 | ATEG_02724 ATEG_02724 ATEG_09907 ATEG_09945 ATEG_09834 ATEG_08334 ATEG_08334 ATEG_04708 ATEG_04708 ATEG_04708 ATEG_04903 ATEG_04903 ATEG_05083 ATEG_05083 ATEG_07920 ATEG_01035 ATEG_04390 ATEG_0490 ATEG_040 ATEG_040 ATEG_0400 A | AC990025000123 AC99005000337 AC99001000207 AC99001000207 AC99001000239 AC99001000039 AC99001000039 AC99001000039 AC99001000039 AC99001000038 AC990110000757 AC99011000053 AC99011000053 AC990112000050 AC990023000053 AC990112000053 AC990112000053 AC99011200055 AC99011200055 AC99011200055 AC99011200055 AC99011200055 AC99011200055 AC99011200055 | AFL26_07119 AFL26_07436 AFL26_07436 AFL26_07436 AFL20_065417 AFL20_0402 AFL20_0402 AFL20_0402 AFL20_04023 AFL26_06239 AFL26_06244 AFL20_07306 AFL20_07306 AFL20_07306 AFL20_07306 | ACLA_049360 ACLA_067520 ACLA_067520 ACLA_052760 ACLA_052760 ACLA_057680 ACLA_077810 ACLA_077810 ACLA_077810 ACLA_061310 ACLA_061310 | NFIA_105910 NFIA_060260 NFIA_018590 NFIA_018590 NFIA_0130800 NFIA_024000 NFIA_024000 NFIA_024000 NFIA_024000 NFIA_02570 NFIA_02570 NFIA_025910 NFIA_025910 | Afu6g14390 Afu8g01970 Afu1g06140 Afu1g06140 Afu8g01490 Afu4g13770 Afu2g1490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14950 | | 2 3 3 3 4 4 4 4 4 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| BGL BGL GLA FAE SF7 GK FAE SF7 GG FAE SF7 PQA FQA PQA FQA PGA ARC ? EVG BXL BXL ABF CO CO URH URH FLY EGL XLIN | bela glucosiase bela glucosiase glucoamyske (returby elatrase glucuropic teatrase glucuropic teatrase contamoglacturonase and ha vytostase ha turostase bela systektase bela systektase bela systektase bela systektase da systektase da systektase bela systektase da systektase da systektase bela systektase da systektase bela systektase da systektase da systektase bela systektase da | GH 61 GH 23 GH 15 GH 15 TakeA CE10 FlakeA CE10 FlakeA CE10 FlakeA GH 25 GH 25 GH 25 GH 25 GH 26 GH 26 | 3 AN1804 5 AN7402 AN0741 5 AN0741 5 AN0741 5 AN0741 5 AN0741 5 AN0741 5 AN0741 5 AN0741 5 AN0505 5 AN0149 5 AN0254 5 AN0255 5 AN025 | 51662 52219 42917 43342 206333 184037 37673 37673 206387 53797 41877 214608 171269 | ATEG_02724 ATEG_08907 ATEG_09807 ATEG_09830 ATEG_0830 ATEG_0830 ATEG_0830 ATEG_04903 ATEG_04903 ATEG_04903 ATEG_04903 ATEG_02882 ATEG_02882 ATEG_02882 ATEG_02892 ATEG_08314 ATEG_02892 | AC990025000123 AC990005000337 AC990001000207 AC99001000207 AC99001000500 AC99001000500 AC99001000500 AC99001000500 AC990012000050 AC990012000050 AC990012000050 AC990012000050 AC990012000050 AC990012000050 AC99001000051 | AFL26,07119 AFL26,0034 AFL26,07436 AFL26,05417 AFL26,05417 AFL20,0469 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0428 AFL20,0448 AFL20,0448 AFL20,0448 | ACLA_049360 ACLA_067520 ACLA_067520 ACLA_057520 ACLA_057520 ACLA_057680 ACLA_076760 ACLA_072870 ACLA_078710 ACLA_078710 ACLA_078710 | NFIA_105910 NFIA_060280 NFIA_018590 NFIA_018590 NFIA_0284050 NFIA_028450 NFIA_064450 NFIA_064450 NFIA_062470 NFIA_08940 NFIA_08940 NFIA_089501 NFIA_085010 | A fu6g14390 A fu8g01970 A fu1g06149 A fu1g06149 A fu8g01490 A fu8g01490 A fu8g14190 A fu8g14190 A fu8g14190 A fu8g1429 A fu8g1429 A fu8g142210 | | 2 2 3 3 3 3 4 4 4 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 | | 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| BGL BGL GLA FAE SF7 GE PLY PGA RGX AXL XX EGL AXC P EXG BNL BNL ABF CO CO URH URH NLH ABF | bela glucosiase bela glucosiase glucoamyske (mulyo starsas) glucounoy festinase exontamogalacturonase exontamogalacturonase abha kyskase baha kyskase baha kyskase baha sizekase bela sylakase bela sylakase amaturted hamogalacturonyi hydroles pelate hyses | GH 61 GH 12 GH 12 GH 15 GH 12 F1 14 F1 1 GH 22 GH 22 GH 22 GH 22 GH 22 GH 22 GH 22 GH 24 GH 25 GH 26 GH 26 G | 3 AN1804 5 AN7402 AN0741 5 AN7402 AN0741 5 AN70741 5 AN5061 AN5061 AN5061 AN3300 5 AN777 5 AN777 5 AN7764 AN3524 AN3524 AN1230 AN1237 AN1237 | 51862 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 171269 38549 38549 | ATEG_02724 ATEG_08807 ATEG_08807 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_06834 ATEG_0780 ATEG_0780 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 | AC990025000123 AC99001500337 AC99001000207 AC99001000207 AC99001000503 AC99002300161 AC9900900470 AC990701000639 AC990001000053 AC9901200050 AC99001200050 AC99001200050 AC99001200050 AC99001200050 AC99001000053 AC99001000053 AC99001000053 AC99001000153 AC99001000153 AC99001000153 | AFL2G, 0034 AFL2G, 0034 AFL2G, 0034 AFL2G, 0034 AFL2G, 01436 AFL2G, 04417 AFL2G, 04417 AFL2G, 04417 AFL2G, 0427 AFL2G, 04514 AFL2G, 04513 AFL2G, 04514 AFL2G, 0451 AFL2G, 0451 AFL2G, 0451 AFL2G, 0451 AFL2G, 0451 AFL2G, 0451 AFL2G, 07347 AFL2G, 07347 AFL2G, 07347 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_057520 ACLA_057680 ACLA_07870 ACLA_07870 ACLA_07870 ACLA_07870 ACLA_078710 ACLA_085410 ACLA_085410 | NFIA_105910 NFIA_060260 NFIA_018590 NFIA_018590 NFIA_028600 NFIA_028600 NFIA_04990 NFIA_04990 NFIA_04990 NFIA_04990 NFIA_05900 NFIA_05900 NFIA_05910 NFIA_05910 NFIA_05910 | Afu6g14390 Afu6g01970 Afu6g01970 Afu6g01970 Afu6g01490 Afu6g1490 Afu6g1450 Afu6g1450 Afu6g1450 Afu6g14100 Afu6g14100 Afu6g14100 Afu6g149 | | 2 3 3 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 | | 33 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| Bisi, Bisi, GLA FAESF7 GE PLY POA ROX AXL XXD EQL, AFC ? ENG BXL BXL ABF CO URH PLY EGL XUN ABF BGL | bets glucostase bets glucostase glucomytes forcivoj estesae potate bysas endopolyakturonase dopolyakturonase dopolyakturonase dopolyakturonase dopolyakturonase dopolyakturonase dobolykotase dela 1 d gluconase bets 1 d gluconase bets 1 d gluconase dobolykotase dobole dopolyakturonase dobole do dopolyakturon dopolyakturonastase celtuse oxtase unasturind rhangaschurony hydrates pectate byase endoplucase endoplucase | GH 61 GH 2 GH 12 GH 12 GH 12 GH 12 GH 22 GH 22 GH 22 GH 24 GH 31 GH 31 GH 31 GH 43 GH 43 GH 45 GH 105 GH 105 GH 105 GH 11 GH 11 GH 12 GH 1 | 3 AN1804 5 AN7402 AN0741 5 AN7402 5 AN7402 5 AN7402 5 AN7402 6 AN0741 6 AN0741 6 AN0549 6 AN1505 6 AN0524 6 AN0525 6 AN0545 6 AN0555 6 AN0555 | 51662 52219 42917 43342 206333 184037 37673 37673 206387 53797 41877 214608 171269 38549 131747 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08844 ATEG_06844 ATEG_06844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08846 ATEG_0 | AC99002600123 AC9900100207 AC99001000207 AC990011000573 AC990023000161 AC990023000161 AC990023000161 AC990023000161 AC990023000500382 AC99012000500 AC99001000115 AC99001000115 | AFL2G, 07119 AFL2G, 07136 AFL2G, 07136 AFL2G, 07136 AFL2G, 07136 AFL2G, 04049 AFL2G, 04049 AFL2G, 04049 AFL2G, 0122 AFL2G, 00517 AFL2G, 00517 AFL2G, 00517 AFL2G, 00514 AFL2G, 00547 AFL2G, 00547 AFL2G, 00547 AFL2G, 05447 AFL2G, 07786 AFL2G, 07786 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_087520 ACLA_057520 ACLA_057680 ACLA_070810 ACLA_070810 ACLA_077810 ACLA_077810 ACLA_025110 ACLA_025110 | NFIA_105910 NFIA_060280 NFIA_018590 NFIA_018590 NFIA_028450 NFIA_028450 NFIA_028450 NFIA_08450 NFIA_08450 NFIA_08940 NFIA_08940 NFIA_08940 NFIA_08940 NFIA_05510 NFIA_05510 NFIA_05510 | Afu6g14390 Afu8g01970 Afu1g06140 Afu1g06140 Afu8g01490 Afu2g1490 Afu2g1490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g14490 Afu2g19520 Afu5g9520 Afu5g9520 | | 2 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 | | 33 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| Bisi, Bisi, GLA FAESF7 GE PLY PGA RGX AXL X08 EGL AXL X08 EGL BNL BNL BNL ABF CO CO CO CO CO CO CO CO BRH BGL BGR | bela glucosiase bela glucosiase glucoamyske (returb) esterse glucoamyske erubje sterse pottab juse contab juse contab glucoame pottab juse scheme scheme scheme scheme bela scheme bela sc | GH 61 GH 2 GH 2 GH 2 GH 2 CE10 FL 1 GH 22 GH 24 GH 26 GH 26 | AN1804 AN1804 AN17402 AN17402 AN17403 AN17404 AN19300 <l< td=""><td>51662 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 171269 38549 131747</td><td>ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08835 ATEG_08085 ATEG_08085 ATEG_08085 ATEG_083144 ATEG_083144 ATEG_083144 ATEG_08314ATEG_08314 ATEG_083</td><td>AC099002600123 AC099001000207 AC099001000277 AC090011000673 AC090023000161 AC0900900470 AC09001000639 AC090010000500382 AC090012000500 AC09001200050 AC0900120050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC090000000000000000000000000000000000</td><td>AFL26,07119 AFL26,07136 AFL26,07136 AFL26,07136 AFL26,07136 AFL26,0409 AFL20,0409 AFL20,0409 AFL20,0409 AFL20,0401 AFL20,</td><td>ACLA_049360 ACLA_087520 ACLA_087520 ACLA_057520 ACLA_05760 ACLA_075760 ACLA_073030 ACLA_073030 ACLA_072870 ACLA_078130 ACLA_085410 ACLA_085410 ACLA_025610</td><td>NFIA_105910 NFIA_060260 NFIA_016590 NFIA_016590 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02870 NFIA_058160 NFIA_05970 NFIA_05970 NFIA_05970</td><td>Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu8g1490 Afu8g1450 Afu2g09350 Afu5g1450 Afu2g14490 Afu2g1490</td><td></td><td>23334445555568888888888888888888888888888</td><td></td><td>33 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20</td></l<> | 51662 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 171269 38549 131747 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08835 ATEG_08085 ATEG_08085 ATEG_08085 ATEG_083144 ATEG_083144 ATEG_083144 ATEG_08314ATEG_08314 ATEG_083 | AC099002600123 AC099001000207 AC099001000277 AC090011000673 AC090023000161 AC0900900470 AC09001000639 AC090010000500382 AC090012000500 AC09001200050 AC0900120050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC0900100050 AC090000000000000000000000000000000000 | AFL26,07119 AFL26,07136 AFL26,07136 AFL26,07136 AFL26,07136 AFL26,0409 AFL20,0409 AFL20,0409 AFL20,0409 AFL20,0401 AFL20, | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_057520 ACLA_05760 ACLA_075760 ACLA_073030 ACLA_073030 ACLA_072870 ACLA_078130 ACLA_085410 ACLA_085410 ACLA_025610 | NFIA_105910 NFIA_060260 NFIA_016590 NFIA_016590 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02860 NFIA_02870 NFIA_058160 NFIA_05970 NFIA_05970 NFIA_05970 | Afu6g14390 Afu8g01970 Afu1g06140 Afu8g01490 Afu8g1490 Afu8g1450 Afu2g09350 Afu5g1450 Afu2g14490 Afu2g1490 | | 23334445555568888888888888888888888888888 | | 33 33 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 |
| BGL BGL GLA FAESF7 GR PLY PGA RGX AVL XG EGL AFC SE BNL BNL BNL BNL ABF CO CO URH PLY EGL SUL BGL | bela glucosiase bela glucosiase glucoamystee (roulyo statrase glucouropi (statrase dipologi (statrase) exontamogalacturonase exontamogalacturonase abita turosiase abita functionase abita functionase bela s/solatase bela s/solatase | GH 61 GH 2 GH 12 GH 12 GH 12 GH 22 GH 22 GH 22 GH 22 GH 31 GH 31 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 102 GH 102 G | 3 AN1804 5 AN7402 AN0741 5 AN7402 5 AN7402 5 AN7402 5 AN7402 5 AN7402 5 AN7402 6 AN1949 5 AN757 6 AN1949 5 AN390 5 A | 51662 52219 42917 43342 206333 184037 37673 206387 53797 216608 171289 38549 38549 131747 128891 | ATEG_02724 ATEG_08907 ATEG_08907 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08844 ATEG_07820 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_07820 ATEG_07820 ATEG_07820 ATEG_07820 ATEG_07820 ATEG_0188 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_08854 ATEG_08854 ATEG_08854 ATEG_08854 ATEG_08854 | AC996025000123 AC990025000337 AC990021000207 AC990021000207 AC990021000207 AC990020001 AC990020001 AC99002000032 AC9900200030 AC9900200000 AC99002000000 AC99002000000 AC990020000000 AC99002000000 AC99002000000 AC99002000000 AC99002000000000000000000000000000000000 | AFL2G, 07119 AFL2G, 07139 AFL2G, 07136 AFL2G, 07136 AFL2G, 07436 AFL2G, 04049 AFL2G, 04049 AFL2G, 04049 AFL2G, 01228 AFL2G, 05484 AFL2G, 01228 AFL2G, 05484 AFL2G, 0128 AFL2G, 00803 AFL2G, 08634 AFL2G, 0756 AFL2G, 0756 AFL2G, 0756 AFL2G, 0756 AFL2G, 0756 | ACLA_049360 ACLA_067520 ACLA_067520 ACLA_067520 ACLA_05760 ACLA_05760 ACLA_076760 ACLA_077610 ACLA_077610 ACLA_077610 ACLA_061310 ACLA_061310 ACLA_061310 ACLA_061310 ACLA_061310 | NFIA_105910 NFIA_060260 NFIA_016590 NFIA_016590 NFIA_02800 NFIA_02800 NFIA_02800 NFIA_028040 NFIA_089670 NFIA_089670 NFIA_015730 NFIA_059670 NFIA_059670 NFIA_059670 NFIA_059670 NFIA_059670 NFIA_059671 | Atu6g14390 Atu6g11970 Atu1g06140 Atu2g01970 Atu2g0930 Atu2g1490 Atu2g14190 Atu2g14190 Atu2g14190 Atu2g14190 Atu2g1490 Atu2g16400 Atu2g09520 Atu5g216900 Atu5g071800 | | 2333344 | | 33 33 333 33 25 25 20 20 20 20 20 20 17 17 17 17 17 17 17 17 14 14 13 |
| BGL BGL GLA FAE SF7 GE PLY PDA ROX AXL XX0 EGL AFC ? ENG BXL BXL AFC CO CO CO CO CO CO URH URH AEF EGL ASL ASF BGL BGL BGL SGE | beta glucostase beta glucostase glucoamystes foruloy salarsase potato hysis andibolygiaturonase andibolygiaturonase andibolygiaturonase andibolygiaturonase andibolygiaturonase andibolygiaturonase andibolygiaturonase abita forostase beta 1,6 gluconase beta 1,6 gluconase endosytamase andibolytam | GH 61 GH 2 GH 1 GH 1 F 1 GH 1 F 1 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 | AN1804 AN1804 SAN7402 S SAN7402 S SAN7402 S SAN7403 S SAN7404 S SAN7405 S SAN741 S SAN5051 S AN25061 S AN25064 S AN25054 AN25054 AN10505 S S AN25241 AN25254 AN25254 AN19505 S S AN12506 AN12507 AN2383 AN1277 AN20183 AN12277 AN2227 AN0452 AN0452 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 171289 131747 129891 52011 | ATEG_02724 ATEG_08907 ATEG_08807 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08314 ATEG_09314 ATEG_09316 | AD99020500123 AD99001500037 AD99001500037 AD99001500037 AD99001500027 AD99001100027 AD99001100027 AD990011000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000059 AD99001000011 AD990001000011 AD99001000011 AD99001000011 | AFL2G, 07119 AFL2G, 07139 AFL2G, 07136 AFL2G, 07136 AFL2G, 07143 AFL2G, 04049 AFL2G, 04049 AFL2G, 04049 AFL2G, 04239 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 04249 AFL2G, 07786 AFL2G, 07786 AFL2G | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_087520 ACLA_052760 ACLA_052760 ACLA_099110 ACLA_097810 ACLA_077810 ACLA_095410 ACLA_025810 ACLA_025810 ACLA_025810 | NFIA_105910 NFIA_060260 NFIA_016560 NFIA_016560 NFIA_02600 NFIA_02600 NFIA_0260400 NFIA_0260400 NFIA_050400 NFIA_050400 NFIA_050400 NFIA_055010 NFIA_055000 NFIA_055000 NFIA_055000 NFIA_0550000000000000000000000000000000000 | Afu6g14380 Afu8g01970 Afu1g06140 Afu2g0350 Afu2g0350 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g1450 Afu5g16800 Afu5g169000 Afu5g169000 Afu5g169000 Afu5g16900 Afu5g16900 Afu5g16 | | | | 33 33 333 33 25 25 20 20 20 20 20 20 17 17 17 17 17 17 14 14 14 14 13 13 |
| Bisl, Bisl, GL, A FAESF7 GR PQA RGX AXL PGA RGX AXL PGA RGX AXL AFC P EXG BXL ABF CO CO CO CO CO CO BGL BGL BGL SGL XO EGL AMY | beta glucositase beta glucositase glucoamytes (forulty) esterates area pectate hyses exontamogischuronase exontamogischuronase exontamogischuronase alpha forsositase alpha forsositase beta sylvalatase beta sylvalatase beta sylvalatase beta sylvalatase alpha forsositase alpha forsositase alpha forsositase alpha forsositase alpha forsositase beta sylvalatase beta glucositase beta glucositase beta glucositase | GH 61 GH 2 GH 12 GH 12 GH 12 GH 12 GH 2 GH 2 GH 2 GH 2 GH 2 GH 3 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 42 GH 10 GH 10 G | A AN1804 AN1804 S ANT402 S | 51662 52219 42917 43342 206333 164037 37673 206387 53797 41877 214680 36549 131747 129881 52011 52011 | ATEG_02724 ATEG_02724 ATEG_08807 ATEG_08807 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_02882 ATEG_02882 ATEG_02882 ATEG_02882 ATEG_02882 ATEG_02882 ATEG_02887 ATEG_02887 ATEG_02887 ATEG_02887 ATEG_02887 ATEG_02887 ATEG_02887 ATEG_0387 ATEG_047 ATEG_0487 ATEG_0487 ATEG_047 ATEG_ | AD99002900123 AD990025000377 AD990025000377 AD990025000377 AD990025000277 AD99002500011000277 AD990011000027 AD990012000030 AD990012000030 AD99001200003 AD99001200003 AD99001200003 AD99001200003 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD99001200005 AD990012005 AD99001005 AD990012005 AD990005 AD990012005 AD99005 | AFL20, D7119 AFL20, D7139 AFL20, D7130 AFL20, D7130 AFL20, D7140 AFL20, D7140 AFL20, D4140 AFL20, D41400 AFL20, D41400 AFL20, D41400 AFL20, D41400A AFL20, D41400A AFL20, D4140A AFL20, D41 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_049310 ACLA_052760 ACLA_052760 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_085410 ACLA_085410 ACLA_029400 ACLA_029400 ACLA_029400 ACLA_029400 ACLA_029400 ACLA_029400 | NFIA_105910 NFIA_060260 NFIA_05520 NFIA_015580 NFIA_015580 NFIA_04550 NFIA_044550 NFIA_054450 NFIA_054450 NFIA_054450 NFIA_054450 NFIA_05510 NFIA_0550 NFIA_050 NFIA_0550 NFIA_0500 NFI | Atu5g14390 Atu5g14390 Atu5g01970 Atu1g06140 Atu5g01490 Atu5g01490 Atu5g1450 Atu5g1450 Atu5g1450 Atu5g14190 Atu5g14430 Atu5g14430 Atu5g14430 Atu5g2012210 Atu5g20190 Atu5g07190 Atu5g07190 Atu5g07190 Atu5g07190 | | | | 33 33 333 33 25 25 20 20 20 20 20 20 20 20 20 20 21 17 17 17 17 17 14 14 13 13 |
| BGL BGL GLA BGL GLA FAESF7 GRE PLY PDA ROX AXL AXL AXL AXL BAL BAL ABF BGL BGL BGL BGL BGL AGT AMY | bela glucosiase bela glucosiase glucoamyske (mulyo stainse) glucounory i seinse diodoolygia (Luronase exontamogalischuronase autopalogia (Luronase autopalogia) abha turosolase bela sylosiadase abha turosolase bela sylosiadase abha manobarted hamogalacturonyi hydrolas pectale yas exotamogalacturonyi hydrolas pectale yas exotapala manobarted hamogalacturonyi hydrolas pectale yas endogluconase endogluconase endogluconase bela glucosiase bela glucosiase bela glucosiase abha ancho etnose bela glucosiase | GH 61 GH 26 GH 26 | AN1804 AN1804 SAN7402 San7402 San7402 San7402 San7402 San7402 San7402 San718 San718 San718 | 51662 52219 42917 43342 2063387 53797 206387 53797 41877 214608 171289 38549 131747 129881 52011 131747 | ATEG_02724 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_00945 ATEG_04786 ATEG_04786 ATEG_04983 ATEG_04983 ATEG_04983 ATEG_04983 ATEG_01035 ATEG_01035 ATEG_01035 ATEG_01035 ATEG_00188 ATEG_0 | AD96005000123 AD9600100037 AD96001000057 | AFL20, 07119 AFL20, 07139 AFL20, 0734 AFL20, 07430 AFL20, 0449 AFL20, 0449 AFL20, 0449 AFL20, 0449 AFL20, 0423 AFL20, 0452 AFL20, 0451 AFL20, 0451 AFL | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_087520 ACLA_052760 ACLA_052760 ACLA_05760 ACLA_077810 ACLA_077810 ACLA_025810 ACLA_025810 ACLA_025810 ACLA_025810 ACLA_04555 ACLA_04555 | NFIA_105910 NFIA_060260 NFIA_095620 NFIA_018590 NFIA_02800 NFIA_02800 NFIA_02800 NFIA_0280490 NFIA_0280490 NFIA_028040 NFIA_028040 NFIA_028040 NFIA_028040 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058010 NFIA_058000 NFIA_0580 | Atu6914390 Atu8901970 Atu1906140 Atu5901490 Atu691490 Atu291450 Atu291450 Atu2914190 Atu2914190 Atu2914190 Atu2914190 Atu29140 | | | | 33 33 333 33 25 25 20 20 20 20 20 20 20 20 20 20 20 20 20 20 20 20 21 17 17 17 17 17 14 14 13 13 13 13 |
| ЫGL BGL GLA FACSF7 GE FACSF7 GE FACSF77 FACSF7 FACSF7 FACSF777 FACSF777 FACSF777 FACSF7777 FACSF777777 FACSF77777777777777777777777777777777777 | bela glucosisse bela glucosisse plucoamyske forulyr sakrease podato hyske kontonie sakrease kontamogalischurnase kontamogalischurnase kontamogalischurnase daha konsolisse bela 15 glucanase bela 15 glucanase to sakreta 15 glucanase to sakre | GH 61 GH 2 GH 1 FaeA CE12 GH 1 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 | AN1804 AN1804 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17403 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN17402 AN11277 AN19103 AN12542 AN16205 AN12277 AN10452 AN10452 AN10452 AN102308 AN102308 | 51662 52219 42917 43342 206333 184037 37673 206387 53797 41877 214608 171269 38549 131747 129691 52011 52011 52011 | ATEG_02724 ATEG_03945 ATEG_08945 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_08844 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 ATEG_0884 | AD96005000123 AD96005000123 AD96005100023 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000053 AD960051000051 AD960051000051 AD960051000051 AD960051000051 AD960051000051 AD960051000051 AD960051000051 | AFL20, 0719 AFL20, 0719 AFL20, 0719 AFL20, 0740 AFL20, 05417 AFL20, 0449 AFL20, 0449 AFL20, 0429 AFL20, 0429 AFL20, 0429 AFL20, 0429 AFL20, 0429 AFL20, 0429 AFL20, 0429 AFL20, 0402 AFL20, 0402 AFL20 | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_087520 ACLA_087580 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_025610 ACLA_025610 ACLA_025610 ACLA_025610 ACLA_049350 ACLA_049350 ACLA_049350 | NFIA_105910 NFIA_060250 NFIA_055020 NFIA_055020 NFIA_054990 NFIA_054990 NFIA_054950 NFIA_054950 NFIA_054950 NFIA_055010 NFIA_055000 NFIA_055000 NFIA_055000 NFIA_055000 NFIA_055000 NFIA_0550000000000000000000000000000000000 | Atu6914390 Atu8901970 Atu1906140 Atu8901490 Atu2909306 Atu291490 Atu2914490 Atu2914490 Atu2914490 Atu2914490 Atu2914490 Atu2914490 Atu2914490 Atu291490 Atu291790 Atu9927190 Atu9907190 Atu9907190 Atu9907190 | | | | 33 33 333 33 25 25 20 20 20 20 20 20 17 17 17 17 17 17 17 17 17 17 13 13 13 13 |
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| BGL BGL GLA FAE SF7 GR FAE SF7 GR FAE SF7 FQA SF0 FQA SF0 SGA SGA SGA AGT | bela glucosiase bela glucosiase glucoamyske (returboj elatense glucoamyske (returboj elatense dipologi elatense exontamogolechuronase akon arbite akon arbite akon arbite dipologi elationese bela sylosiase abha arabite rutomosiase cellutes oxidase bela sylosiase abha arabite rutomosiase cellutes oxidase bela sylosiase abha arabite rutomosiase cellutes oxidase bela sylosiase abha arabite rutomosiase cellutes oxidase bela sylosiase abha arabite rutomosiase bela sylosiase abba arabite rutomosiase bela glucosiase bela glucosiase bela glucosiase abha arabite rutomosiase bela glucosiase abha arabite rutomosiase bela glucosiase abha arabite rutomosiase bela glucosiase abha arabite rutomosiase abha arabite rutomosiase bela glucosiase abha arabite rutomosiase abha arabite rutomosi abha arabite r | GH 61 GH 26 GH 26 | AN1804 AN1804 AN7402 AN17402 AN7402 AN17402 AN7403 AN17402 AN17402 AN17402 AN17403 AN17402 AN17404 AN17402 AN17404 AN1740 | 51662 52219 42917 43342 206333 184037 37673 206387 5377 41877 214608 171269 38549 131747 12869 38549 131747 128691 138469 141677 203143 | ATEG_02724 ATEG_0497 ATEG_04945 ATEG_04945 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04930 ATEG_04390 ATEG_04490 ATEG_04490ATEG_04490 ATEG_04490ATEG_04490 ATEG_04490A | AD9602500123 AD96005100227 AD96005100227 AD96005100227 AD96005100027 AD96005100027 AD96005230014 AD96005230014 AD96005230014 AD96005100027 AD960051000025 AD960051000025 AD960051000005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD96005100005 AD960050005 AD960050005 AD960050005 AD96005000005 AD96005000005 AD960050000005 AD96005000005 AD960050000005 AD960050000005 AD960050000005 AD960050000005 AD960050000005 AD960050000005 AD9600500000005 AD9600500000005 AD9600500000005 AD9600500000005 AD960050000005 AD9600500000005 AD96000000000000000000000000000000000000 | AFL2G, 07119 AFL2G, 07139 AFL2G, 07139 AFL2G, 07130 AFL2G, 07430 AFL2G, 06417 AFL2G, 0449 AFL2G, 0449 AFL2G, 0429 AFL2G, 0429 AFL2G, 0429 AFL2G, 0429 AFL2G, 0427 AFL2G, 0427 AFL2G, 0427 AFL2G, 0447 AFL2G, 0447 | ACLA_049360 ACLA_067520 ACLA_057520 ACLA_057520 ACLA_057520 ACLA_057650 ACLA_073030 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_025610 ACLA_025610 ACLA_049350 ACLA_049350 ACLA_049350 ACLA_049350 ACLA_049350 ACLA_049350 ACLA_052860 ACLA_052860 ACLA_052860 | NFIA_105910 NFIA_060260 NFIA_05520 NFIA_02580 NFIA_012580 NFIA_012680 NFIA_02580 NFIA_02580 NFIA_02580 NFIA_05450 NFIA_05910 NFIA_05 | Atu8g14390 Atu8g01970 Atu1g06149 Atu8g01970 Atu1g06149 Atu8g01490 Atu8g1450 Atu5g14190 Atu5g14190 Atu5g14190 Atu5g14190 Atu5g14190 Atu5g14190 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1490 Atu5g1491 Atu5 | | | | 33 33 333 325 25 20 20 20 20 20 20 20 17 17 17 17 17 17 17 17 13 13 13 13 13 13 13 13 |
| Bisl. Bisl. GLA RAP.SF7 GR RAP.SF8 ROX | bela glucosiase bela glucosiase glucoamyske forulyri sakrase glucoamyske forulyri sakrase sochamogalachuronase exorhamogalachuronase exorhamogalachuronase abha kyskolase bela sykolase bela sykolase bela sykolase bela sykolase bela sykolase bela sykolase bela sykolase celuize oxitase umashuratel rhamogalachuroyi hydroles umashuratel rhamogalachuroyi hydroles endoglucanase bela givase endoglucanase bela givase endoglucanase bela givase endoglucanase endoglu | GH 61 GH 2 GH 1 GH 1 FaeA CE11 PL 1 GH 2 GH 2 GH 2 GH 2 GH 3 GH 4 GH 4 GH 4 GH 10 GH 10 G | AN1804 AN17402 AN12177 AN10452 AN12177 AN10452 AN12170 AN12170 AN12170 AN12170 | 51662 52219 42917 43342 206333 184037 37673 2066387 53797 41877 214608 17129 3549 131747 129891 52011 45304 18459 141677 203143 537797 | ATEG_029274 ATEG_029274 ATEG_00945 ATEG_00945 ATEG_00930 ATEG_00930 ATEG_00930 ATEG_01500 ATEG_01500 ATEG_01500 ATEG_0292 | AD96005000123 AD96005000123 AD96005100024 AD960051000051 AD96005100051 AD960051000051 AD96005100051 AD96005100051 AD960051000051 AD96005 | AFL2G, 07119 AFL2G, 07139 AFL2G, 07139 AFL2G, 07139 AFL2G, 07439 AFL2G, 06417 AFL2G, 04499 AFL2G, 04499 AFL2G, 04629 AFL2G, 06239 AFL2G, 06239 AFL2G, 06239 AFL2G, 07050 AFL2G, 06624 AFL2G, 07050 AFL2G, 06624 AFL2G, 0750 AFL2G, 0750 AF | ACLA_049360 ACLA_067520 ACLA_067520 ACLA_057520 ACLA_057520 ACLA_057580 ACLA_057680 ACLA_097100 ACLA_077810 ACLA_077810 ACLA_077810 ACLA_05140 ACLA_0525610 ACLA_0525610 ACLA_06350 ACLA_05050 ACLA_05 | NFIA_105910 NFIA_050260 NFIA_05520 NFIA_05520 NFIA_012580 NFIA_012580 NFIA_012580 NFIA_05450 NFIA_05970 NFIA_0 | A fuega 4390 A fuega 14390 A fuega 1490 A fuega 1490 A fuega 1490 A fuega 1490 A fuega 1490 A fuega 1490 A fuega 1450 A fu | | | | 3333252520202020202020202020202020202020 |
| Ы4, GA 2007 GA 2007 PLY PLY PLY PLY PLY PLY PLY PLY | beta glucosiase beta glucosiase glucoamyaka foruloy astesase pectate byase endopolyakuronase dopolyakuronase dopolyakuronase apha forcesiase beta 1 d gluconase beta 1 d gluconase beta 1 d gluconase beta 1 d gluconase beta 1 d glucosiase celtuse oxitase celtuse oxitase celtuses oxitase deluses oxitase deluses dostase deluses | GH 61 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 GH 2 | AN1004 AN17402 AN7402 AN7402 AN7402 AN17402 AN7403 AN0741 AN7404 AN0741 AN77402 AN1604 AN0741 AN3604 AN16054 AN1650 AN1654 AN1656 AN1654 AN1656 AN1655 AN1656 AN1654 AN1655 AN1655 AN1655 AN1655 AN1655 AN1654 AN1655 AN1655 AN3030 AN2627 AN4552 AN2654 AN2016 AN2655 AN2657 AN2655 AN2652 | 51682 52219 42917 43342 206333 184037 37673 206387 53797 214608 171289 38549 131747 129891 13747 129891 13747 24408 13747 2577 12788 13747 206387 2577 24408 13747 2577 12788 13747 206387 2577 13747 206387 2577 13747 137 | ATEG_02724 ATEG_02724 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_08834 ATEG_09836 ATEG_09844 ATEG_01980 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_09844 ATEG_0831 | A09602500123 A09600590037 A09600590037 A09600590037 A09600590037 A09600590037 A09600590047 A09600590000000000 | AFL2G, 02149 AFL2G, 02147 AFL2G, 02147 AFL2G | ACLA_049360 ACLA_087520 ACLA_087520 ACLA_067520 ACLA_057680 ACLA_057680 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_072870 ACLA_0285410 ACLA_085410 ACLA_02940 ACLA_02940 ACLA_02940 ACLA_05440 ACLA_05440 ACLA_05440 ACLA_05440 ACLA_05440 | NFIA_105910 NFIA_060260 NFIA_05520 NFIA_015500 NFIA_015500 NFIA_024500 NFIA_024450 NFIA_024450 NFIA_024450 NFIA_027780 NFIA_058400 NFIA_055100 NFIA_05500 NFIA_ | Atu5g14390 Atu5g01970 Atu1g06140 Atu1g06140 Atu2g01970 Atu2g0930 Atu2g19930 Atu2g1490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g14490 Atu2g09400 Atu2g09400 Atu2g09400 Atu2g09400 Atu2g09400 Atu2g094730 Atu2g094730 Atu2g194730 Atu2g194730 Atu2g194730 Atu2g194730 Atu2g194730 Atu2g1420 Atu2g14420 | | | | 33 33 33 33 25 25 20 20 20 20 20 20 20 20 20 20 17 17 17 17 17 17 17 17 13 13 13 13 13 13 13 13 13 13 |
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Chapter 2

Supplemental Table 6D: Detected proteins in cultures grown on sugar beet pulp sorted by number of species that contain an orthologue. The colour codes indicate which percentage of the total number of detected peptides was of the specific protein. (Continued on next page; Please see pdf file for enlargened text).

| | | | 0-0.1% | 0.1-0.5% | 0.5-1% | 1,2% | 2,5% | 5-10% | >10% | | | | |
|-------------|--|--------------|-------------|-----------|------------|-------------------|---------------|-------------|-------------|--------------|------------------|---------------------|---------------------|
| | | | | 0.1 0.010 | 0.0 110 | 1 2 10 | 2 0 10 | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | percentage detected |
| | | | | | | | | | | | no. species with | no. of species with | proteins per no. of |
| enzyme code | function | CAZY | A. nidulans | A. niger | A. terreus | A. oryzae | A. flavus | A. clavatus | A. fischeri | A. fumigatus | detected protein | 1 ortholog of gene | orthologs |
| FAE SF5 | feruloyi esterase | CE 1 | | 011073 | ATEG_01914 | | 15100 00107 | | | | | 1 | 100 |
| PME | pectin methyl esterase | CE 8 | AN3390 | 214857 | | A0090102000010 | AFL2G_09467 | | NFIA_099600 | Afu8g06880 | | 3 6 | 100 |
| RGAE | mamnogalacturonan acetyl esterase | CE 12 | | | | A0090003001268 | AFL2G_01797 | | NEIA 000110 | A 6.0-00400 | 2 | 2 2 | 100 |
| ROAL | hete eluceridane | 04.2 | | | | A0000022000222 | AEL 20, 00022 | | NF04_055110 | A100900400 | | 2 | 100 |
| BGI | beta glucosidase | GH 1 | | | ATEC 07931 | A0030030000223 | AI 220_00020 | | | | | 1 1 | 100 |
| EGL | endoolucanase | GH 5 | | | ATEG 05003 | | | | | | 1 | 1 1 | 100 |
| MAN | endomannanase | GH 5 | AN3297 | | | | | | | | 1 | 1 1 | 100 |
| MAN | endomannanase | GH 5 | AN6427 | | ATEG 09991 | | | | | | 2 | 2 2 | 100 |
| MAN | endomannanase | GH 5 | AN9276 | | | | | | | | 1 | 1 1 | 100 |
| | | GH5 | | | ATEG_03677 | | | | | | 1 | 1 1 | 100 |
| XLN | endoxylanase | GH 10 | | | | A0090001000208 | AFL2G_07437 | | | | 2 | 2 2 | 100 |
| XLN | endoxylanase | GH 11 | AN3613 | | | | | | | | 1 | 1 1 | 100 |
| XG EGL | xyloglucan active endoglucanase | GH 12 | | | ATEG_09894 | | | | | | 1 | 1 1 | 100 |
| AGD | alpha glucosidase | GH 13 | | | | | AFL2G_08694 | | | | 1 | 1 1 | 100 |
| MAN | endomannanase | GH 26 | AN7413 | | | | | | | | 1 | í 1 | 100 |
| AGL | alpha galactosidase | GH 27 | | | ATEG_01905 | | | | NFIA_048850 | Afu6g02560 | 3 | 3 3 | 100 |
| AGL | alpha galactosidase | GH 27 | AN7152 | 41606 | ATEG_02160 | A0090023000151 | AFL2G_04039 | | NFIA_029860 | Afu4g03580 | 7 | 7 | 100 |
| PGA | endopolygalacturonase | GH 28 | | 43957 | | | | | | | | 1 | 100 |
| PGA | endopolygalacturonase | GH 28 | | 182156 | 1750 10050 | | | | | | | 1 1 | 100 |
| ADN | endoarabinanase | OH 32 | | | A166_10255 | 0000000000000004 | AEL 20 00095 | | | | | 2 2 | 100 |
| ABN | endoarabinanase | GH 43 | | | ATEG 03688 | A003000300004 | AT 620_00000 | | | | | 1 | 100 |
| BXL | beta xviosidase | GH 43 | | | ATEG 10193 | | | | | | | 1 | 100 |
| co | cellubse oxidase | GH R1 | | | ATEG 10194 | | | | | | | 1 1 | 100 |
| CO | cellulose oxidase | GH 61 | AN3860 | | | | | | | | 1 | i i | 100 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | AN7908 | | | | | | | | 1 | 1 1 | 100 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | | | ATEG_10379 | | | | | | 1 | 1 1 | 100 |
| RHA | alpha rhamnosidase | GH 78 | 1 | | | | AFL2G_03939 | | | | 1 | 1 1 | 100 |
| ABX | exoarabinanase | GH 93 | AN2060 | | ATEG_06045 | A0090003001017 | AFL2G_02013 | | NFIA_081320 | Afu2G04570 | e | 3 6 | 100 |
| URH | unsaturated rhamnogalacturonyl hydrolas | GH 105 | AN7828 | | | | | | | | 1 | í <u>1</u> | 100 |
| PLY | pectate lyase | PL 3 | AN3337 | | ATEG_06285 | | | | | | 2 | 2 2 | 100 |
| PLY | pectate lyase | PL 3 | AN8453 | | | | | | | | 1 | i 1 | 100 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN3950 | | | | | | | | 1 | 1 1 | 100 |
| PLY | pectate lyase | PLS | | | ATEG_03526 | | | | | | 1 | 1 | 100 |
| RGL | mamnogalacturonan lyase | PL 11 | AN2543 | 170170 | | | | | | | | 1 | 100 |
| PGX | exopolygalacturonase | GH 28 | 410045 | 1/81/2 | | | | | | | | 1 | 100 |
| PGA | exopolygalacturonase | GH 20 | W119042 | | ATEO ANDEE | | | | | | | | 100 |
| DME | apria giocoronidase | OH TIS | AN4900 | 174205 | ATEG_04355 | A 0.0000000000404 | 451.20 10018 | ACLA 025610 | NEIA 020500 | 4.6-2-07650 | | 7 0 | 100 |
| BYL IABE | beta vulosidase/aloba arabinofuranosidar | 011 | AN2217 | 60007 | ATEC 09214 | A0090701000274 | AEL20 05912 | ACLA_033010 | NEIA 020120 | A fuSe07080 | | 7 0 | 00 |
| LAC | beta relactosidase | GH 35 | AN0756 | 51764 | ATEG_00616 | A0090012000445 | AFL20_03352 | ACLA 021260 | NEIA 011250 | Afu1e14170 | | 7 8 | 88 |
| ARE | alpha arabioofuranosidase | GH 54 | AN1571 | 200505 | ATEG 07939 | A0090023000001 | AFI 20, 03901 | ACLA 055470 | NEIA 060630 | Afu6014620 | | 7 8 | 88 |
| AXH | arabinoxylan arabinofuranohydrolase | GH 62 | AN2632 | 55136 | ATEG 10071 | A0090701000885 | AFI 2G 06447 | ACLA 061510 | NEIA 033210 | Afu2e00920 | - | 7 8 | 88 |
| PGX | exopolygalacturonase | GH 28 | AN8761 | 42184 | ATEG 07152 | A0090026000784 | AFL2G 06533 | | NFIA 049320 | Afu6q02980 | 6 | 3 7 | 86 |
| CBH | cellobiohydrolase | GH 6 | AN5282 | 54490 | ATEG_07493 | | | ACLA_062560 | NFIA_002990 | Afu3g01910 | 6 | 5 6 | 83 |
| RGAE | rhamnogalacturonan acetyl esterase | CE 12 | AN2834 | | | A0090113000155 | AFL2G_08631 | | NFIA_092640 | Afu2g17250 | 4 | \$ 5 | 80 |
| ABF | alpha arabinofuranosidase | GH 43 | AN8472 | | | A0090012000356 | AFL2G_03265 | | NFIA_032900 | Afu2g00650 | 4 | \$ 5 | 80 |
| RGL | rhamnogalacturonan lyase | PL 4 | | | ATEG_08610 | A0090138000119 | AFL2G_08794 | | NFIA_094270 | Afu8g00820 | 4 | 4 5 | 80 |
| CBH | cellobiohydrolase | GH 7 | AN5176 | 53159 | ATEG_03727 | A0090012000941 | AFL2G_03805 | ACLA_088870 | NFIA_052720 | Afu6g07070 | 6 | 3 8 | 75 |
| XLN | endoxylanase | GH 10 | AN1818 | 57436 | ATEG_00809 | A0090701000887 | AFL2G_06449 | ACLA_048770 | NFIA_106540 | Afu4g09480 | 6 | 3 8 | 75 |
| XLN | endoxylanase | GH 11 | AN9365 | 52071 | ATEG_07461 | A0090120000026 | AFL2G_08066 | ACLA_063140 | NFIA_000850 | Afu3g00320 | e | 3 8 | 75 |
| AMY | alpha amylase | GH 13 | | 0.000 | 1750 01075 | A0090120000196 | 15:00 11005 | ACLA_094070 | NFIA_032970 | Afu2g00/10 | | 4 | /5 |
| BOX | gucoanyase | OH 15 | AN0004 | 101152 | ATEC 10257 | A0090010000748 | AFL20_11803 | ACLA_054080 | NEIA 006240 | A10200050 | | | 75 |
| NX | exo joulpase | GH 15 | AN11778 | 50004 | ATEG 08155 | A0090701000400 | AFI 20, 06028 | ACLA 094550 | NEIA 033540 | Afi/2n01240 | | , o | 76 |
| SUC | invertase/beta fructofuranosidase | GH 32 | | 198063 | ATEG 07479 | A0090020000640 | AFL2G 10707 | | | | | 3 4 | 75 |
| ABF | alpha arabinofuranosidase | GH 43 | AN7781 | | ATEG 08386 | A0090005000065 | AFL2G 00086 | | | | 1 | 3 4 | 75 |
| PEL | pectin lyase | PL 1 | AN2569 | | ATEG_01216 | A009001000087 | AFL2G_11352 | | | | | 3 4 | 75 |
| LAC | beta galactosidase | GH 35 | AN0980 | 46429 | ATEG_05131 | A0090120000158 | AFL2G_08182 | | NFIA_008690 | Afu1g16700 | 6 | 5 7 | 71 |
| EGL | endoglucanase | GH 5 | AN8068 | | ATEG_09802 | A0090003001341+ | AFL2G_01726 | | NFIA_040280 | Afu5g01830 | 4 | 6 | 67 |
| XLN | endoxylanase | GH 10 | | | ATEG_08906 | A0090103000423 | AFL2G_11983 | | | | 1 | 2 3 | 67 |
| XLN | endoxylanase | GH 10 | | | ATEG_07190 | | | | NFIA_061880 | Afu3g15210 | 2 | 2 3 | 67 |
| XGH | xylogalacturonase | GH 28 | AN3389 | 46065 | | A0090102000011 | AFL2G_09468 | | NFIA_099610 | Afu8g06890 | 4 | - 6 | 67 |
| AGD | aipna glucosidase | GH 31 | AN8953 | | | A0090038000471 | AFL2G_07812 | | | | | 2 3 | 67 |
| XG CBH | xyogucan active cellobiohydrolase | GH 74 | AN1542 | | | | | ACLA 004040 | NFIA_096000 | Atusgu2330 | | 1 3 | 67 |
| PLL DIV | pecan yase | PL 1 | AUG100 | - | ATEO AREAS | 00000028000500 | AEL 20 07020 | ACEA_094210 | NEIA 022470 | Atu200000 | - | 3 | 67 |
| BOI | heta olucosidase | PL 3 QH 3 | AN4102 | 58782 | ATEG 03047 | A0090009000502 | AFL2G_07839 | ACLA 028810 | NEIA 018950 | Afu1005770 | | , b | 67 |
| MAN | endomannanase | GH 4 | AN3358 | 50378 | ATEG 08654 | A0090010000122 | AFL2G_10322 | ACLA 066420 | NEIA 113780 | Afu7c01070 | | , o | 60 |
| CBH | cellobiohydrolase | GH 7 | AN0494 | 51773 | ATEG 05002 | A0090001000348 | AFL2G 07571 | ACLA 085260 | NFIA 057300 | Afu6o11610 | | 5 8 | 63 |
| BXL | beta xylosidase | GH 43 | AN7313 | 122978 | ATEG 01188 | A0090010000562 | AFL2G 11714 | ACLA 043260 | NFIA 096240 | Afu8q02510 | | 5 8 | 63 |
| ABF | alpha arabino furanosidase | GH 51 | AN9439 | 131891 | ATEG_07868 | A0090012000298 | AFL2G_03217 | ACLA_074120 | NFIA_090410 | Afu2g15160 | | 5 8 | 63 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN7135 | 210947 | ATEG_02193 | A0090011000349 | AFL2G_05136 | ACLA_054660 | NFIA_029620 | Afu4g03780 | 5 | 5 8 | 63 |
| RGL | rhamnogalacturonan lyase | PL 4 | AN6395 | 47780 | ATEG_10327 | A0090012000147/ | AFL2G_03075 | ACLA_018320 | NFIA_008140 | Afu1g17230 | 6 | 5 8 | 63 |
| AMY | alpha amylase | GH 13 | AN3402 | 47911 | ATEG_10103 | A0090023000944 | | ACLA_052920 | | | 3 | 3 5 | 60 |
| BXL | beta xylosidase | GH 43 | AN10919 | | ATEG_10072 | A0090010000029 | AFL2G_11296 | | NFIA_093350 | | 3 | 5 | 60 |
| PLY | pectate lyase | PL 1 | AN5333 | | ATEG_05467 | A0090102000072 | AFL2G_09523 | | NFIA_060270 | Afu6g14400 | 3 | 5 | 60 |
| PLY | pectate lyase | PL S | AN2537 | | | A0090038000131 | AFL2G_08953 | | NFIA_062360 | Atu3g14890 | 3 | 5 | 60 |
| AG EGL | xyogucan active endoglucanase | GH 12 | 4140274 | 211053 | ATEG_07420 | A0090026000102 | AFL2G_0/140 | ACLA_00/820 | NEIA_027400 | A10/006150 | | 7 | 57 |
| NGA LAC | exornamiogalacturonase | GH 28 | AN10274 | 194461 | ATEG_09025 | A0090102000139 | AFL2G_09582 | ACLA 099110 | NEIA_027700 | A10/905410 | - | 1 1 | 57 |
| ABN* | endoarabinanase | GH 41 | AN8007 | 197735 | ALCO_07440 | A0090138000055 | AFI 2G 08737 | ACLA 098980 | NFIA 047920 | Afi/6000770 | | 4 7 | 57 |

Supplemental Table 6D Cont: Detected proteins in cultures grown on sugar beet pulp sorted by number of species that contain an orthologue. (Please see pdf file for enlargened text).

| GAL | endogalactanase | GH 53 AN5727 | 187227 | ATEG 02927 | A0090001000492 | AFL2G 09135 | | NEIA 017780 | Afu1o06910 | 4 | 7 57 |
|-----------|--|----------------|---------|-------------|---|---------------|---------------|---------------|--------------|---|----------|
| DEL | enotie hone | DI 4 AM2224 | 41010 | | 40000010000504 | AEL20 11000 | ACLA 012470 | NEIA 070950 | A 6-E-10220 | | |
| FLL | pecalityase | PE I ANZOOT | 41015 | | A0050010000304 | AT1220_11000 | ACCA_013470 | NT PA_070000 | Alasylosoo | | 37 |
| XGEGL | xylogiucan active endogiucanase | GH 12 | 191511 | | | | | NFIA_100330 | | 1 | 2 50 |
| FAE SF5 | feruloyl esterase | CE 1 | | ATEG_06644 | A0090701000884 | AFL2G_06446 | ACLA_061520 | | | 2 | 1 50 |
| RGAE | rhamnopalacturonan acetyl esterase | CE 12 | 51400 | ATEG 10016 | A0090102000092 | AFL2G 09543 | | | | 2 | s 50 |
| DOAE | champeople of uron an each disatoropo | CE 42 ANOE28 | 12005 | ATEC 02511 | 40000701000552 | AEL20, 08177 | ALCI 044070 | NEIA 082750 | A&-2014510 | - | 50 |
| ROAL | mailingalacturonali acetyresterase | CE 12 AN2020 | 1082.04 | AILO_00011 | A0050701000550 | AT 220_00177 | ACCC_041570 | NFPA_002730 | Allosginoto | | 3 30 |
| LAC | beta galactosidase | GH 2 AN2463 | | ATEG_00712 | | | | | | 1 | 2 50 |
| BGL | beta glucosidase | GH 3 AN2612 | | | | | | | Afu7g00240 | 1 | 2 50 |
| EGL | endoglucanase | GH 5 AN1285 | 205580 | ATEG 05002 | | | ACLA 085250 | NFIA 057290 | Afu6o11600 | 3 | 5 50 |
| AMOV | elabe emplose | CH 12 AN2200 | | ATEC 02515 | | | | | | | |
| AUNT | aipila alliyiase | GIT 15 AN5500 | | ATEG_02313 | | | | | | | L 30 |
| AGL | alpha galactosidase | GH 27 AN7624 | 207264 | ATEG_09830 | A0090003001305 | AFL2G_01765 | ACLA_003130 | NFIA_039990 | Afu5g02130 | 4 | 3 50 |
| PGA | endopolygalacturonase | GH 28 AN8327 | 46255 | | | | | NFIA_008150 | Afu1g17220 | 2 | ۱ 50 |
| BXI | beta vylosidase | GH 43 AN2664 | | ATEG 06107 | 40090012000350 | AFI 2G 03257 | | NEIA 072780 | Afr/5014190 | 3 | 50 |
| 000 | hate or dealdean | 011 40 4110477 | | ATEO 04000 | | 45100.00074 | | | | | |
| DAL | beta xylosidase | GR 45 ANO477 | | ATEG_01292 | | AFL2G_00974 | ACLA_010100 | | | 2 | 50 |
| ABF | alpha arabinofuranosidase | GH 51 | 50975 | ATEG_03540 | | | | | | 1 | 2 50 |
| CO | cellubse oxidase | GH 61 AN6428 | | ATEG 06077 | | | | | | 1 | 2 50 |
| AGII | alpha olucuropidase | GH 67 AN9286 | 56619 | ATEG 06085 | 40090026000127 | AFI 2G 07114 | ACI A 017270 | NEIA 072510 | Afr/5o14380 | 4 | 3 50 |
| NO FOI | a de ale ano a altra de ale anos | 011 74 4115004 | 000000 | ATEO 04700 | | | 401.4.044040 | 100100 | 10.0000000 | | |
| AG EGE | xylogiucan active endogiucanase | GH 74 ANS001 | 206333 | ATEG_04706 | | | ACEA_044310 | MLM/034330 | Allogo1490 | 3 | o ou |
| ABX | exoarabinanase | GH 93 | | | A0090011000141 | AFL2G_04929 | | | | 1 | 2 50 |
| AFC | alpha fucosidase | GH 95 AN6673 | 53702 | ATEG 09768 | A009000900086 | AFL2G 10565 | ACLA 004070 | NFIA 040960 | Afu5o01190 | 4 | 3 50 |
| IIDM | upsaturated champonalacturopyd bydrolar | GH 105 AN10505 | | - | 0.0000001000062 | AEL 20, 07308 | ACLA 072870 | NEIA 029240 | A6-2014E20 | 1 | 50 |
| UDU | unautorated mannogalactorony hydrolaz | | 44077 | ATT:0.00000 | 400000000000000000000000000000000000000 | AFL20_01000 | ACLA_072010 | 111 14_003040 | Allazy14030 | | 500 |
| UKM | unsaturated mamnogalacturonyl hydrolas | GR 105 AR9365 | 910// | ATEG_02092 | A0090113000146 | AFL2G_00024 | ACEA_077610 | | | 3 | o ou |
| AGU | alpha glucuronidase | GH115 | | | A0090001000267 | AFL2G_07498 | | | | 1 | 2 50 |
| PLY | pectate lvase | PL 3 AN6748 | | ATEG 06314 | A0090005000472 | AFL2G 00461 | | NFIA 027690 | Afu7o06400 | 3 | 3 50 |
| BOI | beta olucosidase | GH 3 AN2828 | | ATEC 07419 | 0.0090701000841 | AEL20, 06408 | ACLA 007810 | NEIA 027390 | 0.6-7-06140 | 1 | 1 43 |
| DIG (A DE | beta glacidada fabba ambia Amaraida | 011.0 4110404 | | ATEO CONTRO | 40000000000000 | AFL20_00400 | ACLA_COTOTO | NEW_021000 | A 6-2-02000 | | |
| DAUADE | beta xylosidasevalpna arabinoturanosidas | GH 5 AND401 | | ATEG_09052 | A0090103000120 | AFL2G_12202 | ACEA_062400 | NFM_003100 | Miubg020a0 | 3 | 40 |
| XLN | endoxylanase | GH 10 AN7401 | | ATEG_03410 | A0090103000326 | AFL2G_12071 | ACLA_086910 | NFIA_059570 | Afu6g13610 | 3 | 7 43 |
| PGA | endopolygalacturonase | GH 28 | 172944 | ATEG 01601 | A0090005001400 | AFL2G 01310 | ACLA 036670 | NFIA 068440 | Afu3o08680 | 3 | 43 |
| PI V | nantata kaza | DI 1 AM7646 | 45021 | ATEG 08123 | 0000701000021 | AEL20.05954 | | NEIA 033040 | A6-2x00760 | 1 | 41 |
| 101 | precision (y doc | PL 1 AN/040 | +3021 | ATEG_00123 | | Art20_00954 | | NEW 033040 | A 6 5 4 2020 | 3 | 43 |
| AGL | aipna gaiactosidase | GH 27 | 37736 | ATEG_04382 | | | ACLA_016820 | NFIA_073100 | Atu5013830 | 2 | 40 |
| ABF | alpha arabinofuranosidase | GH 43 AN2533 | | | A0090701000838 | AFL2G_06400 | | NFIA_002750 | Afu3g01660 | 2 | 5 40 |
| PLY | nertate lyage | DI 3 AN2542 | | | A0090010000706 | AEL2G 11846 | ACLA 059210 | NEIA 098670 | Afu8005910 | 2 | 5 40 |
| EAE CEE | familed asternas | CE 4 AMEDRE | 49706 | ATEC 08113 | A 000000000000000 | AEL20_04047 | ACLA 055050 | NEIA 080720 | A6-0014E30 | - | 10 |
| FAE DED | reruioyresterase | CE T ANS207 | 43703 | AIEG_00112 | A0050023000156 | AFL20_04047 | ACCA_055050 | NFIA_009720 | A102014030 | 3 | 3 30 |
| PME | pectin methyl esterase | CE 8 | 44585 | | A0090012000749 | AFL2G_03618 | ACLA_044240 | NFIA_095020 | Afu8g01520 | 2 | 3 33 |
| BGL | beta glucosidase | GH 3 AN1804 | | | A0090026000123 | AFL2G_07119 | | | | 1 | 3 33 |
| BXI | heta vulnaidase | GH 3 AN2359 | 205870 | ATEG 05108 | 40090005000988 | AEL2G_00957 | | | Afu1016920 | 2 | 1 33 |
| 0702 | cola Ny coloudo | 0110 7412000 | 200010 | 1750 07000 | 10000000000000000 | 15100 01000 | | | And Igroome | | |
| BXL | beta xylosidase | GH 3 | | ATEG_07383 | A0090011000140 | AFL2G_04928 | | | | 1 | 3 33 |
| MAN | endomannanase | GH 5 AN7639 | | | A0090038000444 | AFL2G_07781 | ACLA_044470 | NFIA_099770 | Afu8g07030 | 2 | 3 33 |
| EGL | endoolucanase | GH 7 | | ATEG 08705 | | | ACLA 098940 | NFIA 047960 | Afu6q01800 | 1 | 3 33 |
| GI A | nlucnamulase | GH 15 AN7402 | | | | | ACLA 049360 | NEIA 105910 | | 1 | 33 |
| DOM | geocenigace | 01110 | | 1750 00100 | | 15100 00074 | 100-00000 | 100010 | | | |
| RGX | exornamnogalacturonase | GH 20 | _ | ATEG_06408 | A0090113000199 | AFL2G_00671 | | | | 1 | 3 33 |
| ABF | alpha arabinofuranosidase | GH 51 AN2541 | 206387 | ATEG_02882 | A0090124000023 | AFL2G_08019 | ACLA_099110 | | | 2 | 3 33 |
| 00 | celulose oxidase | GH 61 | | ATEG 05418 | 40090023000787 | AFI 2G 04596 | ACLA 047220 | NEIA 108320 | Afu4n07850 | 2 | 3 33 |
| AVH | arabigoodan arabigofuranohudrolang | 04.62 | | ATEC 00198 | 0.0000102000022 | AEL20, 12281 | ACLA 071560 | MEIA 097000 | A6/2012770 | 2 | 22 |
| | arabitoxylari arabitoturanonyurolase | 01102 | | AIL0_00100 | A0030103000000 | MIL20_12201 | ACCA_071000 | MIPA_007800 | Allozyiziro | 4 | |
| RHA | alpha rhamnosidase | GH 78 | 42916 | ATEG_03018 | A0090009000471 | AFL2G_10227 | | NFIA_018620 | Atu1g06130 | 2 | 5 33 |
| RHA | alpha rhamnosidase | GH 78 AN7151 | | ATEG 02922 | A0090010000561 | AFL2G 11713 | | NFIA 057930 | Afu6q12030 | 2 | 3 33 |
| ABY | exparabinanase | GH 93 | | ATEC 00891 | | | | NEIA 058080 | A6-6-12120 | 4 | 1 11 |
| 000 | bete elseveraldese | 011.0 | 50444 | ATEO 04004 | 4.00000000000000 | A 51 20 42050 | | NELA 000700 | A6-0+14500 | | |
| 005 | beta glucuronidase | GH 2 AN2395 | 52111 | AIEG_01031 | A0090023000055 | AFL2G_03956 | | MLM_009100 | A102014520 | 4 | 23 |
| BGL | beta glucosidase | GH 3 AN7396 | 179265 | ATEG_10320 | A0090012000135 | AFL2G_03066 | | NFIA_007920 | Afu1g17410 | 2 | 7 29 |
| EGL | endoglucanase | GH 5 AN5214 | 209376 | | A0090005001553 | AFL2G 01447 | ACLA 081650 | NFIA 053150 | Afu6q07480 | 2 | 7 29 |
| GLN. | evo 1.6 oslactanase | GH 5 AND166 | 194447 | ATEG 10242 | 0.0090012000046 | AEL2G_02982 | - | NEIA 072400 | A6:5014580 | 2 | 7 29 |
| 000 | exterio guadanase | | 400000 | ATEO 00402 | 4 000000000000000 | AFL20 02002 | A CL A | NELA DACCOO | Alabyitoot | - | |
| Con | Cellobionydrolase | GH 6 ANTZ73 | 100000 | AIEG_00195 | A0090036000439 | AFL2G_0///6 | ACLA_025560 | NFIA_013000 | | 4 | 23 |
| EGL | endoglucanase | GH 7 AN3418 | | ATEG_08700 | A0090010000314 | ALF2G_11497 | ACLA_066030 | NFIA_114250 | Afu7g01540 | 2 | 7 29 |
| XLN | endoxvianase | GH 11 | 171265 | ATEG 04943 | A0090001000111 | AEL2G 07347 | ACLA 085410 | NEIA 058160 | Afu6012210 | 2 | 7 29 |
| BYI | hate vulneideea | GH 43 AN7275 | | ATEG 06643 | A0090701000888 | AEL2G 08448 | ACLA 078600 | NEIA 033220 | A6/2000930 | 2 | 7 29 |
| | bela xylosidase | 01140 201210 | | AILO_00040 | | | ACCA_010000 | IN PA COULLO | Allazgoosso | | |
| AGU | alpha glucuronidase | GH115 AN9329 | | ATEG_09974 | A0090010000038 | AFL2G_11304 | ACLA_006360 | NFIA_025630 | Atu/g04680 | 2 | 29 |
| RHG | endorhamnogalacturonase | GH 28 | 211163 | ATEG_07607 | A0090010000484 | AFL2G_11646 | | | | 1 | 1 25 |
| AXE | acetyl xylan esterase | CE 1 AN6093 | 211544 | ATEG 09843 | A0090011000745 | AFL2G 05471 | ACLA 081220 | NEIA 099230 | Afu8006570 | 2 | 3 25 |
| GE | ducuropovi esterase | CE15 | 2.1011 | ATEG 00945 | | | ACLA 087520 | NEIA 060260 | Afu6o14390 | 4 | 1 25 |
| 00 | bate elucereldese | 0010 4040100 | 2000000 | ATEO 00040 | 4.0000000000000000000000000000000000000 | 451.00 00107 | ACLA 007020 | NEW_000200 | 44-0-00700 | | 20 |
| DOL | beta glucosidase | GH 3 AN10482 | 208871 | ATEG_06617 | A0090001000544 | AFL2G_09187 | ACLA_083710 | NFIA_054350 | A106908700 | 2 | 25 |
| GLA | glucoamylase | GH 15 | | A/EG_05980 | AU090003000321 | AFL2G_02658 | ACLA_089470 | | | 1 | 25 |
| PGA | endopolygalacturonase | GH 28 AN4372 | 141677 | ATEG_04991 | A0090023000401 | AFL2G_04252 | ACLA_052860 | NFIA_102450 | Afu4g13920 | 2 | 3 25 |
| AGD | alpha olucosidase | GH 31 AN2017 | 214233 | ATEG 00723 | A0090003001209 | AFL2G 01842 | ACLA 049370 | NFIA 105900 | Afu4o10150 | 2 | 3 25 |
| 400 | alpha olycopidana | QH 21 AM0041 | 110950 | ATEC 05177 | 0000005001084 | AEL 20 01028 | ACLA 019200 | NEIA 000190 | Afrita16250 | 2 | 20 |
| 140 | angina galadadada | 01101 00091 | 13030 | | | 100 01030 | | NEW_000100 | 10.000000 | 4 | 20 |
| LAG | ueta galactosidase | GH 35 | 41910 | | | AFL2G_03616 | | ner #A_000910 | Alaban | 1 | 25 |
| ABN | endoarabinanase | GH 43 AN2534 | 203143 | ATEG_03520 | A0090701000481 | AFL2G_06106 | ACLA_042100 | NFIA_062660 | Afu3g14620 | 2 | 3 25 |
| BXL | beta xylosidase | GH 43 AN1870 | 179682 | ATEG_06059 | A0090003000239 | AFL2G_02739 | ACLA_090090 | NFIA_081240 | Afu2g04480 | 2 | 3 25 |
| 00 | cellulose ovidase | GH 61 AN1044 | 52000 | ATEG 00449 | 40090001000221 | AFI 20 07454 | ACLA 022090 | NEIA 012990 | Afr:1012560 | 2 | 2 26 |
| 00 | pellulase puidese | 00.01 01000 | 100400 | ATEC 07700 | 0000005000521 | AEL20_07404 | ACLA_050700 | NEIA 000510 | A 6-9-00920 | 2 | 20 |
| ~~ | Celuluse 0XIOBSE | GR 01 AN1602 | 102430 | A16G_07/90 | M0090005000531 | AFL2G_00532 | MCEN_029/80 | ne #4_099510 | Minodineoon | 2 | 25 |
| PLY | pectate lyase | PL 1 AN0741 | | ATEG_08834 | A0090011000673 | AFL2G_05417 | | | | 1 | 25 |
| CO | cellulose oxidase | GH 61 AN7891 | 194765 | | A0090138000004 | AFL2G_08699 | | | | 1 | 25 |
| AXI | alpha vylosidase | GH 31 | 43345 | ATEG 08200 | 40090701000639 | AFI 2G 06229 | | NEIA 032690 | | 4 | 20 |
| no a | and and and a strength of the | 011.00 | 40044 | | 4.0000000000000000000000000000000000000 | 15100 00239 | | NEW 000440 | | | 20 |
| MUA | endopolygalacturonase | GH 28 AN6656 | 50161 | | AU090005000186 | AFL2G_00201 | | NFIA_099410 | | 1 | 20 |
| RGX | exorhamnogalacturonase | GH 28 | 42917 | | A0090009000470 | AFL2G_10228 | | NFIA_018590 | Afu1g06140 | 1 | 5 20 |
| AFC | alpha fucosidase | GH 95 AN8149 | 184037 | ATEG 01580 | A0090005000382 | AFL2G 00373 | | | | 1 | 5 20 |
| PGA | endonolygalacturopase | GH 28 | 52210 | | A0090023000161 | AFI 2G 04049 | | NEIA 095620 | Afr:8001970 | - | 20 |
| | encopolyganacteronase | 011.0 | 52213 | 4750 44575 | A0000023000101 | | A 01 A 050555 | NEW_000020 | A100901370 | | 20 |
| r | | GH 3 AN3360 | 37673 | AIEG_04963 | | | ACLA_052760 | NFIA_102600 | Atu4g13770 | 1 | 17 |
| CO | cellulose oxidase | GH 61 AN9524 | 53797 | ATEG_07920 | A0090012000090 | AFL2G_03026 | | NFIA_044390 | | 1 | 5 17 |
| RHA | alpha rhamnosidase | GH 78 AN10277 | 170173 | | A0090003001291 | AFL2G 01780 | | NFIA 022970 | Afu1o01660 | 1 | 3 17 |
| EVO | beta 1.6 okranase | CH 6 AN2777 | | ATEC 00044 | 0.0000011000757 | AEL20 05494 | | NEIA 084850 | A 6:2009350 | | |
| EAG | ueia i,o glucanase | GH 5 AN3777 | | ATEG_09844 | A0090011000757 | AFL2G_05484 | | wr #A_084850 | A102909350 | 1 | 17 |
| BGL | beta glucosidase | GH 1 AN9183 | 131747 | A/EG_02657 | AU090120000075 | AFL2G_08111 | | NEIA_099670 | Atu8g06970 | 1 | 14 |
| EGL | endoglucanase | GH 5 | 214608 | ATEG_04390 | A0090011000715 | AFL2G_05447 | ACLA_081310 | NFIA_085010 | Afu2g09520 | 1 | 14 |
| AGI | alpha palactosidase | GH 27 AN0022 | 172233 | ATEG 03427 | A0090005000217 | AEL2G 00225 | | NEIA 023390 | Afu1001200 | 4 | 7 14 |
| 4.01 | and a second sec | 011 42 | 483405 | ATEC 07847 | 4.0000000000000000 | AFL20_00220 | ACLA 070700 | NEW 000000 | 44-2-44760 | | |
| MON | enuuaraultanase | GH 43 | 182100 | AIEG_07817 | A0090026000804 | AFL2G_06513 | ACLA_072730 | NF 4A_089980 | A102914750 | 1 | 14 |
| EXG | exo 1,3 galactanase | GH 5 AN4052 | 202490 | ATEG_03849 | A0090003000990 | AFL2G_02039 | ACLA_031040 | NFIA_021060 | Afu1g03600 | 1 | 3 13 |
| AGT | 4 alpha glucanotransferase | GH 13 AN3308 | 188489 | ATEG 03623 | A0090003001498 | AFL2G 01594 | ACLA 063440 | NFIA 001710 | Afu3q00900 | 1 | 3 13 |
| AGD | alpha olucosidase | GH 31 AN0280 | 55410 | ATEG 02528 | A0090005000767 | AEL2G 00750 | ACLA 031260 | NEIA 021450 | Afu1003140 | 4 | 13 |
| 100 | alpha geodoluoso | 0H 38 ANR100 | 00918 | ATEO 07000 | A 00000000000000000 | AFL20_00750 | ACLA 044000 | NEW_021450 | A6-8-04430 | - | 13 |
| AGL | aipina gaidCt05i0ase | GH 36 ANS138 | 212736 | AIEG_0/929 | A0090010000684 | AFL2G_11824 | ACLA_044620 | NF 94_094580 | A108901130 | 1 | 13 |
| BXL | beta xylosidase | GH 43 AN7864 | 47677 | ATEG_01292 | A0090102000331 | AFL2G_09750 | ACLA_072000 | NFIA_088370 | Afu2g13190 | 1 | 3 13 |
| 11110 | hale measurabless | 04.2 444742 | 100070 | 1750 00000 | 100000010000550 | 15100 00001 | 1.01.1.000570 | NEW DEALOO | 16-0-00040 | | 42 |

Chapter 3

Analysis of the molecular basis for the aberrant phenotype of *Aspergillus vadensis* compared to other black Aspergilli

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Abstract

In the recent past, much research has been applied to the development of *Aspergillus*, most notably *A. niger* and *A. oryzae*, as hosts for recombinant protein production. Recently *A. vadensis*, a close relative of *A. niger*, has been suggested as a suitable and more favourable alternative for recombinant protein production as it does not acidify the culture medium and produces very low levels of extracellular proteases. Generated growth profiles of this species on a variety of different carbon sources revealed further phenotypical differences, with *A. vadensis* possessing a distinct inability to degrade maltose or starch compared to the other black Aspergilli. In this study we examined the molecular differences between *A. vadensis* and six other species of black Aspergilli with a particular focus on the *prtT* and *amyR* Zn2Cys6 regulatory gene loci on chromosome VI to examine if the lack of protease and amylase gene expression is due to a mutation/deletion in this area.

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Introduction

The black Aspergilli (Aspergillus section Nigri) form an important subgroup of the genus Aspergillus, of which A. vadensis is a recently identified and novel member. Over the past 25 years, much research has been dedicated to the development of Aspergillus, most notably A. niger and A. oryzae, as hosts for the industrial production of recombinant proteins. To date however, yields for heterologous protein production still remain low due in part, to the acidification of the media due to the production of organic acids in the case of A. niger but also due to the presence of high levels of secreted proteases which effectively degrade many heterologous proteins [33]. Despite considerable progress being made in the development of host strains with reduced levels of secreted proteases [5, 21], these issues remain of significant importance in the area of fungal strain development. As a result, much research has been applied to the identification and assessment of the regulatory pathways involved in the induction of protease gene expression [13, 14, 16, 31]. Based on the available genome sequences from various Aspergillus species, the number of protease genes found in the fungal genome was between 100-200 [23], the regulation of which was revealed to be very complicated and interrelated with the global carbon, nitrogen and pH regulatory circuits [5, 15, 16]. Recently, however, Punt et. al. (2008) identified the PrtT regulatory protein, a homologue of which was found to be present in many Aspergillus species and which was shown to govern expression of many secreted protease genes, including the major alkaline protease *alpA* and neutral protease *Np1* in *A. oryzae* and the aspergillopepsin encoding gene pepA in A. niger [25]. Furthermore, in all Aspergillus species carrying a prtT orthologue, a gene cluster related to starch degradation, including the Zn2Cys6 regulatory gene amyR, is present directly upstream of the *prtT* locus [25].

A. vadensis, a close relative of A. niger, has been suggested as a suitable and more favourable alternative to both A. niger and A. oryzae for recombinant protein production as it does not acidify the culture medium and produces very low levels of extracellular proteases thus facilitating heterologous protein production and many downstream processes [9]. To date, the use of His-tag affinity chromatography in fungal systems has not been either efficient or practical in fungal systems due to the difficulties experienced with degradation of the Histidine residues by extracellular proteases. Purification of recombinant enzymes from A. vadensis through His-tag affinity chromatography has been shown to be successful (Culleton et al. 2014), suggesting its potential as a versatile host in the fundamental research of proteins and for industrial enzyme production.

In this study we examined the molecular and phenotypical differences between *A. vadensis* and six other species of black Aspergilli i.e. *A. niger, A. acidus, A. aculeatus, A. carbonarius, A. brasiliensis* and *A. tubingensis.* We analysed the generated growth

profiles of the individual species on a variety of carbon sources to determine if A. *vadensis* has a similar ability for plant biomass degradation as the other black Aspergilli or if its unique phenotype exceeds its protease deficiency. Finally, we examine the *prtT* and *amyR* Zn2Cys6 regulatory gene loci on chromosome VI to determine if the lack of protease expression is due to a mutation/deletion in this area.

Materials and Methods

Strains, media and culture conditions

A. vadensis CBS 113365, A. niger ATCC 1015, A. acidus CBS 106.47, A. aculeatus CBS 172.66, A. carbonarius ATCC MYA-4641, A. brasiliensis CBS 101740 and A. tubingensis CBS 134.48 were used in the growth profiles of this study. A. vadensis CBS 137441 and A. niger N402 [4] were used for all remaining experiments. All strains were taken from glycerol stocks stored at -45°C and grown on MEA (Malt Extract Agar) prior to use. All plates were grown at 30°C.

Aspergillus minimal medium (MM) and complete medium (CM) were described previously [9]. Agar was added at 2% (w/v) for solid medium. All monomeric and oligomeric carbon sources were added to a final concentration of 25 mM, while pure polymeric substrates were added to a final concentration of 1%. The pH of the medium was adjusted to 6.0. For plate growth, the centre of the plates was inoculated with 2 μ l of a suspension of 500 spores/ μ l and plates were incubated for 5 days. All species were grown on MM with 13 different carbon sources including monosaccharides (glucose, fructose, mannose and xylose), oligosaccharides (cellobiose, maltose and sucrose), pure plant polysaccharides (starch, inulin, beechwood xylan, apple pectin and galactomannan) and phosphoprotein substrate casein. Growth on 25 mM D-glucose was used as a reference for growth rate and growth on the other substrates relative to growth on glucose was then compared among the species. Growth on plates was then analyzed by visual inspection after incubation at 30°C for 5 days. For the analysis of enzyme activities, 400 mL liquid cultures (2.5 L baffled flasks) of MM + 1% (w/v) maltose / 1% (w/v) soluble starch were inoculated in duplicate with 1 x 10^6 spores/mL (final) and incubated at 30°C in an orbital shaker at 250 rpm.

For gene expression studies, *A. vadensis* was pre-grown in CM for 16 h at 30°C and 250 rpm after which the mycelium was harvest over a büchner funnel without suction and washed with MM without carbon source. Aliquots (approx. 1 g wet weight) of the mycelium were transferred to 50 ml MM with either 25 mM sucrose or 25 mM maltose and incubated for 2 h at 30°C and 250 rpm after which mycelium was harvested and frozen immediately in liquid nitrogen.
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Enzyme profile comparison by SDS-PAGE

400 ml liquid cultures were inoculated with 1 x 10^6 spores/mL (final concentration) and were grown in duplicate in 2.5 L baffled flasks at 30°C and 250 rpm for 3 days, taking samples on a daily basis. The mycelia were removed from the culture filtrate samples by filtration over a Büchner funnel with nylon gauze. SDS-PAGE was done using a 12% polyacrylamide gel containing 0.1% (w/v) SDS with 15 µL culture filtrate samples being loaded for each expression strain. Protein bands were detected by Coomassie Blue staining [18].

Enzyme activity measurement

For the measurement of enzyme activities from the A. vadensis and A. niger liquid cultures, an initial 1:10 dilution of the culture filtrates was carried out in 100 mM sodium acetate buffer, pH 4.5 including BSA (1 mg/mL). Para-nitrophenol (pNP) assays were used for the measurement of α -/ β -galactosidase and α -/ β -glucosidase activities. Initial α -/ β -galactosidase assays were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 10 mM p-nitrophenyl α -Dgalactopyranoside (pH 4.5) / β -D-galactopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. Similarly, for the initial measurement of α -/ β glucosidase activities, 0.2 mL of diluted enzyme was added in duplicate to 0.2 mL of 10 mM p-nitrophenyl α -D-glucopyranoside (pH 4.5) / β -D-glucopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. The reactions were stopped with the addition of 3 mL of 2 % (w/v) tri-sodium orthophosphate, pH 12.0 and the absorbance was measured at 400 nm. Activities were expressed as Units/ml where one unit is defined as 1 micromole of *p*-nitrophenol liberated per minute per millilitre of culture filtrate. Depending on results obtained, further time-points were conducted to ensure that activity rates were linear.

Para-nitrophenol (*p*NP) assays were also used for the measurement of α -/ β amylase activities. Initial α -amylase assays were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 10 mM blocked *p*-nitrophenyl- α -Dmaltoheptaoside + 2.5 U (final) thermostable α -glucosidase (pH 4.5) and incubated at 40°C for 60 min. Initial β -amylase activities were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 10 mM blocked *p*-nitrophenyl- β -Dmaltotrioside + 1.0 U (final) thermostable β -glucosidase (pH 4.5) and incubated at 40°C for 60 min. The reactions were stopped with the addition of 3 mL of 2 % (w/v) tri-sodium orthophosphate, pH 12.0 and the absorbance was measured at 400 nm. Enzyme blanks were prepared by adding Stopping Solution to enzyme dilution before the addition of substrate. Activities were expressed as Units/ml where one unit is defined as 1 micromole of *p*-nitrophenol liberated per minute per millilitre of culture filtrate. Depending on results obtained, further time-points were conducted to ensure that activity rates were linear.

The Megazyme D-Glucose (glucose oxidase/peroxidase; GOPOD) assay kit was used for the measurement of glucoamylase activities using soluble starch (Sigma; Cat. No. S-9765) as the substrate. Initial glucoamylase assays were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.5 mL of 10 mg/mL (final) soluble starch (pH 4.5) and incubated at 40° C for 60 min. The reactions were terminated by placing in a boiling water bath for 2 min. All samples were then treated with 3.0 mL of GOPOD reagent (glucose oxidase, peroxidase and 4-aminoantipyrine (pH 7.4) from Megazyme; Cat. No. KC-GLUC1 + KC-GLUC2) and incubated at 40°C for 20 min before absorbance was read at 510 nm. Enzyme blanks were prepared by boiling enzyme dilution before the addition of substrate. Sugar standards were prepared in quadruplicate by adding 50 µL of 1.0 mg/mL D-glucose (Megazyme; Cat. No. KC-GLUC3) and 150 µL of 100 mM sodium acetate buffer (pH 4.5) to 0.2 mL of 10 mg/mL (final) soluble starch (pH 4.5) and treating as enzyme reactions (above). Activities were expressed as Units/ml where one unit is defined as 1 micromole of glucose liberated per minute per millilitre of culture filtrate. Depending on results obtained, further time-points were conducted to ensure that activity rates were linear.

Genome mining

The genome of A. vadensis CBS 137441 was sampled using both short Illumina GAII reads and long PacBio RS reads for the purpose of hybrid assembly by ServiceXS B.V. The Illumina reads were filtered based on their sequence quality by the Q25 phred score (corresponding to a chance of one error in 333 bases). Only reads longer than 45bp after filtering were kept. The filtering was done by the in-house developed Fastq Filter v.2.05 from ServiceXS. The PacBio long read data was generated from two SMRT cells by one 90 minute movie run and one 45 minute run. PacBio SMRT Portal v1.4 was used to process the raw data. Adapter sequences were removed from the reads, which were then split into sub-reads and filtered by overall quality cut-off value of 0.75 and sequence length 50bps. The first attempt of hybrid assembly was performed by PacBio's AHA hybrid assembler and scaffolder. Cerulean v1.0 [10] was then used to merge assembled short read contigs from Abyss [28] (k-mer size of 3) and PacBio long reads. The PacBio reads were not error-corrected and the input file consisted of the data from two runs. In-house scripts were written at the end, to combine the two versions of assemblies. Augustus v3.0.2 [29] and Genemark v2.2a [20] were used to call the potential gene models from the final assembly.

Genome comparison was performed by Mugsy v.1.2.2 [2] and visualized by gmaj [3] and IGV [32]. Comparative genomics analysis of *A. vadensis* and other Aspergilli was done by OrthoMCL [12] with option 1e-20, inflation size 1, and sequence

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coverage 60%. Multiple gene alignment was done by BLASTP [1] and Muscle [11], and was visualized by ClustalOmega [27]. Cross-species gene clustering was performed by MultiGeneBlast [22].

RNA isolation and gene expression analysis

Molecular biology methods were performed according to standard procedures [26], unless stated otherwise. Frozen mycelium was ground using a TissueLyser II (Qiagen). RNA was extracted using TRIzol reagent (Invitrogen) [7] and purified with NucleoSpin RNA II (Macherey-Nagel) with DNase treatment. The quantity and quality of RNA samples were checked by NanoDrop-1000 spectrophotometer and by RNA gel electrophoresis. Total RNA in amount of 0.25 μ g was converted into cDNA (ThermoScriptTM RT-PCR System, Invitrogen) according to the instructions of the manufacturer and the obtained cDNA was diluted 10x and used in qPCR reaction.

Gene expression was assayed by real-time qPCR (Applied Biosystems 7500 Realtime PCR system) using ABI Fast SYBR Master Mix (Applied Biosystems) according to the supplier's instructions. The primers were designed using Primer Express[®] 3.0 software (Applied Biosystems), optimized and had amplification efficiencies > 97.5 and <102.7% (Supplemental Table 1). Expression levels were normalized against the histon H2B gene expression and calculated according to relative quantification $2^{-\Delta CT}$ method [19].

Results and Discussion

Comparative growth profiles on different carbon sources

The growth profiles of the different Aspergillus species showed notable differences between A. vadensis and some of the other black Aspergilli (Fig. 1; www.funggrowth.org). All strains grew well on MM + glucose and glucose was therefore used as an internal reference to compare the strains, to avoid misleading differences caused by general differences in growth speed between the species. Growth on the other substrates relative to growth on glucose was then compared between the species. All species had similar growth on the monosaccharides glucose, fructose, mannose and xylose, with the exception of A. carbonarius, which was slow to grow on all carbon sources, with especially poor growth on xylose. Growth on cellobiose and sucrose was similar to growth on glucose for all strains, while growth on the pure plant polysaccharides exceeded that generated on the simple sugars in all cases. The most notable growth differences for A. vadensis, were observed on maltose and starch, where no growth was observed on either carbon source. This was significantly lower than for the other Aspergilli, with the exception of A. carbonarius, which had poor growth on maltose and no growth on starch. The genes involved in maltose and starch degradation, such as glucoamylases (glaA), α -glucosidases (agdA) and to a lesser extent α -amylase (*amyA*, *amyB*) are all regulated by the amylolytic regulator, AmyR [24, 30]. The unusual growth profile for *A. vadensis* when grown on these carbon sources could be explained by a mutation/deletion of the *amyR* regulatory gene. Interestingly, the *prtT* locus is found close to the *amyR* locus, with only two genes



Figure 1: Growth profiles of *A. vadensis* compared to six other black Aspergilli when grown on 13 different carbon sources. All strains were grown on *Aspergillus* Minimal Medium (MM) + 25 mM / 1% carbon sources, pH 6.0 as indicated in Materials and Methods and incubated at 30°C for 5 days.



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(*agdA/aglU* and *amyA*) between the two regulators, both of which are target genes of AmyR in *A.niger*. Further analysis of the *prtT/amyR* region of the genome revealed high conservation in the black Aspergilli (Fig. 2), as supported by the efficient maltose/starch degradation abilities observed for the other black Aspergilli. Growth on casein however, was comparably poor for most the species tested, with the exception of *A. niger*, indicating that this more common protease deficiency may be accounted for at a transcriptional level.

Extracellular enzyme profile and activity comparison

Liquid cultures of A. vadensis and A. niger were performed to compare enzyme production levels for both species during growth on maltose and starch. Culture filtrate samples were taken on a daily basis to monitor extracellular enzyme production and activities, with Day 3 giving optimum production as determined by SDS-PAGE (Fig. 3). A. niger demonstrated a clear increase in enzyme production levels over the 3 days when grown on both maltose and soluble starch compared to A. vadensis, which failed to show any detectable protein bands over this incubation period. This was in correlation with the growth for both of these species, with A. niger generating more dense mycelia in both maltose and starch liquid cultures compared to A. vadensis. Further evaluation of the enzyme profiles in A. niger showed an intense band at approximately 80 kDa, which likely corresponds to the extracellular glucoamylase (GlaA), for which the calculated molecular mass in A. niger CBS 513.88 (An03g06550) is ~70 kDa [6], but for which molecular weights of between 70-91 kDa in other strains have been reported [17]. The other faint protein band produced by A. *niger* on both maltose and starch is consequently expected to be α -glucosidase (AgdA), for which a calculated molecular mass in A. niger CBS 513.88 (An04g06920) is ~110 kDa [6].



Figure 3: SDS-PAGE profile of *A. vadensis* (CBS 137441) and *A. niger* (N402) culture filtrate samples after 3 days of growth. M = 1% (w/v) maltose; SS = 1% (w/v) soluble starch. Cultures were grown in biological duplicates (1 & 2). Low molecular weight (LMW) ladder produced in house.



Figure 4: Measured activity values obtained from Day 3 culture filtrate samples from the indicated *A. vadensis* **and** *A. niger* **strains.** Error bars calculated on standard deviations between technical duplicates (White/Black). All polysaccharide substrates and *p*-nitrophenol substrates were tested at 40°C, pH 4.5 under conditions as described in Materials and Methods.

In combination with the visual profiles of extracellular enzyme production during growth on maltose/soluble starch, α -amylase, glucoamylase and α -glucosidase activities were measured and compared for both strains. The activities of α - and β galactosidase and β -glucosidase were also measured as a control and indicative of the level of change in production of un-associated enzyme activities between both species. In correlation to what was discovered with the enzyme profiles, the overall levels of maltose/starch associated enzyme activities were much higher for A. niger than for A. vadensis on both carbon sources (Fig. 4). This was especially the case for glucoamylase with A. niger producing ~6 times the levels of A. vadensis on maltose (134 mU/mL vs. 21 mU/L) and ~200 times the levels on soluble starch (494 mU/mL vs. 2mU/mL). This again was in correlation with the growth for both of these species. with A. niger generating more dense mycelia in both maltose and especially starch liquid cultures compared to A. vadensis, demonstrating a greater degradation and utilisation potential for both carbon sources. Interestingly and in contrast to the general activity profile trend observed, the levels of α -galactosidase and β -glucosidase were higher for A. vadensis on maltose but not on soluble starch. Maltose is comprised of α -1,4-linked glucose molecules but is not highly branched with α -1,6-linked subunits as is the case with starch so only requires the action of α -glucosidase to hydrolyse it into glucose molecules. As indicated by the recorded enzyme activities (Fig. 3), some α glucosidase expression was evident in A. vadensis cultures, especially when grown on maltose.



Figure 5: Measured gene expression levels for *agdA*, *amyA*, *amyR* and *ptrT* when grown on maltose and the control substrate, sucrose.

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Maltose is degraded by the enzymatic action of this enzyme to give glucose molecules. AmyR controls genes involved in starch degradation but it has been reported for *A. niger* that it also regulates other genes, including those encoding α -galactosidases and β -glucosidases [34]. While starch and maltose are the most common inducers of this regulator, glucose also induces AmyR activation in *A. niger* [34]. In *A. niger* fructose is a relatively weak repressing carbon source [8], while glucose at higher concentrations can cause significant repression of many genes encoding poly- and oligosaccharide degrading enzymes. The increased levels of α -galactosidase and β -glucosidase on maltose for *A. vadensis* could be the result of regulation from a different regulator to AmyR that does not affect amylase gene expression.

Aspergillus vadensis genome and gene expression analysis

Liquid cultures of *A. vadensis* were performed on maltose and sucrose to compare gene expression levels for *amyR* and *prtT* as well as for *agdA* and *amyA* that are lying in between them on the genome and are regulated by *amyR* (Figure 5). Sucrose was used as a control, because it supports good growth of *A. vadensis* and *amyR* and its associated genes should not be up-regulated on this carbon source. In correlation to what was observed in both the growth profiles and liquid expression studies, *amyR*, *prtT* and *amyA* are all very poorly expressed on maltose, giving signals comparable to that observed on sucrose. Interestingly, *agdA* is expressed specifically on maltose and this correlates with the observed α -glucosidase activities observed in *A. vadensis* when grown on maltose (Fig. 3).

Analysis of the *A. vadensis* genome demonstrated that the region of +/-15k bp around *prtT* is well preserved, with no missing or additional genes and no large insertions of non-coding regions. The amino acid sequences of PrtT and AmyR are highly similar to those of the other black Aspergilli and do not contain internal STOP codons (data not shown). This combined data suggests that the phenotype of *A. vadensis* is therefore most likely caused by the low expression of *prtT* and *amyR* and not a mutation or deletion of these regulators. The cause of this low expression however, remains unclear at this point, but this is likely due to a dysfunctional signal transduction, activation or sensing mechanism.

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Analysis of the molecular basis for the aberrant phenotype of Aspergillus vadensis compared to other black Aspergilli

| Primers | Sequence | Direction | Opt. Conc. |
|---------|------------------------|-----------|---------------|
| | | | (nM) |
| agdA_F | TTGGCCGCTCAACTTTCG | Forward | 900 |
| agdA_R | ATTGTCACCGCCCAGTGT | Reverse | 900 |
| amyA_F | CCGCAACATTCACCATCCTA | Forward | 900 |
| amyA_R | TGCTCCTGACCGGCGTAT | Reverse | 900 |
| amyR_F | TCCACCGGGAATCAAGTTATG | Forward | 900 |
| amyR_R | ACCCTGCGCCGTTCCT | Reverse | 900 |
| prtT_F | GCATCTCTCCCCCAACGA | Forward | 300 |
| prtT_R | GGCGACCATGCACGAAA | Reverse | 300 |
| H2B_F | GGTATCTCGACTCGCGCTATGT | Forward | 300 |
| H2B_R | TCGCGACACGCTCAAAGATAT | Reverse | 900 |

Supplemental Table 1: Primers used in this study.

Chapter 4

New promoters to improve heterologous protein production in *Aspergillus vadensis*

Culleton, H.M., Bouzid. O., McKie, V.A. and de Vries, R.P. 2014. Curr Biotechnol. 3: 1-8.

Abstract

Aspergillus is a widely used host organism for the industrial production of homologous and heterologous proteins. Although Aspergillus niger is most commonly used, a close relative of this species, Aspergillus vadensis, has been suggested as a suitable and more favourable alternative due in part, to the low levels of extracellular proteases which it produces. Despite much progress being made in the hyper production of homologous proteins, the yields obtained for heterologous proteins have still not reached a comparable level. Genetic strategies, including the development of strong constitutive promoters have been shown to lead to an increase in the levels of recombinant protein production. In this study, six novel constitutive promoters from A. niger (pef1 α , ptktA, pef1 β , ptal1, pcetA and ppgkA) and a further five from A. vadensis (pefla, prps31, pgpdA, pubil and poliC) were tested in A. vadensis using a gene encoding a secreted arabinofuranosidase from Fusarium oxysporum as a reporter for heterologous protein production. Remarkably, 9 of the 11 promoter constructs tested all resulted in higher ABF activity than the commonly used *gpdA* promoter. While this could partly be assigned to the number of copies of the expression cassette in the transformants, clear differences in productivity of the promoters could be observed.

Introduction

Over the past 25 years, much research has been dedicated to the development of filamentous fungi, most notably Aspergillus, as hosts for the industrial production of recombinant proteins. Although many Aspergillus species have the capacity to grow at high rates and to high biomass densities, the protein secretion levels observed in Aspergillus niger and Aspergillus oryzae make them the most commonly used industrial hosts among the Aspergilli [7]. While significant progress has been made in the hyper-production of heterologous proteins, yields have not yet reached the levels obtained for homologous proteins [3]. This may in part, be caused by the acidification of the media due to the production of organic acids in the case of A. niger but also by the presence of high levels of secreted proteases which effectively degrade heterologous proteins [25]. Aspergillus vadensis, a close relative of A. niger, has been suggested as a suitable and more favourable alternative for recombinant protein production as it does not acidify the culture medium and produces very low levels of extracellular proteases [5]. In fact, this species has been shown to produce higher levels of A. niger FaeB than A. niger itself [1] and also produced a basidiomycete esterase that could not be produced in A. niger [6].

Studying gene expression in filamentous fungi has greatly increased our understanding of the molecular mechanisms controlling transcription initiation and/or regulation of homologous proteins. Such genetic strategies, including the use of strong homologous promoters, have been developed to improve the yields associated with heterologous protein production in fungi. To date, the set of promoters which have been described for recombinant gene expression in A. niger have included several highly inducible or strong constitutively active promoters with the focus being on those which have shown high expression levels of their target genes [9]. Inducible expression systems, of which the glucoamylase (glaA) promoter from A. niger [11], the TAKA-amylase A (amyB) promoter from A. oryzae [13] and the alcohol dehydrogenase (alcA) from A. nidulans [27] are the most commonly used, allow for high and low expression from the same construct but are usually dependent on strictly defined conditions and media requirements. Expression of the *glaA* gene for example, is repressed in the presence of xylose but is highly induced if maltose or starch is used as the single carbon source [11]. Although transcriptional control under such inducible promoters allows for the fine-tuned regulation of expression yields and subsequent high biomass formation, the necessity for certain media components or expensive inducers in conjunction with a finely tuned timing of induction can make it both inflexible and costly for some industrial applications. The more favoured option often involves the use of constitutive promoters e.g. the glyceraldehyde-3-phosphate dehydrogenase promoter (gpdA) from A. nidulans [22], the protein kinase A (pkiA) and the alcohol dehydrogenase (adhA) promoters from A. niger [23] and the glutamate dehydrogenase promoter (*gdhA*) from *A. awamori* [19], which allow for the development of an expression cassette which is not media/component dependent. In recent studies, Blumhoff *et. al.* (2013) identified six novel constitutive promoters in *A. niger*, of which a promoter with a strong similarity to multiprotein bridging factor 1 (*Mbf1*) from *S. cerevisiae* (*PmbfA*) was shown to give higher expression levels of the heterologous *gusA* reporter gene than the *gpdA* promoter, which is the most common promoter of choice for heterologous gene expression in *Aspergillus* [8, 10, 17, 18].

To further improve protein production in *A. vadensis*, six novel constitutive promoters from *A. niger* and a further five from *A. vadensis* were selected and tested in *A. vadensis* in comparison to the *gpdA* promoter from *A. nidulans*. A gene encoding a secreted α -arabinofuranosidase from *Fusarium oxysporum* was used as a reporter for protein production.

Materials and Methods

Strains, media and culture conditions

Escherchia coli DH5 α chemically competent strain (Invitrogen) was used as a host for recombinant DNA manipulation. Microarray analysis was carried out on RNA obtained from *A. niger* CBS 120.49 (N400) and *A. vadensis* Ref 7 and promoter constructs were obtained by PCR from the gDNA of *A. niger* N402 [2] and from *A. vadensis* Ref 7. *A. vadensis* CBS 113365 (*pyrG* -) was used as the parental strain for transformation. All other *A. vadensis* strains were derived from the parental strain and are listed in Table 1.

Aspergillus minimal medium (MM) and complete medium (CM) were described previously [5]. Agar was added at 2% (w/v) for solid medium. Pre-cultures for protoplast formation were grown overnight at 30°C in 200 mL MM supplemented with 0.5% (w/v) yeast extract, 0.2% (w/v) casamino acids, 2% (w/v) glucose and 1.22 mg/mL (final) uridine after inoculating with 5 x 10⁶ spores/mL. For the analysis of promoter activities, 50 mL liquid cultures (250 mL flasks) of MM + sucrose (2%) were inoculated with 10⁶ spores/mL (final) and incubated at 30°C in an orbital shaker at 250 rpm. For chromosomal DNA isolation, *A. niger* and *A. vadensis* transformants were cultivated in CM supplemented with 1% glucose.

Molecular biology methods

Standard methods were used for DNA manipulations, subcloning, DNA digestion reactions, DNA isolations and Southern Analysis [24]. Chromosomal DNA was isolated as previously described by de Graaff *et al.* 1988 [12].

Table 1: Strains used for this study. *A. niger* CBS 120.49 (N400) and *A. vadensis* CBS Ref 7 were used in microarray analysis to identify promoter constructs. gDNA from *A. niger* N402 and *A. vadensis* CBS Ref 7 were used in PCR reactions to obtain promoter constructs. *A. vadensis* CBS 113365 was used as the parental strain for transformation. All other *A. vadensis* strains marked with an asterisk (*) were derived from the parental strain as listed.

| Species | Strain | Description | Reference |
|-------------|-----------|---|-----------------------|
| A. niger | CBS120.49 | | |
| A. niger | N402 | cspA1 mutant of CBS120.49 | Bos et al., 1988 |
| A. vadensis | CBS113365 | <i>pyrA5</i> mutant | de Vries et al., 2004 |
| A. vadensis | Ref7 | Reference strain | This study * |
| A. vadensis | FP-401 | pef1a of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-402 | ptktA of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-403 | pef1 β of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-404 | ptal of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-405 | pcetA of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-406 | ppgkA of A. niger, abf of F. oxysporum | This study * |
| A. vadensis | FP-407 | pgpdA of A. nidulans, abf of F. oxysporum | This study * |
| A. vadensis | FP-450 | pefla of A. vadensis, abf of F. oxysporum | This study * |
| A. vadensis | FP-451 | prps31 of A. vadensis, abf of F. oxysporum | This study * |
| A. vadensis | FP-452 | pgpdA of A. vadensis, abf of F. oxysporum | This study * |
| A. vadensis | FP-453 | pubil of A. vadensis, abf of F. oxysporum | This study * |
| A. vadensis | FP-454 | poliC of A. vadensis, abf of F. oxysporum | This study * |

Selection of constitutive promoters for A. niger and A. vadensis using microarray expression data

A. vadensis Ref 7 was grown in a 200 mL MM + sucrose (2%) culture at 30°C and 250 rpm. After 48, 72 and 96 hrs the mycelia were gently harvested by filtration over a Büchner funnel with nylon gauze, dried, frozen in liquid nitrogen and stored at -80°C. RNA for microarray analysis was extracted using TRIzol reagent (Invitrogen) and purified using TRIzol® Plus RNA Purification Kit (Sigma-Aldrich) according to the instructions of the manufacturer. The quality of the RNA was analysed with an Agilent 2100 bioanalyzer using an RNA6000 LabChip kit (Agilent Technology). Expression analysed data was using the Bioconductor Affy tool package (http://www.bioconductor.org) under the statistical environment R. The probe intensities were normalized by using the Robust Multiarray Average (RMA) algorithm for the Affimetrix. CEL files, quartiles algorithm was used to perform normalization and gene expression values were calculated by the medianpolish summary method (Bolstad BM, unpublished) with only the perfect match (PM) probes. The normalized data was analysed manually for genes with a high expression value. These were then compared to an in-house dataset of expression data from *A. niger* to identify genes that were highly expressed in *A. vadensis* as well as under most growth conditions in *A. niger*. This resulted in a selection of five genes for *A. vadensis*. In addition, six *A. niger* genes that were highly expressed under all conditions of the in-house dataset were also selected.

PCR and expression vectors

Promoters of the selected genes were amplified from *A. niger* and *A. vadensis* genomic DNA using primers targeting regions 1-1.3Kb upstream of the genes (Supplemental Table 1). PCR's were carried out using AccuTaqTM from Sigma and conditions supplied. PCR products were inserted into pGEMT easy vector (Promega) and plasmids were verified by sequencing.

The pGPDGFP expression vector was used to test the individual promoters in this study. As described by Lagopodi *et al.* (2002) [16], the pGPDGFP vector is composed of the *gfp* gene under the control of the *gpdA* promoter [22] and terminated with the *trpC* terminator [20]. A new expression vector was built based on the pGPDGFP vector where *gfp* was replaced with the arabinofuranosidase gene (*abf*) from *Fusarium oxysporum* using restriction enzymes *Ncol/HindIII*. The *gpdA* promoter was then replaced with the new promoter candidates using restrictions enzymes *Notl/Ncol* (Fig. 1).



Figure 1: Expression plasmid construction steps. (1) Replacement of green fluorescence protein gene (sgfp) by arabinofuranosidase from *Fusarium oxysporum* (*sabf*). (2) Insertion of *A. niger* or *A. vadensis* selected promoters in place of glyceraldehydes-3-phosphate dehydrogenase promoter (*pgpdA*) from *A. nidulans*.

Transformation of A. vadensis and selection of expression strains

The formation of protoplasts by Aspergillus strains was based on the protocols by Peraza et al. (2003) [21] and de Bekker et al. (2009) [4]. Strains were grown for 16 hrs, after which time the mycelia were gently harvested by filtration over a Büchner funnel with nylon gauze. After washing with 0.9% NaCl (w/v), 2.5 g (wet weight) mycelium was resuspended in 20 mL stabilization buffer (0.2 M phosphate buffer (pH 6.0) and 0.8 M sorbitol). Lytic enzymes were added to the following final concentrations; 5mg/mL lysing enzymes from Trichoderma harzianum (L1412, Sigma), 460 units/mL β -glucuronidase from *Helix pomatia* (G0751, Sigma) and 0.15 units/mL chitinase from Streptomyces griseus (C6137, Sigma). The mixture was incubated in an orbital shaker at 37°C for 1 to 2 hrs with gentle shaking (120 rpm). Protoplasts were separated from the mycelium by filtering over glass wool. The protoplasts were recovered by centrifugation in a swing-out rotor (10 min 2,200 rpm) and were washed twice with STC (1.33 M sorbitol, 50 mM CaCl₂ and 10 mM Tris/HCL, pH 7.5). Transformation was performed as described by Kusters-van Someren et al. (1991) [15], with 2 x 10⁶ protoplasts, 0.5 µg of pGW635 (carrying the A. niger pyrG gene for selection) and 20 µg of the different expression vectors (carrying *Fox-abf* under the control of the studied promoters).

To test the resulting transformants for *abfB* expression, a fluorimetric ABF screen based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -L-arabinopyranoside (0.02 mg/mL (final) in MM + 2% (w/v) sucrose + 2% (w/v) agar) when 1000 spores (2µL) from each transformant were grown at 30°C for 2 days and viewed under UV light. Based on fluorescence, two transformants per strain were brought forward for liquid expression studies.

α -Arabinofuranosidase activity measurement

Liquid cultures were inoculated with 1 x 10^6 spores/mL (final concentration) and grown at 30°C and 250 rpm with aliquot samples being taken on a daily basis for up to 5 days, centrifuged to remove mycelium and stored at -20°C. For the measurement of α -arabinofuranosidase (ABF) activity, culture filtrate samples (20 µL) were incubated with 10 µL of a 0.1% (w/v) *p*-nitrophenyl α -L-arabinofuranoside solution, 50 µL of a sodium acetate buffer (pH 5.0) and 20 µL water for 1 hr at 30°C in microtiter plates. The reaction was stopped by the addition of 100 µL 0.25 M bisodium carbonate and the optical density at 405 nm was measured on a microplate reader. Activities were expressed as nanomoles of *p*-nitrophenol liberated per minute per millilitre of culture filtrate.

Southern blotting

For each of the selected strains, 5 μ g of chromosomal DNA was digested with *ApaI* and with *XhoI* for 6hr at 37°C. *ApaI* has two restriction sites in the *abf* gene of *F*. *oxysporum*, so was used as a target. *XhoI* has two restriction sites in the pectin lyase gene (*pel*) of *A. vadensis*, which is present in a single copy in the *A. vadensis* genome and was therefore used as a control. For Southern analysis, digested DNA was transferred to a Hybond-N+ membrane (Amersham Biosciences). The hybridization of the DIG-labelled probes was performed according to the DIG user's manual.

Results

Selection of constitutive promoters for A. niger and A. vadensis

Preliminary data demonstrated that during growth on MM with sucrose, very few proteins are produced by *A. vadensis* (data not shown), so this condition was selected for heterologous protein production. Microarray analysis from RNA isolated from sucrose grown mycelium was performed to identify highly expressed genes. Mycelium was harvested at 48, 72 and 96 hrs to select for genes that were continuously highly expressed. The resulting genes were then used to identify the orthologs from *A. niger* and the expression of these orthologs was then evaluated in an in-house micro array database for constitutively high expression. Based on this analysis five promoters from *A. vadensis* and a further six from *A. niger* were selected to be tested as promoters to drive gene expression and protein production (Table 2).

| Source | Promoter | Acc/gene No. | Description | |
|------------------------------|---------------|--------------|--|--|
| <i>A. niger</i> CBS513.88 | peflα | An18g04840 | Similar to elongation factor 1 alpha | |
| | p <i>tktA</i> | An08g06570 | Putative transketolase | |
| | p <i>eflβ</i> | An08g03490 | Elongation factor 1 beta | |
| | ptal1 | An07g03850 | Putative transaldolase | |
| | pcetA | An16g03330 | Secreted thaumatin like protein | |
| | p <i>pgkA</i> | An08g02260 | Phosphoglycerate kinase | |
| A. vadensis Ref 7 | peflα | KJ420614 | Similar to elongation factor 1 alpha | |
| | prps31 | KJ420615 | Similar to cytoplasmic ribosomal subunit | |
| | p <i>gpdA</i> | KJ420616 | Glyceraldehyde-3-phosphate dehydrogenase | |
| | pubi1 | KJ420617 | Similar to ubiquitin | |
| | p <i>oliC</i> | KJ420618 | Similar to mitochondrial ATP synthase | |

Table 2: Promoters tested in this study.

Development of expression strains

Of the genes identified from the microarrays, 1-1.3 kb fragments upstream of the coding regions were PCR amplified, inserted into pGEMT easy vector (Promega) and sent for sequencing. Sequencing results confirmed that the correct promoter regions were amplified. To test the efficiency of the selected promoters, the corresponding fragments were cloned into a vector containing an α -arabinofuranosidase encoding gene (*abf*) from *Fusarium oxysporum* as the reporter for protein production. This approach was adopted to enable us to determine the strength of the selected promoters by accurate measurement of the expression of a heterologous gene. Once cloned the expression vector containing the promoter sequence was then transformed into *A. vadensis* CBS113365 (Table 2). To test the resulting transformants for *abf* expression, a fluorimetric screen based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -L-arabinopyranoside was applied to select the strongest expressers for each strain (Fig. 2).



Figure 2: Fluorimetric screen to select the strongest ABF producers among transformants containing *pgpdA* from *A. nidulans*. The screen was based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -*L*- arabinopyranoside (0.02 mg/mL (final)) when 1000 spores (2 μ L) from each transformant were grown at 30°C for 2 days and viewed under UV light. CBS 137441 was grown as a negative control. In this instance colonies no. 7 and no. 10 gave the strongest fluorescence and hence were chosen for liquid expression studies. Fluorimetric screens were conducted in the same way for the other promoter constructs (Fig. not shown).

Promoter characterization using α -arabinofuranosidase (abf) from Fusarium oxysporum as a reporter for protein production.

Of the transformants screened for ABF expression, two different transformants i.e. biological duplicates (labelled 01 and 02 in Fig. 3) from both the *A. vadensis* and *A. niger* promoter strains were chosen for liquid expression studies and grown in duplicate, with samples being taken on a daily basis for up to 5 days. Arabinofuranosidase (ABF) activities were measured for all samples, with day 4 samples showing optimum activity for both *A. niger* and *A. vadensis* transformants and activities being compared to FP-407 strain which contained *abf* under the control of pgpdA from *A. nidulans* (Fig. 3).



Figure 3: Comparison of arabinofuranosidase (ABF) activity levels in *A. vadensis* transformants strains expressing the heterologous *abf* of *Fusarium oxysporum* under the control of the selected promoters from both *A. niger* and *A. vadensis*. Cultures grown at 30°C and at 250 rpm, with culture filtrate samples being taken on day 4 (optimum) and assayed against *p*-nitrophenyl- α -L-arabinofuranoside substrate with absorbance measured at 405 nm. Activity levels expressed as nmol *p*-nitrophenol released per minute per mL of culture filtrate. FP-407 contains *abf* under the control of pgpdA from *A. nidulans*. *A. vadensis* CBS 137441 and CBS 113365 (*pyrG*-) strains were grown and assayed as negative controls.

ABF activity was detected for all transformants tested and was significantly above the basal level of the parental strains which were grown as negative controls (Fig. 3). Expression duplicates were measured and the average obtained to give a standard deviation for each transformant and strain. The gpdA promoter from A. nidulans (FP-407) was used as a positive control and comparative standard in both sets of expression studies. Remarkably, all 6 selected promoters from A. niger and 3 of the 5 promoters selected from A. vadensis resulted in higher ABF activity than that expressed under the gpdA promoter. In the case of the A. niger promoters, the activity levels obtained portray the order of $pef1\alpha$ (FP-401) > $pef1\beta$ (FP-403) > ptal (FP-404) > pcetA (FP-405) > ptktA (FP-402) > ppgkA (FP-406) > pgpdA (FP-407). ABF activities of the perla transformants were more than 4 times the ABF activity of the pgpdA transformants. The A. vadensis promoters resulted in ABF activity in the order of pgpdA (FP-452) > polic (FP-454) > pefla (FP-450) > pgpdA (FP-407) > pubil (FP-453 > prps31 (FP-451) where the highest producer also displayed over four times the ABF activity of the lowest. In this instance, pgpdA did not result in the lowest level of ABF activity, as the levels of the *pubil* and *prps31* transformants were approximately 14% and 19% lower, respectively. The pgpdA (FP-407) control gave consistent activity levels in both sets of expression studies with pefla from both A. niger (FP-401) and A. vadensis (FP-450) also giving similar results in both sets of expression studies signifying the close relationship between the two species. The pgpdA promoter from A. vadensis itself (FP-452) gave approximately three times the level of ABF activity of the pgpdA promoter from A. nidulans indicating a possible preference between the two promoter constructs. The corresponding activity levels for the two different transformants of each promoter construct were generally quite comparable in both sets of expression studies with over half of the promoter constructs giving < 30%variance between biological duplicates, however, in some cases e.g. the pgpdA (FP-407) control presented with ~ 50% difference in monitored *abf* activity levels between both strains. An exceptional difference of 64% was also noted for poliC (FP-454) in the A. vadensis expression study with one transformant generating 10.3 nmol pnitrophenol released/min/mL and the other giving a value of 28 nmol p-nitrophenol released/min/mL.

Southern blots

To confirm the presence and determine the copy number of the promoter constructs in the genome of *A. vadensis*, the chromosomal DNA from selected transformants was digested with *ApaI* and *XhoI*. *ApaI* has two restriction sites in the *abf* gene of *F. oxysporum* at positions 534/530 and 1216/1212, which when separated generates two specific target bands. *XhoI* has two restriction sites in the pectin lyase gene (*pel*) of *A. vadensis* at positions 433/437 and 1152/1156, also generating two specific bands when

analysed. As mentioned above, the pectin lyase gene (*pel*) of *A. vadensis* is present in a single copy in the *A. vadensis* genome and was therefore used as a control to estimate the copy number of expression constructs present in each of the transformants (Fig. 4). The additional bands observed in the *A. vadensis* blot are likely due to heterologous hybridisation to other *pel* genes.



Figure 4: Estimated copy number of expression constructs based on Southern analysis of *A. vadensis* transformants FP401 - FP407 (*A. niger* promoters) and FP450 - FP454 (*A. vadensis* promoters), using an *abf* probe fragment of *Fusarium oxysporum*. CBS 113365 (R) was used as a negative control. A fragment of the *pel* gene (encoding a pectin lyase) was used as an internal control for *A. vadensis*. Copy-numbers were determined by comparing the intensity of the *abf* signal to that of the *pel* signal.

Discussion

To date, many strategies including the use of strong homologous promoters have been applied for the development of filamentous fungi as hosts for the industrial production of recombinant proteins. In this study we identified six novel constitutive promoters from A. niger, namely pefla, ptktA, $pefl\beta$, ptall, pcetA and ppgkA and a further five from A. vadensis; pef1a, prps31, pgpdA, pubil and poliC, and tested them in A. vadensis using a gene encoding a secreted α -arabinofuranosidase from Fusarium oxysporum as a reporter for heterologous protein production. In both sets of expression studies comparing the levels of ABF activity produced under firstly, the A. niger and secondly, the A. vadensis promoters, all A. niger promoters and 3 out of 5 A. vadensis promoters resulted in higher ABF activity levels than those observed under the control of the widely characterized *gpdA* promoter, which is currently the promoter of choice for recombinant gene expression in Aspergillus [8, 10, 17, 18]. In the case of the A. *niger* promoters, the highest expressing promoter, $pefl\alpha$, gave greater than 4 times the level of *abf* activity of pgpdA. Interestingly, when transformed into A. vadensis, the pgpdA promoter from A. vadensis itself gave approximately three times the level of ABF activity of the pgpdA promoter from A. niger, indicating the possibility of species differences and preferences between the two promoter constructs. The $pefl\alpha$ from both A. niger (FP-401) and A. vadensis (FP-450) gave comparable results in both sets of expression studies, signifying not only the close relationship between the two species but also demonstrating that this gene is likely expressed similarly in both species as well.

Expression vectors for recombinant protein expression are generally integrated into the chromosomal DNA of Aspergillus by either homologous or non-homologous recombination [9]. Multiple insertions of up to and greater than 10 copies are common and are often found to increase recombinant protein production in Aspergillus [26], but this is not always the case possibly due to the pleiotropic effect of random integrations or the titration of endogenous transcription factors [14]. To gain a better understanding of the actual strength of the promoter constructs tested in this study, we felt it was necessary to determine the copy number of expression vectors transformed into the individual strains and see if the differences in ABF activity between biological duplicates and promoter constructs could be explained this way. In the A. niger expression study pef1a (FP-401), ppgkA (FP-406) and pgpdA (FP-407) all contain low copy numbers of the expression vector compared to ptktA (FP-402) where the duplicates both contain ~10 copies and pcetA (FP-405) which has an average of ~15 copies. With this information the promoter strengths can now be put into the order of pefla (FP-401) > ptal (FP-404) >> ppgkA (FP-406) > pgpdA (FP-407) > $pefl\beta$ (FP-403 > pcetA (FP-405) > ptktA (FP-402) with pefIa expressing a 10 fold increase in ABF activity per copy number compared to ptktA. The A. vadensis strains presented much more variances in copy numbers between the biological duplicates and promoter constructs which goes a long way to explain the noteworthy differences in activity levels measured between transformant duplicates in this study. Taking copy number into account the *A. vadensis* promoter constructs can be written in the order of pefla (FP-450) > poliC (FP-454) > prps31 (FP-451) > pgpdA (FP-407) > pgpdA (FP-452) > pubi1 (FP-453). Taking the new orders of strength into consideration we can see a different pattern forming than what was portrayed from the measurement of ABF activity alone. pefla is now not only performing comparably between both sets of expression study but is also the strongest expressing promoter of both the *A. niger* and *A. vadensis* promoters tested. pgpdA from *A. nidulans* also remains consistent as a control between both sets of data but now we see a much closer association in ABF expression per copy number between it and pgpdA from *A. vadensis* (FP-452) which are now both lying mid-table in terms of promoter strength.

In summary, we identified six novel constitutive promoters from *A. niger* and a further five from *A. vadensis* and tested them in *A. vadensis* using the *abf* gene from *Fusarium oxysporum* as a reporter for heterologous protein production. Of the promoters tested, 3 from *A. niger* (pef1a, ptal and ppgkA) and 3 from *A. vadensis* (pef1a, poliC and prps31) all resulted in higher ABF activity than for that of the commonly used *gpdA* promoter from *A. nidulans* signifying their potential for the industrial production of recombinant proteins.

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| Primers | Second second | D: (; * | Amplicon | |
|---------------|-----------------------------------|-------------------------|----------|--|
| [A. niger] | Sequence | Direction* | ** | |
| An18g04840F | GCGGCCGCATGGCAAAAAAAGACG | Forward 1022 | | |
| An18g04840R | CCATGGGATGACGGTTGTGAATGAAC | Reverse | 1055 | |
| An08g06570F | GCGGCCGCCTGGCTTGAAAATTGG | Forward Reverse 1138 | | |
| An08g06570R | CCATGGGATTGCTGTGTCTAGAGAAGG | | | |
| An08g03490F | GCGGCCGCTTCCCAAGCCAAGTAAG | Forward 1017 | | |
| An08g03490R | CCATGGTGTGAATGTGTGTGTTTGGGGGG | Reverse | 1017 | |
| An07g03850F | GCGGCCGCCACTGTCACAATGCTG | Forward 1021 | | |
| An07g03850R | CCATGGGGTGGAGGTAGAGAATGAG | Reverse | rse | |
| An16g03330F | GCGGCCGCGTAAAAGAAAGGAACG | Forward | 1043 | |
| An16g03330R | CCATGGTTTGATGAGATTTGTTTTAAGAGATTG | Reverse | | |
| An08g02260F | GCGGCCGCGCTAATATTATTACCAGG | Forward | d 1117 | |
| An08g02260R | CCATGGGATGAACGACGGTTCTAC | Reverse 1117 | | |
| Primers | Security | Direction* | Amplicon | |
| [A. vadensis] | Sequence | Direction | ** | |
| KJ420614F | GGGCGGCCGCAAAAAAGACGTGG | Forward | 1125 | |
| KJ420614R | GGCCATGGCCGTTAGCCCATGTCC | Reverse | | |
| KJ420615F | GGGCGGCCGCGAAGCGTCAAGCTCC | Forward | 1033 | |
| KJ420615R | GGCCATGGTGACTGTCTGATGTAGC | Reverse | | |
| KJ420616F | GGGCGGCCGCAGAGGCCAGAATAATAAG | Forward | 1052 | |
| KJ420616R | GGCCATGGTTTAGATGTGTCTATG | Reverse. | | |
| KJ420617F | GGGCGGCCGCATGGAGCCCGGCGATTTG | Forward | 1038 | |
| KJ420617R | GGCCATGGTGATGAAGGTCTGG | Reverse | | |
| KJ420618F | GGGCGGCCGCAGGGACCCACCCGCACG | Forward | 1007 | |
| KJ420618R | GGCCATGGTGATGGTTTGTGG | Reverse | | |

Supplemental Table 1: Primers used in this study.

* Forward primers contain a restriction site of *Not*I and reverse primers contain a restriction site of *Nco*I

** Amplification size upstream of gene

Chapter 5

Overexpression, purification and characterization of homologous α -L-arabinofuranosidase and *endo*-1,4- β -D-glucanase in *Aspergillus vadensis*

Culleton, H.M., McKie, V.A. and de Vries, R.P. 2014. Ind. Micro. & Biotech. **41**: 1697-1708

Abstract

In the recent past, much research has been applied to the development of *Aspergillus*, most notably A. niger and A. oryzae, as hosts for recombinant protein production. In this study, the potential of another species, A. vadensis, was examined. The full length gDNA encoding two plant biomass degrading enzymes i.e. α -L-arabinofuranosidase (abfB) (GH54) and endo-1,4- β -D-glucanase (eglA) (GH12) from A. vadensis were successfully expressed using the gpdA promoter from A. vadensis. Both enzymes were produced extracellularly in A. vadensis as soluble proteins and successfully purified by affinity chromatography. The effect of culture conditions on the expression of abfB in A. vadensis were examined and optimised to give a yield of 30 mg/L when grown on a complex carbon source such as wheat bran. Characterization of the purified α -Larabinofuranosidase from A. vadensis showed an optimum pH and temperature of pH 3.5 and 60°C which concur with those previously reported for A. niger AbfB. Comparative analysis to A. niger AbfA demonstrated interesting differences in temperate optima, pH stability and substrate specificities. The *endo*-1,4- β -D-glucanase from A. vadensis exhibited a pH and temperature optimum of pH 4.5 and 50°C respectively. Comparative biochemical analysis to the orthologous EglA from A. niger presented similar pH and substrate specificity profiles. However, significant differences in temperature optima and stability were noted.

Overexpression, purification and characterization of homologous α -Larabinofuranosidase and endo-1,4- β -D-glucanase in Aspergillus vadensis

Introduction

Over the past two decades, Aspergilli have become the most widely studied group of filamentous fungi due, in part, to their potential in many industrial applications [3, 4, 7, 11, 30]. Aspergilli have adapted to their lifestyle as common soil fungi that are found in many different environments by producing an extensive set of enzyme mixes in order to degrade the broad range of plant polysaccharides which they encounter [8]. Plant cell walls consist mainly of the polysaccharides cellulose, hemicelluloses (xyloglucans, xylan and galacto(gluco)mannan) and pectin which interact with each other as well as the aromatic polymer lignin to form a network of polymers with linkages and hydrogen bonds that give the plant cell wall its rigidity [13]. Fungi such as Aspergilli degrade these polysaccharides extracellularly by secreting diverse enzymatic mixtures which release utilizable oligo- and monosaccharides from the polysaccharide that is present [20]. The complete degradation of cellulose for instance, requires the action of at least three enzymes: β -1,4-D-glucosidase, cellobiohydrolase and β -1,4-D-endoglucanase. In contrast, the hydrolysis of xylan requires the combined action of at least nine different enzymes: α -L-arabinofuranosidase, α -1,4-Darabinoxylan galactosidase, α -glucuronidase, acetylxylan esterase. arabinofuranohydrolase, β -1,4-D-xylosidase, feruloyl esterase, β -1,4-D-galactosidase and β -1.4-D-endoxylanase [9]. The ability to produce such a broad range of enzymes combined with their good fermentation capabilities have resulted in many studies being dedicated to the development of Aspergillus as hosts for the industrial production of recombinant proteins [15]. To date, much of this research was performed with A. niger and A. oryzae, but recently A. vadensis, a close relative of A. niger, has been suggested as a possibly more favourable alternative due to the low levels of extracellular proteases which it produces and the fact that it does not acidify the culture medium [14]. In this study, the potential of A. vadensis as a host for recombinant protein production was examined by cloning and expressing two homologous genes encoding cell wall polysaccharide degrading enzymes i.e. α -Larabinofuranosidase (*abfB*) (GH54) and *endo*-1,4- β -D-glucanase (*eglA*) (GH12) in this prospective industrial strain.

 α -L-arabinofuranosidases (non-reducing end α -L-arabinofuranosidases; EC 3.2.1.55) act by hydrolysing the terminal non-reducing α -L-1,2-, α -L-1,3-, and α -L-1,5-arabinofuranosyl residues in α -L-arabinosides and can act synergistically with other hemicellulases and pectic enzymes for the complete hydrolysis of xylans and pectins [26]. In recent years α -L-arabinofuranosidases have attracted considerable interest due to their potential in industrial applications such as in oligosaccharide synthesis [34, 35], the pre-treatment of lignocelluloses for bioethanol production [17, 37] and in the chlorination of paper products [19, 25]. To date, α -L-arabinofuranosidases have been isolated from various bacterial and fungal organisms

such as *Streptomyces sp.*[39], *Thermotoga maritima* [27] and *A. niger* [36] and their genes have been cloned and expressed in developed expression systems. *A. niger* itself produces two main extracellular α -L-arabinofuranosidases; α -L-arabinofuranosidase A (AbfA) which is specifically active towards *p*-nitrophenyl- α -L-arabinofuranoside and 1,5- α -L-arabinofuranose oligosaccharides, and α -L-arabinofuranosidase B (AbfB) which has a broader activity range and is active on both these substrates but also has activity towards 1,5- α -L-arabinofuranosidase B (AbfB) which has a broader activity range and is active on both these substrates but also has activity towards 1,5- α -L-arabinofuranosidase B (AbfB) Additional genes encoding putative α -L-arabinofuranosidases were detected in the genome of *A. niger*, but have not been biochemically characterised [8]. In this study, a homologous α -L-arabinofuranosidase B encoding gene (*abfB*) from glycosyl hydrolase family 54 (GH54) was cloned and expressed in *A. vadensis* to examine the potential of this expression system. This α -L-arabinofuranosidase B (AvAbfB) was then characterized and compared to the commercially available and biochemically different α -L-arabinofuranosidase A from *A. niger* (AnAbfA) (Megazyme; Cat. No. E-AFASE) from glycosyl hydrolase family 51 (GH51).

Endoglucanases (*endo*-1,4- β -D-glucanases, EC 3.2.1.4) are a group of enzymes which combined with cellobiohydrolases (EC 3.2.1.91) and β -glucosidases (EC 3.2.1.21) are responsible for the effective degradation of cellulose into glucose. Cellulose exists as highly ordered linear polymers of β -1,4-linked D-glucose residues which are bundled together in microfibrils via hydrogen bonds [18]. It is believed that endoglucanases act by initiating random attacks at multiple sites in the non-crystalline regions of the cellulose fibre [33]. This in turn, opens up sites for subsequent attack by cellobiohydrolases, which cleave cellulose chains at the ends and release cellobiose. These oligosaccharides are then further degraded into D-glucose molecules by the action of β -glucosidases and exoglucanases [9, 33]. Among the most efficient producers of cellulolytic enzymes, in particular *endo*-1,4- β -D-glucanase, is the filamentous fungus A. niger which has gained it significant interest especially in the food, textile and pharmaceutical industries [13]. Genes encoding endoglucanases from A. niger, such as eglA, eglB and eglC, have been cloned and characterized, with EglA demonstrating the highest activity towards β -glucan compared to EglB and EglC [21, 40]. Thus, for this study the *endo*-1,4- β -D-glucanase A encoding gene (*eglA*) from glycosyl hydrolase family 12 (GH12) was cloned and expressed in A. vadensis and the activity of the corresponding enzyme (AvEgIA) was compared to the commercially available orthologous enzyme, AnEglA from A. niger (Megazyme; Cat. No. E-CELAN), which was also previously expressed through Pichia and described by Quay et.al. (2011) [33].
Materials and Methods

Strains, media and culture conditions

Escherchia coli XL1-Blue chemically competent strain (Fisher Scientific) was used as a host for recombinant DNA manipulation. Gene constructs were obtained by PCR from the gDNA of *Aspergillus vadensis* CBS 137441. *Aspergillus vadensis* CBS 113365 (*pyrG* -) was used as the parental strain for transformation.

Aspergillus minimal medium (MM) and complete medium (CM) were described previously [14]. Agar was added at 2% (w/v) for solid medium. Pre-cultures for protoplast formation were grown overnight at 30°C in 200 mL MM supplemented with 0.5% (w/v) yeast extract, 0.2% (w/v) casamino acids, 2% (w/v) glucose and 1.22 mg/mL (final) uridine after inoculating with 5 x 10⁶ spores/mL. For the analysis and characterization of enzyme activities, 400 mL liquid cultures (2.5 L baffled flasks) of MM + 1% (w/v) wheat bran (for production of AvAbfB) or 2% (w/v) sucrose (for production of AvEgIA) + 0.1% (w/v) TWEEN[®] 80 were inoculated with 1 x 10⁶ spores/mL (final) and incubated at 35°C in an orbital shaker at 250 rpm.

Molecular biology methods

Standard methods were used for DNA manipulations, subcloning, DNA digestion reactions and DNA isolations [38].

PCR and expression vectors

Genes were amplified from the then partially sequenced A. vadensis genome (Culleton and de Vries, unpublished data) with primers including 5' NcoI and 3' AfIII restriction sites necessary for the cloning of the α -L-arabinofuranosidase encoding *abfB* gene (1500 bp) and 5' SphI and 3' AfIII restriction sites necessary for the endo-1,4- β -Dglucanase encoding eglA gene (836 bp). The nucleotide sequences of the primers utilised (incorporating the restriction sites indicated) are as follows: *abfB* 5' end oligonucleotide 5'-GGCCATGGTCTCCCGCCGAAACC-3' and abfB 3' oligonucleotide 3'-GGCTTAAGCGAAGAAAACGCCGTCTC-5'. eglA 5' end oligonucleotide 5'-GGGCATGCAGCTCGCAGTGACAC-3' 3' and eglA oligonucleotide 3'-GGCTTAAGGTTGACACTAGCGGTCC-5'. PCR reactions were carried out using KOD DNA Polymerase (Merck Biosciences) and conditions supplied. PCR products were inserted into pCR2.1 TOPO vector (Invitrogen) following instructions provided and plasmids were verified by sequencing.

The pGPDGFP expression vector was used to make the new expression constructs in this study. As described by Lagopodi *et al.* (2002) [24], the pGPDGFP vector is composed of the *gfp* gene under the control of the *gpdA* promoter from *A. nidulans* [31] and terminated with the *trpC* terminator [28]. A new expression vector was built based on the pGPDGFP vector where pgpdA from *A. nidulans* was replaced with pgpdA from A. vadensis [10] using restrictions enzymes NotI/NcoI. Due to the presence of an internal HindIII within pgpdA from A. vadensis, the HindIII site at the 3' end of the gfp gene was replaced with AfIII and a 6 X Histidine tag and stop codon were added to assist in the downstream purification of the enzymes once expressed. Due to the presence of an NcoI site within the gene sequence of AveglA, an additional SphI site was added alongside the NcoI site at 3' end of promoter to facilitate the cloning on of this gene. The gfp gene was then replaced with the new gene candidate from A. vadensis using restriction enzymes NcoI/AfIII for AvabfB and SphI/AfIII for AveglA (Fig. 1).

Transformation of A. vadensis

The formation of protoplasts by Aspergillus strains was based on the protocols by Peraza et al. (2003) [29] and de Bekker et al. (2009) [12]. Strains were grown for 16 hrs, after which time the mycelia were gently harvested by filtration over a Büchner funnel with nylon gauze. After washing with 0.9% NaCl (w/v), 2.5 g (wet weight) mycelium was resuspended in 20 mL stabilization buffer (0.2 M phosphate buffer (pH 6.0), 0.8 M sorbitol). Lytic enzymes were added to the following final concentrations: 5 mg/mL lysing enzymes from Trichoderma harzianum (Sigma; Cat. No. L1412), 460 units/mL β -glucuronidase from *Helix pomatia* (Sigma; Cat. No. G0751) and 0.15 units/mL chitinase from Streptomyces griseus (Sigma; Cat. No. C6137). The mixture was incubated in an orbital shaker at 37°C for 1 to 2 hrs with gentle shaking (120 rpm). Protoplasts were separated from the mycelium by filtering over glass wool. The protoplasts were recovered by centrifugation in a swing-out rotor (10 min; ~ 800 rcf) and were washed twice with STC (1.33 M sorbitol, 50 mM CaCl2 and 10 mM Tris/HCl, pH 7.5). Transformation was performed as described by Kusters-van Someren et al. (1991) [22], with 2 x 10⁶ protoplasts, 0.5 µg of pGW635 (carrying the A. niger pyrG gene for selection) and 20 µg of the different expression vectors (carrying the studied genes under the control of the pgpdA from A. vadensis).



Figure 1: Construction of the new expression plasmid. (1) Replacement of glyceraldehyde-3-phosphate dehydrogenase promoter (p*gpdA*) from *A. nidulans* with p*gpdA* from *A. vadensis.* (2) Replacement of *Hind*III site at 3' end of gene construct with *Afl*II and addition of 6 x His-tag. (3) Insertion of *Sph*I site alongside *Nco*I site and *A. vadensis* genes (*sAVX*) in place of green fluorescence protein gene (*sgfp*).

Screening of transformants and selection of expression strains

To test the resulting transformants for *abfB* expression, a fluorimetric ABF screen based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -Larabinopyranoside (0.02 mg/mL (final) in MM + 2% (w/v) sucrose + 2% (w/v) agar) when 1000 spores (2 μ L) from each transformant were grown at 30°C for 2 days and viewed under UV light. To test for *eglA* expression, resulting transformants were grown on agar plates containing high purity dyed and crosslinked insoluble AZCL-Barley beta-Glucan (Megazyme; Cat. No. I-AZBGL) (0.1% (w/v) in MM + 2% (w/v) sucrose + 2% (w/v) agar) when 1000 spores (2 μ L) from each transformant were grown at 30°C for 2 days. In both screens, CBS 137441 was grown as a negative control.

Production and purification of recombinant enzymes

400 ml liquid cultures were inoculated with 1 x 10^6 spores/mL (final) and grown in 2.5L baffled flasks at 35°C, 250 rpm with production levels being monitored daily by SDS polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was done using a 12% polyacrylamide gel containing 0.1% (w/v) SDS and protein bands were detected by Coomassie Blue staining [23]. Cultures were grown for the time indicated in Results, after which time the mycelium was removed by filtration over a Büchner funnel with nylon gauze. Culture filtrates were purified by His-tag affinity chromatography, equilibrated with buffer (10mM imidazole, 10 mM HEPES and 500 mM NaCl, pH 7.5) and eluted with a stepwise gradient in same buffer containing 10 mM to 500mM imidazole. Eluent from the column was monitored for levels of protein concentration by testing 10 µL of sample in 150 µL of Bio-Rad protein assay dye reagent (Bio-Rad; Cat. No. 500-0006). 15 µL samples of culture filtrates (C.F.) and column fractions were viewed by SDS-PAGE (as above) and fractions containing purified protein were pooled and precipitated with 50% ammonium sulphate. Enzyme fractions were then concentrated by centrifugation and the enzyme pellet was resuspended in 3.2 M ammonium sulphate solution for characterization.

Enzyme activity measurement

For the initial measurement of the *A. vadensis* α -arabinofuranosidase B (AvAbfB) and *A. niger* α -arabinofuranosidase A (AnAbfA) activities, serial dilutions of the purified enzymes were carried out in 100 mM sodium acetate buffer, pH 4.0 including BSA (1 mg/mL). 0.2 mL of diluted enzyme were added to 0.2 mL of 10 mM *p*-nitrophenyl α -L-arabinofuranoside (pH 4.0) and incubated at 40 °C for 10 min. The reaction was stopped with the addition of 3 mL of 2 % (w/v) tri-sodium orthophosphate, pH 12.0 and the absorbance was measured at 400 nm. Activities were expressed as Units/mg where one unit is defined as 1 micromole of *p*-nitrophenol liberated per minute per milligram of enzyme.

For the initial measurement of the *A. vadensis endo*-1,4- β -D-glucanase A (AvEglA) and the orthologous *A. niger endo*-1,4- β -D-glucanase A (AnEglA) activities, serial dilutions of the purified enzymes were performed in 100 mM sodium acetate buffer, pH 4.5 including BSA (1 mg/mL). Nelson-Somogyi reducing sugar assays were performed by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 10 mg/mL Barley β -glucan (Megazyme; Cat. No. P-BGBM) (pH 4.5) and incubated at 40°C for 10 min. The reaction was stopped by the addition of 0.5 mL of Stopping Solution (25 mL of Solution A (2.5% (w/v) sodium carbonate anhydrous, 2.5% (w/v) potassium sodium tartrate and 20% (w/v) sodium sulphate), to which 1 mL of Solution B (3% (w/v) copper sulphate pentahydrate) was added). Enzyme reactions were then boiled for 20 min and allowed to cool to room temperature for 5 min before the addition of 3.0 mL of a 1:5 dilution of Solution C (5% (w/v) ammonium molybdate, 4.2% (v/v) concentrated sulphuric acid and 0.6% (w/v) sodium arsenate heptahydrate).

pH optima assays were conducted in duplicate measurements using the optimum enzyme dilution as determined from the initial activity assays and using the following pH buffers covering from pH 1.0 to pH 9.0 including BSA (1 mg/mL); potassium chloride (pH 1.0 and pH 2.0), glycine (pH 2.0, pH 2.5, pH 3.0), citrate phosphate (pH 3.0, pH 3.5, pH 4.0, pH 4.5, pH 5.0, pH 5.5, pH 6.0, pH 6.5 and pH 7.0) and sodium phosphate (pH 6.0, pH 7.0, pH 8.0 and pH 9.0). The remaining assay conditions were consistent with the assay conditions used for the measurement of initial activity. For pH stability assays an initial 10-fold dilution of the enzyme was carried out in the buffers outlined above (pH 1.0 – pH 9.0) including BSA (1 mg/mL) and incubated at 4°C for 48 hrs. Activity assays were then conducted in duplicate measurements using the optimum enzyme concentration and pH as determined by previous assays with the enzyme activity at the optimum pH set at 100%.

For temperature stability assays an initial 10-fold dilution of the enzyme in optimum pH buffer was performed and aliquots incubated at the following temperatures for 15 min; 25°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C. Following incubation, the enzyme was further diluted in optimum pH buffer to an optimum concentration and assayed in duplicate as described above. Temperature optima assays were performed in duplicate measurements using optimum enzyme concentration and pH as determined in previous assays and at temperatures that the enzyme proved stable.

A. vadensis α -arabinofuranosidase B (AvAbfB) and A. niger α -arabinofuranosidase A (AnAbfA) substrate specificity assays were performed using the following polysaccharide substrates at a final concentration of 5 mg/mL; wheat flour arabinoxylan (Megazyme; Cat. No. P-WAXYL), sugar beet arabinan (Megazyme; Cat. No. P-ARAB) and debranched sugar beet arabinan (Megazyme; Cat. No. P-DBAR). Activities were determined under optimum conditions using Nelson-Somogyi reducing

sugar assays as described above. To test *A. vadensis* (AvEglA) and *A. niger* (AnEglA) endoglucanase A for substrate specificity, the following polysaccharide substrates and *p*-nitrophenol substrates were used at final concentrations of 5 mg/mL and 5 mM, respectively; carboxymethyl cellulose 4M (Megazyme; Cat. No. P-CMC4M), galactomannan (Megazyme; Cat. No. P-GALML), glucomannan (Megazyme; Cat. No. P-GLCML), pachyman (Megazyme; Cat. No. P-CMPAC), soluble starch (Sigma; Cat. No. S9765), xyloglucan (Megazyme; Cat. No. P-XYGLN), *p*-nitrophenol α -D-glucopyranoside, *p*-nitrophenol β -D-glucopyranoside and *p*-nitrophenol β -D-glucopyranoside. Assays were performed under optimum pH and temperature conditions as determined in previous assays and using methods as described above.

Results

Development of expression strains

The selected genes were PCR amplified from *A. vadensis* gDNA, inserted into pCR2.1 TOPO vector (Invitrogen) and sequenced using the Sanger method at LGC Genomics. Sequencing results confirmed that the correct gene regions were amplified. Identification and comparison of the corresponding α -L-arabinofuranosidase B protein (499 amino acids) and *endo*-1,4- β -D-glucanase A protein (239 amino acids) from *A. vadensis* with other characterized protein sequences was performed using the BLAST program at NCBI. The AvAbfB protein sequence has 99% identity to *Aspergillus kawachii* IFO 4308 (Q8NK89.1), *Aspergillus awamori* (Q9C4B1.1) and *Aspergillus niger* AbfB (XP_001396769.1). On the basis of the similarities to these other α -L-arabinofuranosidases, *A. vadensis abfB* was assigned to GH54 (CAZy-http://www.cazy.org) [5]. The AvEgIA protein sequence showed high identity (96%) to *A. niger* CBS 513.88 (XP_001400902.1) and 95% to *Aspergillus usamii* (AEL12376.1), thus providing basis for its inclusion into the GH12 family.

For expression and characterization of the selected genes, the corresponding fragments were cloned behind the *gpdA* promoter from *A. vadensis* [10]. The resulting expression vectors containing the gene sequence were then transformed into *A. vadensis* CBS113365. To test the resulting transformants for *abfB* expression, a fluorimetric screen based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -L-arabinopyranoside was applied to select the strongest expressing transformants (Fig. 2A) i.e. transformant no. 7 (*abfB*_t7). To test for *eglA* expression, resulting transformants were grown on agar plates containing high purity dyed and crosslinked insoluble AZCL-Barley beta-Glucan (Megazyme; Cat. No. I-AZBGL), from which the strongest expressing clones were selected based on ability to hydrolyse the AZCL-Barley beta-Glucan substrate (Fig. 2B) i.e. transformant no. 9 (*eglA*_t9).

Effect of culture conditions on the production of α -arabinofuranosidase (AbfB)

A. vadensis abfB_t7 was cultivated under several different conditions for investigation of α -arabinofuranosidase production. Glucose, mannose and sucrose were used as simple carbon sources for comparison purposes, with sucrose giving optimum *abfB* expression with yields of ~7 mg/L. The addition of 0.1% (w/v) TWEEN[®] 80 to media doubled these yields to ~15 mg/L. Growth temperatures from 25°C - 37°C were examined with *A. vadensis abfB_t7* favouring higher incubation temperatures i.e. 35°C - 37°C, for the expression of recombinant α -arabinofuranosidase. The use of a complex carbon source such as wheat bran (Odlums) under optimum conditions increased native and recombinant protein production alike, giving a purified AvAbfB yield of 30 mg/L.



Figure 2: Substrate plate screens to select the strongest AbfB producers (A) and EgIA producers (B). Fluorimetric ABF screen based on the conversion of the fluorogenic substrate 4-methylumbelliferyl- α -L-arabinopyranoside (0.02 mg/mL (final)) when 1000 spores (2µL) from each transformant were grown at 30°C for 2 days and viewed under UV light. EGL screen based on the ability to hydrolyse the dye particles of the crosslinked and insoluble AZCL-Barley beta-Glucan (I-AZBGL, Megazyme) (0.1% (w/v)) when 1000 spores (2µL) from each transformant were grown at 30°C for 2 days. CBS 137441 was grown as a negative control. In this study colony no. 7 (*abfB*_t7) was shown to give the strongest fluorescence and colony no. 9 (*eglA*_t9) was shown to give the greatest amount of dye release and hence both were chosen for liquid expression studies.

α -arabinofuranosidase (AbfB) production and characterization

Liquid cultures were inoculated with 1 x 10^6 spores/mL (final) from chosen transformants, grown at 35°C and 250 rpm and expression levels were monitored on a daily basis by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Optimum AvAbfB production was observed after 3 days incubation and the enzyme was purified from these culture filtrates using His-tag affinity chromatography. 15 µL samples of culture filtrates (C.F.) and column fractions were viewed by SDS-PAGE (Figure 3A) with purified protein visible in the eluent fractions containing 50 mM, 100 mM and 500 mM imidazole. These were pooled and precipitated with 50% ammonium sulphate. Enzyme fractions were then concentrated and quantified giving a total yield of 30 mg/L purified protein (Table 1A).



Figure 3: SDS-PAGE analysis and purification of *A. vadensis* α -L-arabinofuranosidase B (AvAbfB) (A) compared to increasing amounts i.e. 1 µg, 5µg and 10µg, of industrially purified α -L-arabinofuranosidase A from *A. niger* (AnAbfA) (B) and *endo*-1,4- β -D-glucanase (AvEglA) (C) compared to increasing amounts i.e. 1 µg, 5µg and 10µg, of industrially purified and orthologous *A. niger* EglA (D). Low molecular weight (LMW) ladder produced in house. Culture filtrates (C.F.) were purified by affinity chromatography, equilibrated with buffer (10mM imidazole, 10 mM HEPES and 500 mM NaCl, pH 7.5) and eluted with a stepwise gradient in same buffer containing 10 mM to 500mM imidazole. Flow through (F.T.) contained proteins which had no specific binding capacity to the resin.

The enzymatic properties of the recombinant α -arabinofuranosidase B from A. *vadensis* (AvAbfB) compared to that of the commercially available and industrially purified α -arabinofuranosidase A from A. *niger* (AnAbfA) were examined. The optimal pH for both enzymes was pH 3.5 (activity measured at pH 3.5 equalled that at pH 4.0 for AnAbfA) with both retaining \geq 90% activity at pH 3.0 and pH 4.0 (Figure 4A). AvAbfB demonstrated greater pH stability than AnAbfA, with \geq 92% of AvAbfB activity remaining at all pH's tested i.e. pH 1.0 – pH 9.0, compared to AnAbfA which resulted in 0% activity at pH 1.0, even with immediate testing after 1 hr incubation at this pH (Figure 4B).



Figure 4: Biochemical properties of purified recombinant α -arabinofuranosidase from A. *vadensis* (AvAbfB) compared to those of commercially available α -arabinofuranosidase A from A. *niger* (AnAbfA). Values expressed as relative specific activity (%) of maximum activity obtained for that assay. In the case of pH optima and stability, average values were taken where there was an overlap in pH with the use of different buffers. Error bars calculated on standard deviations between technical duplicates. (A) pH optima of AvAbfB and AnAbfA; (B) pH stability of AvAbfB and AnAbfA; (C) Temperature optima of AvAbfB and AnAbfA; (D) Temperature stability of AvAbfB and AnAbfA.

AvAbfB was also more stable at higher temperatures than AnAbfA, with 100% of activity being preserved for both enzymes up to 50°C but with 77% of AvAbfB activity being maintained after a 15 min incubation at 60°C compared to just 58% in the case of AnAbfA (Figure 4D). The temperature optima for these enzymes therefore differed with AvAbfB giving maximum activity at 60°C and AnAbfA having an optimum of 50°C (Figure 4C). Specific activities for both enzymes in 100mM citrate phosphate buffer pH 3.5 at 40°C were 44 U/mg for AvAbfB and 127 U/mg for AnAbfA. These values increased to 100 U/mg for AvAbfB and 221 U/mg for AnAbfA when assayed under optimum pH and at 50°C.

Additional enzyme activities were also measured for both enzymes on wheat flour arabinoxylan, sugar beet arabinan and debranched sugar beet arabinan relative to the specific activities obtained on *p*-nitrophenyl α -L-arabinofuranoside at 40°C (Table 2). In these experiments AvAbfB demonstrated greater activity on all three tested polysaccharides than AnAbfA.

endo-1,4-β-D-glucanase (EglA) production and characterization

Liquid cultures were inoculated with 1 x 10^6 spores/mL from chosen transformants, grown at 35°C and 250 rpm and expression levels were monitored on a daily basis by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Optimum AvEglA production was observed after 4 days incubation and resulting culture filtrates were purified by

Table 1: Purification of α -L-arabinofuranosidase (AbfB) and *endo*-1,4- β -D-glucanase (EglA) from *Aspergillus vadensis*. Specific activities calculated at 40°C / 50°C under optimum conditions for each enzyme as outlined in Material and Methods.

| A) Purification of α-L-arabinofuranosidase (AbfB) | | | | | | |
|--|-----------------------|--------------------------|--------------------------|--|--|--|
| | Purification- Fold | Concentration (mg/mL) | Specific Yield (mg/L) | Specific Activity (U/mg) 40°C / 50°C | | |
| Crude (1500 mL) | - | - | - | - | | |
| Purified (20 mL) | 75 | 2.27 | 30 | 44 / 100 | | |
| B) Purification of <i>endo</i> -1,4-β-D-glucanase (EglA) | | | | | | |
| | Purification- Fold | Concentration (mg/mL) | Specific Yield (mg/L) | Specific Activity (U/mg) 40°C / 50°C | | |
| Crude (370 mL) | - | - | - | - | | |
| Purified (10 mL) | 37 | 0.92 | 25 | 204 / 280 | | |

His-tag affinity chromatography. 15μ L samples of culture filtrates (C.F.) and column fractions were viewed by SDS-PAGE (Figure 3B) with the 100 mM and 500 mM fractions containing purified protein. These were pooled and precipitated with 50% ammonium sulphate. Enzyme fractions were then concentrated and quantified giving a total yield of 25 mg/L purified protein (Table 1B).

The enzymatic properties of the recombinant *endo*-1,4- β -D-glucanase from *A. vadensis* (AvEglA) were compared to the commercially available and industrially purified orthologous *endo*-1,4- β -D-glucanase from *A. niger* (AnEglA). The optimal pH for both enzymes peaked at pH 4.5 with AvEglA demonstrating a narrower optima curve than AnEglA, losing 55% / 40% relative activity at pH 4.0 / pH 5.0 respectively compared to AnEglA which lost 25% / 6% activity at same (Fig. 5A). Both enzymes showed comparable stability at all pH's tested i.e. stable at pH 1.0 – pH 9.0 after 48 hrs incubation at this pH (Fig. 5B).



Figure 5: Biochemical properties of purified recombinant endoglucanase from *A. vadensis* (AvEgIA) compared to those of commercially available *endo*-1,4- β -D-glucanase from *A. niger* (AnEgIA). Values expressed as relative specific activity (%) of maximum activity obtained for that assay. In the case of pH optima and stability, average values were taken where there was an overlap in pH with the use of different buffers. Error bars calculated on standard deviations between technical duplicates. (A) pH optima of AvEgIA and AnEgIA; (B) pH stability of AvEgIA and AnEgIA; (C) Temperature optima of AvEgIA and AnEgIA; (D) Temperature stability of AvEgIA and AnEgIA and AnEgIA activity.

The temperature optimum of AvEglA was 50°C, while AnEglA gave a broader optimum curve i.e. between 50°C and 60°C but which favoured the higher temperature of 60°C (50°C giving ~2.5% less relative specific activity compared to 60°C). AnEglA also demonstrating a greater stability profile in maintaining 91% relative activity at 60°C compared to AvEglA where activity at this temperature measured only 5% of that obtained at 50°C (Fig. 5D and 5C).

Specific activities for both enzymes in 100 mM sodium acetate buffer pH 4.5 at 40°C were 204 U/mg for AvEglA and 179 U/mg for AnEglA. These values increased to 280 U/mg for AvEglA and 230 U/mg for AnEglA when assayed under optimum pH at 50°C. No activity was detected for either enzyme on galactomannan, glucomannan, pachyman, soluble starch, xyloglucan, *p*-nitrophenol α - D-glucopyranoside, p-nitrophenol β -D-glucopyranoside and *p*-nitrophenol β -D-xylopyranoside. A lower activity on cellulose was observed for AvEglA than for AnEglA relative to the specific activities obtained on Barley β -glucan at 40°C (Table 2).

Table 2: Relative activities of the purified α -arabinofuranosidase and *endo*-glucanase against different substrates. All polysaccharide substrates and *p*-nitrophenol substrates were tested at 40°C and at concentrations of 5 mg/mL (w/v final) and 5 mM (final) respectively, under conditions as described in Materials and Methods.

| Substrate | Relative activity (%) | | | |
|---|------------------------------|---------------|--|--|
| | A. vadensis AbfB | A. niger AbfA | | |
| <i>p</i> -nitrophenol α -L-arabinofuranoside | 100.0 | 100.0 | | |
| Wheat flour arabinoxylan | 1.5 | 1.4 | | |
| Sugar beet arabinan | 19.2 | 5.9 | | |
| Debranched sugar beet arabinan | 3.2 | 1.0 | | |
| Substrate | A. vadensis EglA | A. niger EglA | | |
| Barley β -Glucan | 100.0 | 100.0 | | |
| Cellulose | 24.0 | 65.0 | | |
| Galactomannan | 0.0 | 0.0 | | |
| Glucomannan | 0.2 | 0.2 | | |
| Pachyman | 0.0 | 0.0 | | |
| Soluble Starch | 0.0 | 0.0 | | |
| Xyloglucan | 0.0 | 0.0 | | |
| <i>p</i> -nitrophenol α -D-glucopyranoside | 0.0 | 0.0 | | |
| <i>p</i> -nitrophenol β -D-glucopyranoside | 0.0 | 0.0 | | |
| <i>p</i> -nitrophenol β -D-xylopyranoside | 0.0 | 0.0 | | |

Discussion

Over the past 25 years, much research has been devoted to the development of *Aspergillus* as a host for homologous and heterologous protein production. Not only does *Aspergillus* growth excel under fermentation conditions with an exceptional capacity of secreting high levels of homologous product but their potential in plant polysaccharide degradation and the extensive set of enzyme mixes which they secrete for this purpose is also well recognized [9]. To date, much of this research has been focused on *A. niger* and *A. oryzae*, while the capacity of *A. vadensis*, a close relative of *A. niger*, has remained largely unexplored. In this study, the potential of *A. vadensis* as a host for recombinant protein production was examined by cloning and over-expressing two homologous genes encoding plant biomass degrading enzymes i.e. *a*-L-arabinofuranosidase (*abfB*) and *endo-1*,4- β -D-glucanase (*eglA*).

As shown in Figure 3A the A. vadensis α -L-arabinofuranosidase B (AvAbfB) protein was purified at a molecular weight of ~ 53 kDa, which corresponds to the calculated predicted mass of 52.5 kDa, based on the obtained amino acid sequence information and to that of the previously recorded 51.0 kDa for the orthologous AbfB enzyme in A. niger [16]. Earlier studies reported that the α -L-arabinofuranosidases from various Aspergillus species had a molecular weight of about 30 to 118 kDa, depending on the level of N- and O-glycosylation sites [2]. This appears to be the case for α -L-arabinofuranosidase A (AnAbfA) which has a molecular weight of ~ 62 kDa and is visualised as a single major band on SDS-electrophoresis (Figure 3B) as is described on the product's data sheet (http://secure.megazyme.com/Alpha-L-Arabinofuranosidase_A._niger). The biochemical properties of A. vadensis α -Larabinofuranosidase B (AvAbfB) were examined and found to match those of the previously identified orthologous AbfB from A. niger with a pH and temperature optimum of pH 3.5 and 60°C but interestingly, is stable at pH's as low as pH 1.0, which other α -L-arabinofuranosidases from A. niger were not [2]. Comparative biochemical analysis with A. niger α -L-arabinofuranosidase A (AnAbfA) demonstrated that AnAbfA had more specific activity towards p-nitrophenyl a-Larabinofuranoside but ~ 3 times less than AvAbfB on both the branched and debranched sugar beet arabinan. Sugar beet arabinan consists of a 1.5- α -linked backbone to which $1,3-\alpha$ -linked (and possibly some $1,2-\alpha$ -linked) L-arabinofuranosyl residues are attached. In previous studies, AbfA from A. niger was reported to be incapable of splitting $1,3-\alpha$ -L - or $1,2-\alpha$ -L linked arabinose substituents or arabinose from longer 1,5- α -L-linked arabinose residues whereas AbfB can [36].

Figure 3C shows the purification of *A. vadensis endo*-1,4- β -D-glucanase (AvEglA) at a molecular weight of 26 kDa which too, is in keeping with the calculated predicted mass of 27.72 kDa, based on the obtained amino acid sequence information and with that previously obtained for the purification of the orthologous protein from *A. niger*

[33]. This is confirmed with A. niger endo-1.4- β -D-glucanase (AnEglA) being visualised at a molecular weight of ~ 27 kDa and as a single band on SDSelectrophoresis (Figure 3D), as is described on the products data sheet (http://secure.megazyme.com/Cellulase endo-1-4-Beta-D-glucanase A. niger). The biochemical characteristics of A. vadensis endo-1.4- β -D-glucanase (AvEglA) are similar to those previously identified in the orthologous enzyme from A. niger [33]. Comparative biochemical analysis of AvEgIA to the orthologous endo-1.4- β -Dglucanase from A. niger (AnEglA) showed similar characteristics, both giving equivalent pH optima (pH 4.5) and pH stability (pH 1 - pH 9) profiles. Interestingly, a notable difference was displayed for temperature optima and stability however, with AvEglA having a temperature optimum of 50°C, compared to AnEglA which gave a broader optimum curve between 50°C and 60°C but which favoured the higher temperature of 60°C. AnEgIA also demonstrating a greater stability profile in maintaining 91% relative activity at 60°C compared to AvEglA where activity at this temperature measured only 5% of that obtained at 50°C. Both AvEgIA and AnEgIA demonstrated little to no activity on the additional substrates tested, with the exception of cellulose, where AnEglA demonstrated ~ 3 times greater activity against this substrate than AvEgIA. Cellulose is a polymer of $1.4-\beta$ -linked D-glucosyl residues which both *endo*-1,4- β -D-glucanases should be able to degrade with similar efficiency. Sequence alignments for both EglA orthologs studied show a 5% difference at the amino acid level (Supplemental Figure 1B) which may lead to slight variations in the kinetic properties of the corresponding enzymes. Although both enzymes are visually pure by SDS comparison (Figures 3C and 3D), differences in production hosts, glycosylation patterns and purification methods may contribute to the variations recorded.

This study has demonstrated the potential of *A. vadensis* as a host for recombinant protein expression. Not only does *A. vadensis* not acidify the culture medium but it also produces very low levels of extracellular proteases and so facilitates many downstream processes. To date, the use of His-tag affinity chromatography in fungal systems has not been either efficient or practical in fungal systems due to the difficulties experienced with degradation of the Histidine residues by extracellular proteases. Purification of recombinant enzymes from *A. vadensis* through His-tag affinity chromatography have worked well thus far, suggesting its potential as a versatile host in the fundamental research of proteins and for industrial enzyme production. With an initial expression yield of ~ 30 mg/L, *A. vadensis* shows positive signs of becoming an industrially significant contender but considerable improvements will be required to rival the secretion abilities of commercial production strains such as *A. niger* and *T. reesei*. Strategies for improving recombinant protein production in fungi, including the use of strong promoters and effective secretion signals or gene

fusions to genes associated with well-expressed and secreted proteins have been implemented successfully to other systems in the past [32, 41]. Successes have also been obtained using a bioprocessing approach of optimizing fungal morphology, mycelia immobilization and culture conditions [1, 6]. It should be noted however, that the *A. vadensis* strain used in these experiments is near wild type and has not undergone the extensive strain improvement strategies that have resulted in the commercial *A. niger* and *T. reesei* strains that secrete much higher levels of enzyme, suggesting room for improvement.

The results of this study demonstrate significant variations in biochemical characteristics in the different α -arabinofuranosidases, AnAbfA and AvAbfB but also in the orthologous *endo*-1.4- β -D-glucanases (EgIA) when produced from A. *niger* and A. vadensis, despite these organisms being taxonomically very close. Despite AnAbfA (GH51) and AvAbfB (GH54) having just 22% identity at an amino acid level (Supplemental Figure 1A) and being assigned to different CAZy families, the biochemical differences observed between these two enzymes are not significantly greater than that observed in the orthologous EglA comparison which have 95% identity. In particular, the difference in temperature optima and stability for these orthologous enzymes is noteworthy and would directly impact the applications of these enzymes. Screening of orthologs from related fungi may therefore be worthwhile for many industrial applications and suggests that it is not always necessary to go to distantly related species or enzyme classifications to get significant changes in biochemical properties. Detailed structural comparison of these orthologous enzymes may reveal insight into the molecular basis of this difference in stability and is worth pursuing in follow-up studies.

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Supplemental Figure 1A: Protein Sequence Alignment for *A. vadensis* α-Larabinofuranosidase B (AvAbfB) and *A. niger* α-arabinofuranosidase A (AnAbfA).

А.

| AvAbfB AnAbfA | MFSRRNLLALGLAATVSAGPCDIYEAGDTPCVAAHSTTRALYSSFSGALYQLQRGSDDTT MFSSTL-VSLAVSWLAGQALAIELTV-SKTGGNSSSPLLYGVMFEDINNSGDGG *** ::*.: : ** . **:: **::: . ** |
|------------------|--|
| AvAbfB AnAbfA | TTISPLTAGGVA-DASAQDTFCANTTCLITIIYDQSGNGNHLTQAPPGGFDG IHGQVLRNNGFQGDDPGLTAYSAVGNVTISQDTANPLSSAITSTLKVAVPSGATGYVG . * *. * . ::.* . :** * * *. * *: * |
| AvAbfB AnAbfA | PDTDGYDNLASAIGAPVTLNGQKAYGVFMSPGTGYRNNEATGTATGDEAEGMYAVLDG FANEGYDGVPVLGTDYDNYFYMKGDYSGTVNLRLVGNSSGIIYADHNI .:*** : * * * :* * *::: :** : |
| AvAbfB AnAbfA | THYNDACCFDYGNAETSSTDTGAGHMEAIYLGNSTTWGYGAGDGPWIMVDME- TVASTSSNFTYYETSFSSSVSSDAHNVWQLRFDASKVAGSSLNFGLVQLFP * . :. * * ::. **: :* : ::::: |
| AvAbfB AnAbfA | NNLFSGADEGYNSGDPSISYSFVTAAVKGGAD PTYNSRYNGLRDDVASFLADIKPSFLRFPGGNNLEGATPSDRWKWNETIGPVVDRPGREG *. :.* : * .*. **: * |
| AvAbfB AnAbfA | KWAIRGGNAASGSLSTYYSGARPDYSGYNPMSKEGAIILGIGGDNSNGAQGTFYE DWTYPNTDALGLDEYLQWCEDMDMEPLLAVWSGLSLGGGIVSGSALDPYVD .*: :* .* :* |
| AvAbfB AnAbfA | GVMTSGYPSDDTENSVQENIVAAKYVVGSLVSGPSFTSGEVVSLRVTTPGYTTRYIAHTD DILNELEQYVLGSADTTYGSLRAKNGRTEPWDVKYLEVGN : *. :**:** : * *.: : * : : : * : : : : |
| AvAbfB AnAbfA | T-TVNTQVVDDDSSTTLKEEASWTVVTGLANSQCFSFESVDTPGSYIRHY EDNLNSGCGTYANRFTLIYDAVHAAYPNLTIVASTTDTSCLPSTIPDGVITDIHHYLTPD .:*: . ** . *::::::*: * : *:** |
| AvAbfB AnAbfA | NFELLINANDGTKQFHEDATFCPQAALNGEGTSLRSWSYPTRYF EFIDLFDEWDNWSRDWPILVGEYASTTGNDGSTTYWSYMQGSCSEAVYMIGMERNSD :* *:: .:: .* . *: .* * *** |
| AvAbfB AnAbfA | RHYDNVLYAASNGGVQTFDSKTSFNNDVSFEIETAFSS IVKMASFAPLLEHFDMAEWSPDLFGLDSSPDSVTGSTSYYVQKMFSTNRGSTVLPVNT .*:* . :: . *::: * **: :: . *:: |
| AvAbfB AnAbfA | TADFDPLYWVASVSDEGTYYVKLANYGSSSQNVTVNIEGTTSGQLQMLSGGETVSNYPHD |
| AvAbfB AnAbfA | VSITTQSSTVSGSGSFTVDMPAWAVAVLAVS |

22% amino acid sequence identity.

Chapter 5

Supplemental Figure 1B: Protein Sequence Alignment for A. vadensis endo-1,4- β -D-glucanase A (AvEglA) and A. niger endo-1,4- β -D-glucanase A (AnEglA).

B.

| AvEglA AnEglA | MKLAVTLSMLAATAMGQTMCSQYDSASSPPYSVNQNLWGEYQGTGSQCVYVDKLSSSGAS MKLPVSLAMLAATAMGQTMCSQYDSASSPPYSVNQNLWGEYQGTGSQCVYVDKLSSSGAS ***.*: |
|------------------|--|
| AvEglA AnEglA | WHTKWTWSGGEGTVKSYSNSGLTFDKKLVSDVSSIPTSVEWKQDNTNVNADVAYDLFTAA WHTEWTWSGGEGTVKSYSNSGVTFNKKLVSDVSSIPTSVEWKQDNTNVNADVAYDLFTAA ***:******************************** |
| AvEglA AnEglA | NVDHATSSGDYELMIWLARYGYIQPIGKQIATATVGGKSWEVWYGTSIQAGAEQRTYSFV NVDHATSSGDYELMIWLARYGNIQPIGKQIATATVGGKSWEVWYGSTTQAGAEQRTYSFV ************************************ |
| AvEglA AnEglA | SESPINSYSGDINAFFSYLTQNQGFPASSQYLINLQFGTEPFTGGPATFTVDNWTASVN SESPINSYSGDINAFFSYLTQNQGFPASSQYLINLQFGTEAFTGGPATFTVDNWTASVN ************************************ |

95% amino acid sequence identity.

Chapter 6

An evolutionary screen to increase inulinase production in *Aspergillus oryzae*

Culleton, H.M., Majoor, E., McKie, V.A. and de Vries, R.P. 2014. In preparation.

Abstract

Inulin is found widely distributed in nature as a storage polysaccharide and consists of a linear polymer of β -1,2-linked D-fructose molecules which can be hydrolyzed by *endo-/exo*-inulinases and fructofuranosidase (invertase) to give D-fructose and fructooligosaccharides. Fructose and fructooligosaccharides have gained considerable interest recently as important ingredients in the food and pharmaceutical industry due to their high sweetening capacity combined with their many functional and nutritional properties. Conventional methods of fructose production have proven to be both costly and inefficient with attention now being directed towards the use of microbial inulinases as a more promising approach for obtaining optimum D-fructose yields. In this study we aimed to improve the inulin degradation potential of *Aspergillus oryzae* through the upregulation of *exo*-inulinase production using an evolutionary screening method. As an organism with no predicted *endo*-inulinase function, improved inulin degradation would be largely dependent on the overproduction of this enzyme. Subsequent generation growth of *Aspergillus oryzae* (Rib40) on inulin for 9 weeks successfully resulted in *exo*-inulinase overproducing mutants.

Introduction

Inulin is widely distributed in nature as a storage carbohydrate in the roots and tubers of several plants such as dandelion, chicory, dahlia, Jerusalem artichoke and burdock [15]. It contains a linear polymer of β -1,2-linked D-fructose, connected to a terminal sucrose residue [11] and can be hydrolyzed by inulinases (endo- / exo-) [5] and fructofuranosidase (invertase) [17], resulting mainly in the formation of D-fructose and fructooligosaccharides. Fructose is the sweetest, naturally occurring sugar compound identified to date and consequently is emerging fast as an important ingredient in the food and pharmaceutical industry. Fructose is a more favourable alternative sweetener to sucrose due to its higher sweetening capacity, low viscosity and beneficial effects associated with diabetic patients and iron absorption in children [12]. Furthermore, fructooligosaccharides have many positive functional and nutritional properties i.e. reduced caloric value, stimulation of intestinal bifidobacteria and as a source of dietary fibre in food preparations [9, 14]. Conventional methods of fructose production involving the enzymatic degradation of starch followed by the conversion of Dglucose to D-fructose using glucose isomerase yields only ~42% D-fructose, with the remaining end-products consisting of glucose (~50%) and oligosaccharides (~8%) [14]. Other techniques, such as ion exchange chromatography, have been developed for fortification of fructose but all add to the production cost [18]. The use of microbial inulinases has therefore been proposed as a more favourable and promising approach for obtaining optimum D-fructose yields.

In a recent study, the plant polysaccharide degrading potential was compared for three different Aspergillus species i.e. A. nidulans, A. niger and A. oryzae [2]. Comparison of the CAZy (Carbohydrate-Active enzyme database (CAZyhttp://www.cazy.org) [8]) content of the genomes of these fungi demonstrated significant differences, with the number of genes related to particular polysaccharide degradation varying substantially between the three species. A. niger has the highest number of inulin-related genes in its genome with 5 open reading frames (ORF's), compared to A. nidulans and A. oryzae, which have 2 ORF's and 4 ORF's respectively. Growth profiles for these species on inulin, compared on the Fungal Growth Database website (www.fung-growth.org), showed a correlation with genome content, with growth of A. niger exceeding that of both A. nidulans and A. oryzae on this carbon source. While such poor growth could be expected for A. nidulans, the genome of which had less than half the number of ORF's of A. niger, the results were somewhat surprising for A. oryzae. Further analysis into the function predictions of these ORF's showed that of the three species, A. niger was the only one to contain an endo-inulinase within its genome, thus enhancing its ability for efficient inulin degradation. Based on this information, A. nidulans and A. oryzae would be required to over-produce *exo*-inulinase in order to utilise this substrate efficiently, thus resulting in an increased yield of D-fructose. This suggests that these Aspergilli, although closely related, have a unique biological approach towards the enzymatic degradation of the same substrate and presents the question if this approach could be optimised through prolonged cultivation on inulin rather than by genetic manipulation.

The use of this type of evolutionary screen has been very effective in obtaining (industrial) microorganisms with improved phenotypes, such as an expanded substrate range, increased stress tolerance and efficient substrate utilisation [10, 13]. In this study we have looked at an evolutionary approach to develop a strain with increased *exo*-inulinase activity. We have taken the filamentous fungi, *A. oryzae* and *A. nidulans* and generationally grown them on inulin for 9 weeks to obtain *exo*-inulinase overproducing mutants.

Materials and Methods

Strains, media and culture conditions

Aspergillus nidulans (FGSCA4) and Aspergillus oryzae (Rib40) were used in this study. Both strains were taken from glycerol stocks stored at -45° C and grown on MEA (Malt Extract Agar) prior to the evolution experiments. All plates were grown at 30° C.

Aspergillus minimal medium (MM) was described previously [4]. Agar was added at 2% (w/v) for solid medium. For the evolution experiments, MM solid agar was supplemented with 1% (w/v) inulin (dahlia tubers) (Sigma; Cat. No. I3754). For the analysis and characterization of enzyme activities, 400 mL liquid cultures (2.5 L baffled flasks) of MM + 1% (w/v) inulin (dahlia tubers) (Sigma; Cat. No. I3754) / inulin (chicory) (Sigma: Cat. No. I2255) were inoculated with 1 x 10⁶ spores/mL (final) and incubated at 30°C in an orbital shaker at 250 rpm for 4 days.

Preparation of evolution strains

From the initial regeneration on MEA agar, spores from *A. nidulans* and *A. oryzae* strains were harvested in 10 ml ACES (*N*-(2-Acetamido)-2-aminoethanesulfonic acid) buffer (0.2% (w/v) ACES + 0.02% (w/v) TWEEN[®] 80, pH 6.0) and 200 μ L was used to inoculate the evolution specific agar (MM + 1% (w/v) inulin) in triplicate for each strain. All plates were incubated at 30°C. After one week, growth was examined and 100-200 μ L of spores was used to inoculate new inulin plates. This process was repeated for a period of 9 weeks after which time the strains were purified on inulin by diluted plating and selecting the best growing colony. The purified strains were stored in 30% (w/v) glycerol at -80°C.

Enzyme profile comparison by SDS-PAGE

400 ml liquid cultures were inoculated with 1 x 10^6 spores/mL (final) and were grown in 2.5 L baffled flasks at 30°C and 250 rpm for 4 days. Following incubation, the mycelia were removed from the culture filtrate by filtration over a Büchner funnel with nylon gauze. SDS-PAGE was done using a 12% polyacrylamide gel containing 0.1% (w/v) SDS with 15 µL culture filtrate samples being loaded for each expression strain. Protein bands were detected by Coomassie Blue staining [7].

Enzyme activity measurements

For the measurement of enzyme activities from the *A. oryzae* generation strains, an initial 1:10 dilution of the culture filtrates was carried out in 100 mM sodium acetate buffer, pH 4.5 including BSA (1 mg/mL). Para-nitrophenol (*p*NP) assays were used for the measurement of α -/ β -galactosidase and α -/ β -glucosidase activities. Initial α -/ β -galactosidase assays were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 10 mM *p*-nitrophenyl α -D-galactopyranoside (pH 4.5) / β -D-galactopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. Similarly, for the initial measurement of α -/ β -glucosidase activities, 0.2 mL of diluted enzyme was added in duplicate to 0.2 mL of 10 mM *p*-nitrophenyl α -D-glucopyranoside (pH 4.5) / β -D-glucopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. Similarly, for the initial measurement of α -/ β -glucosidase activities, 0.2 mL of diluted enzyme was added in duplicate to 0.2 mL of 10 mM *p*-nitrophenyl α -D-glucopyranoside (pH 4.5) / β -D-glucopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. Similarly, for the initial measurement of α -/ β -glucosidase activities, 0.2 mL of diluted enzyme was added in duplicate to 0.2 mL of 10 mM *p*-nitrophenyl α -D-glucopyranoside (pH 4.5) / β -D-glucopyranoside (pH 4.5) respectively and incubated at 40 °C for 60 min. The reactions were stopped with the addition of 3 mL of 2 % (w/v) tri-sodium orthophosphate, pH 12.0 and the absorbance was measured at 400 nm. Activities were expressed as Units/ml where one unit is defined as 1 micromole of *p*-nitrophenol liberated per minute per millilitre of culture filtrate. Depending on results obtained, further time-points were conducted to ensure that activity rates were linear.

Nelson-Somogyi reducing sugar assays were used for the measurement of *exo*inulinase activities. Two different substrates were used in these experiments for comparison purposes; High MW (Molecular Weight) Inulin (Raftiline; HP Inulin) and Kestose (Megazyme; Cat. No. O-KTR). Initial *exo*-inulinase assays were performed in duplicate by the addition of 0.2 mL of diluted enzyme to 0.2 mL of 20 mg/mL (final) Inulin (pH 4.5) / 5 mg/mL (final) Kestose (pH 4.5) respectively and incubated at 40°C for 60 min. The reactions were stopped by the addition of 0.5 mL of Stopping Solution (25 mL of Solution A (2.5% (w/v) sodium carbonate anhydrous, 2.5% (w/v) potassium sodium tartrate and 20% (w/v) sodium sulphate), to which 1 mL of Solution B (3% (w/v) copper sulphate pentahydrate) was added). Enzyme reactions were then boiled for 20 min and allowed to cool to room temperature for 5 min before the addition of 3.0 mL of a 1:5 dilution of Solution C (5% (w/v) ammonium molybdate, 4.2% (v/v) concentrated sulphuric acid and 0.6% (w/v) sodium arsenate heptahydrate). Depending on results obtained, further time-points were conducted to ensure that activity rates were linear.

Results and Discussion

Development of evolution strains

Aspergillus nidulans (FGSCA4) and Aspergillus oryzae (Rib40) were grown on inulin for 9 weeks with weekly re-inoculation over which time the growth rate of *A. oryzae* increased (data not shown). This effect was not seen for *A. nidulans*, indicating that the basal levels of enzymes required to degrade this carbon source had not increased to the same degree as they had in *A. oryzae*.

To further demonstrate this hypothesis, the *exo*-inulinase activity levels for both strains at the beginning and end of the generation experiment were tested. *A. oryzae* week 1 (*Ao*1.1) and 9 (*Ao*9.1) and *A. nidulans* week 1 (*An*1.1) and 9 (*An*9.1) generation strains were chosen, with the same biological clone being used for both time points to give an accurate comparison. Strains were grown in media containing inulin from dahlia tubers and incubated at 30°C for 4 days. Initial *exo*-inulinase activity levels for both generations of each strain were compared, with *Ao*9.1 giving higher than double the activity levels (~125 mU/min/mL) of that obtained for *Ao*1.1 (~60 mU/min/mL). The activity levels measured for *An*1.1 and *An*9.1 remained unchanged and consistent with that obtained for *Ao*1.1, providing a strong correlation with the growth profiles.

| | AoRib40 (D) | AoRib40 (C) | Ao9.1 (D) | Ao9.1 (C) | Ao9.2 (D) | Ao9.2 (C) | Ao9.3 (D) | Ao9.3 (C) |
|-------------------------|----------------|----------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 66.0 kDa | | | | | | | | |
| 52.3 kDa | | | | | | | | |
| 41.0 kDa | | | | | | | | |
| 30.0 kDa | | | | | | | | |
| 2 <mark>6.</mark> 0 kDa | | | | | | | | |
| 21.9 kDa | | | | | | | | |

Figure 1: SDS-PAGE analysis of *A. oryzae* (Rib40) and week 9 evolved biological triplicate strains (*Ao*9.1, *Ao*9.2 and *Ao*9.3) when grown on MM + 1% inulin (dahlia tubers (D)/chicory (C)) at 30°C, 250 rpm for 4 days. Low molecular weight (LMW) ladder produced in house.

Extracellular enzyme profile and activity comparison

Based on the results obtained from the initial enzyme activity measurements, the extracellular activities in the *A. oryzae* mutant strains were examined in more detail. *A. oryzae* (Rib40) and the biological triplicates generated from week 9 (*Ao*9.1, *Ao*9.2 and *Ao*9.3) were chosen for this study, with *A. oryzae* (Rib40) acting as a control and indicative of the basal levels of enzyme activities expected when *A. oryzae* wild type (WT) is grown on inulin as a carbon source. Inulin from both dahlia tubers and chicory were examined to determine whether the precise structure of the inulin affects the enzyme mixtures produced by the mutants.

After 4 days of growth, the extracellular enzyme profiles generated for each strain were visualised by SDS-PAGE (Fig. 1). The A. oryzae mutant strains all demonstrated a clear increase in enzyme production levels when grown on inulin compared to the wild type (Rib40) with notable differences also being present between the three mutants. This again, was in correlation with the growth profiles of these strains with Ao9.3 generating more dense mycelia and a more concentrated enzyme profile in inulin rich liquid cultures than the other strains. Further evaluation of the enzyme profiles showed an intense band at approximately 52 kDa (Fig. 1), which likely corresponds to the extracellular exo-inulinase (InuE), for which the calculated molecular mass in A. oryzae Rib40 (AO090701000400) is ~55 kDa. The other visible protein bands are consequently expected to be multiple fructofuranosidases (invertases) i.e. AO090020000640, AO090701000038 and AO090103000043, the molecular weights which are ~64 kDa, 69 kDa and 121 kDa respectively [2]. Interestingly, higher enzyme levels were generated on inulin from chicory than from dahlia tubers, the latter being the source of inulin used for the generation studies. Previous studies using analytical HPLC to determine the chain lengths of both these commercial preparations of inulin showed that inulin from dahlia contains a higher molecular weight range which in turn has been known to affect solubility [6]. This indicates that while the basic structure of both carbon sources are identical and require the same enzyme actions for degradation, the inulin from chicory may be more easily utilised by the fungal cell.

In combination with the visual profiles of extracellular enzyme production during growth on inulin, the levels of *exo*-inulinase activity were measured in duplicate and compared for the different strains. The activities of α - and β -galactosidase and α - and β -glucosidase were also measured in duplicate as a control and indicative of the basal level of change in production of un-associated enzyme activities. In correlation to what was discovered with the enzyme profiles, the overall levels of all enzyme activities were higher for the *A. oryzae* mutant strains than for the wild type (Fig. 2). This was especially the case for *Ao*9.3, which gave a 4 and 9 fold increase in *exo*-inulinase activity on inulin and kestose, respectively. Kestose contains more terminal ends than



Figure 2: Measured activity values obtained from Day 4 culture filtrate samples from the indicated week 9 *A. oryzae* generation strains. *Ao*Rib40 was used as a control and indicative of the basal levels of activities generated when *A. oryzae* wild type (WT) is grown on inulin as a carbon source. Error bars calculated on standard deviations between technical duplicates. All polysaccharide substrates and *p*-nitrophenol substrates were tested at 40°C, pH 4.5 under conditions as described in Materials and Methods. White = Dahlia inulin, Black = chicory inulin.

inulin, thus confirming the *exo*-acting properties of the enzyme being over-produced. An average of a 12 fold increase in activities for the control enzymes was also noted indicating that the evolution screening not only increased the levels of the required enzyme activities i.e. *exo*-inulinase, but also the overall growth rate and secretion levels of these strains when grown on inulin. This could indicate that instead of a specific *exo*-inulinase increase in the mutants, enzyme secretion as whole has been improved in these strains.

Another hypothesis involves the up-regulation of genes associated with AmyR in response to the complete degradation of inulin to fructose and glucose. AmyR controls genes involved in starch degradation but it has been noted in *A. niger* that it also regulates genes encoding *beta*-glucosidases and *alpha*- and *beta*-galactosidases [16]. While starch and maltose are the most common inducers of this regulator, glucose also induces AmyR activation in *A. niger* and *A. oryzae* [1, 16]. If, like in *A. niger*, AmyR is also responsible for the regulation of these otherwise un-associated genes in *A. oryzae*, the complete degradation of inulin may then also cause an increase in the associated enzyme activities. In *A. niger* fructose is a relatively weak repressing carbon source [3], while glucose at higher concentrations can cause significant repression of many genes encoding poly- and oligosaccharide degrading enzymes. Degradation.

The mutants were maintained for several generations on MEA and then tested again on inulin without losing their improved properties, signifying that the changes were genetically stable and not dependent on a continuous selection. Future studies will analyse the nature of the mutations in more detail.

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Chapter 7

Summary and general discussion

Plant biomass is the most abundant and usable carbon source for most fungal species on the planet. Primarily consisting of plant cell walls, it contains two main groups of polysaccharides: plant cell wall polysaccharides (cellulose, hemicelluloses [xyloglucans, xylan, galacto(gluo)mannan] and pectin) and storage polysaccharides (e.g. starch and inulin). Plant cell wall polysaccharides interact with each other as well as the aromatic polymer, lignin, to form a network of linkages and hydrogen bonds that give the plant cell wall its rigidity. Fungi degrade these polysaccharides extracellularly by secreting enzymes which release oligo- and monosaccharides which can then be utilized by the fungal cell [15]. Fungi have optimized this process by producing diverse enzymatic mixtures, specific to the polysaccharides which they encounter. The fine-tuned production of such diverse enzyme sets is controlled through a set of transcriptional regulators, of which there is predicted to be between 400-600 in Aspergillus alone, that either activate (e.g. AmyR, XlnR, AraR, InuR, GalR/GalX, RhaR, ManR, ClbR) or repress (CreA) the expression of the genes encoding plant biomass degrading enzymes [25].

Aspergilli have become one of the most widely studied group of filamentous fungi due to their potential in plant biomass degradation and in many industrial applications [4, 5, 9, 13, 35]. As common soil fungi, found in many different environments, Aspergilli, are capable of producing an extensive set of enzyme mixes and degrade a very broad range of polysaccharides [10]. Much research over the past two decades has been applied to the development of Aspergillus, most notably A. niger and A. oryzae, as hosts for recombinant protein production. Their natural ability to grow at high rates and to high biomass densities and their exceptional capacity to secrete high levels of homologous product are well recognized [10, 14]. While significant progress has been made in the hyper-production of heterologous proteins, yields have not yet reached the levels obtained for homologous proteins [12]. This may in part be caused by the acidification of the media due to the production of organic acids in the case of A. niger, different native protein glycosylation patterns or by the presence of high levels of secreted proteases which effectively degrade heterologous proteins [41]. Regardless of the broad use of other fungal systems e.g. cellulase production in Penicillium sp. [1, 8] and Trichoderma reesei [18, 27] and development of new industrial fungal enzyme producers, such as Myceliophthora thermophila [40, 44], Aspergillus has remained of major interest for applied research. This is reflected by the number of species with a published genome sequence [3, 22, 30, 32-34, 39, 46]; http://genome.jgi-psf.org/programs/fungi/genome-releases.jsf,

http://www.ncbi.nlm.nih.gov/ bioproject] and the recent initiation of a project to sequence every species of the genus, as well as comparative studies of these genomes addressing plant biomass degradation [10], **Chapter 2**].

Plant biomass degradation in Aspergillus

Plant polysaccharide degradation by fungi has been a topic of study for many decades due to its relevance for many industrial applications, such as paper & pulp [9], food [35], feed [5], beverages, textiles [4] and detergents. More recently, the increasing interest in biomass-based fuels and chemicals has accelerated research into fungal decomposition of plant biomass. The availability of an increasing number of fungal genome sequences has encouraged genome mining of many species to identify new and/or better enzymes than those produced by established enzyme producers, such as *A. niger* and *T. reesei* [11]. By comparison, less attention has been given to the diverse strategies i.e. both enzyme-encoding genes and regulatory systems [16], employed by fungi themselves to efficiently degrade plant biomass.

In Chapter 2, eight Aspergillus species were compared with respect to their genomic ability to degrade plant cell wall biomass. While all tested Aspergilli had a similar potential to degrade plant biomass, results showed that even in closely related species, their strategies differed markedly. The variation in CAZy content of the genome of these species was relatively small, likely due to their close phylogenetic relationships and their similar habitats. Hierarchical clustering of the plant polysaccharide degrading enzymes of these species demonstrated that species with the most similar CAZy content in their genome were also taxonomically close. In contrast to this correlation, a high level of variation was detected in the enzymes produced by the tested species, especially during growth on sugar beet pulp. Sugar beet pulp contains mainly cellulose and pectin and therefore unsurprisingly, pectinases and cellulases were found to be the main enzymes produced on this substrate. The noted differences in pectinolytic gene content between the species could partially be explained by pH preference of the associated enzymes i.e. A. niger, which strongly acidifies the culture medium [34], has an evolutionary preference to pectin hydrolase production (acidic pH optima) over pectin lyases (neutral to alkali pH optima). This genomic pH preference to pectin degradation was also found to be prevalent in the thermophilic fungi Myceliophthora thermophila and Thielavia terrestris, where T. terrestris, which has 3.5 times more pectin hydrolases than lyases in its genome, was found to grow best on pectin at an acidic pH, while *M. thermophila*, which has 3.5 times more lyases than hydrolases in its genome, was found to favour a neutral to alkaline pH for growth on pectin [6]. However, as A. niger was the only species in this study to strongly acidify the medium, the variability associated with enzyme production for the remaining species was more likely be due to regulatory differences at the transcriptional level, suggesting that specific regulator function and/or range of target genes could be unique to different Aspergillus species. Recent research by Meijer et. al. (2011) suggests that this variability and uniqueness between different Aspergillus species is indeed accurate. In a study that compared growth and extracellular enzyme profiles of wild type *A. niger* isolates from different biotopes all over the world and type strains of other black Aspergilli, it was found that irrespective of the environment and carbon source that a particular strain was conditioned to, adaptation to the natural environment does not occur at the genetic level for *A. niger* and its ability to utilise various carbon sources remains consistent across the isolates of this species. In contrast, interspecies variation with respect to carbon source utilisation was significant, especially on D-galactose where *A. brasiliensis* was unique in its ability to grow on this substrate suggesting a distinct difference in sugar transport mechanisms [42] or metabolism.

The unique phenotype of A. vadensis compared to other black Aspergilli

A. vadensis, a recently identified member of the black Aspergilli and a close relative of *A. niger*, has been suggested as being beneficial for recombinant protein production as it does not acidify the culture medium and produces very low levels of extracellular proteases [17]. In recent studies, Punt *et. al.* (2008) identified the PrtT regulatory protein, a homologue of which was found to be present in many *Aspergillus* species and which was shown to govern expression of many secreted protease genes, including the major alkaline protease *alpA* and neutral protease *Np1* in *A. oryzae* and the aspergillopepsin encoding gene *pepA* in *A. niger*. In addition, a gene cluster related to starch degradation, including the *amyR* regulatory gene, is present directly upstream of the *prtT* locus, separated by only two genes (*agdA/aglU* and *amyA*), both of which are target genes of AmyR in *A. niger* [37].

In Chapter 3, the molecular and phenotypical differences between A. vadensis and six other species of black Aspergilli i.e. A. niger, A. acidus, A. aculeatus, A. carbonarius, A. brasiliensis and A. tubingensis were examined. Growth profiles showed significant and unique differences between A. vadensis and the other black Aspergilli with no growth being observed for A. vadensis on maltose or starch. The unusual growth profile for A. vadensis when grown on these carbon sources could be explained by a mutation/deletion within the *amyR* regulatory gene. Growth on casein however, was comparably poor for all the black Aspergilli, with the exception of A. *niger*, indicating that this more common protease deficiency may be accounted for at a transcriptional level. Genome analysis, however, demonstrated that the *prtT* and *amyR* regulatory loci and also the *agdA/aglU* and *amyA* genes were well conserved among all the black Aspergilli, including A. vadensis. Combined with gene expression data indicating very poor levels of expression for both prtT and amyR, along with the associated *amyA* gene, the aberrant phenotype of *A. vadensis* is likely caused by the low expression of prtT and amyR regulators or their associated genes and not a mutation or deletion of the structural part of these genes as was originally concluded,
thus reinforcing the unique abilities and adaptations of individual and often closely related *Aspergillus* species in the efficient degradation of plant biomass.

Optimisation of recombinant enzyme production in A. vadensis

Despite the common use of several *Aspergillus* species, most notably *A. niger* and *A. oryzae* for the industrial production of proteins [19], the yields for heterologous protein production still remain low. This is in part, due to different native protein glycosylation patterns and the presence of high levels of secreted proteases which effectively degrade many heterologous proteins [41]. Genetic strategies, including the development of highly inducible or strong constitutively active promoters, have been shown to lead to an increase in the levels of recombinant protein production.

In **Chapter 4**, six novel constitutive promoters from A. niger (pefla, ptktA, pefl β , ptall, pcetA and ppgkA) and a further five from A. vadensis (pefIa, prps31, pgpdA, pubil and poliC) were tested in A. vadensis using a gene encoding a secreted arabinofuranosidase from Fusarium oxysporum as a reporter for heterologous protein production. When comparing just the ABF expression levels obtained under the control of the different promoters, it was found that 9 out of the 11 promoters tested resulted in higher ABF activity levels than those observed under the control of the widely characterized gpdA promoter from A. nidulans [20, 21, 28, 29]. However, as multiple insertions of up to and greater than 10 copies are common in Aspergillus and are found to affect recombinant protein production, both positively [43] and negatively [24], it was necessary to determine the copy number of expression vectors transformed into the individual strains in order to gain a complete understanding of the actual strength of the tested promoter constructs. Interestingly, the order of promoter strength altered notably with 3 of the promoters from A. niger ($pefl\alpha > ptal > ppgkA$) and 3 from A. vadensis (pefla > poliC > prps31) then resulting in higher ABF activity than the gpdA promoter from A. nidulans.

The over-production of target genes by different promoters in *Aspergillus* is also receiving attention in other studies. Only recently, Li *et.al.* (2014) published research accounting the over-expression of β -glucuronidase (GUS) (*uidA*) when fused to the promoter region encoding the β -glucosidase II gene (*bglII*), with reported GUS specific activity of 189 U/mg. Interestingly however, when fused with a smaller segment of this promoter region, much higher GUS specific activity of 448 U/mg was reported [26]. This method of utilising sections of promoter regions is not a new one however with Minetoki *et. al.* (1998) describing a similar method whereby multiple copies of the conserved sequence region III in the promoter regions of the amylase-encoding genes (*amyB*, *glaA* and *agdA*) of *A. oryzae* were introduced into the *agdA* promoter, resulting in both a significant increase in promoter activity at the transcriptional level and in expression of the *agdA* target gene [31].

Chapter 7

In Chapter 5, the full length gDNA encoding two plant biomass degrading enzymes i.e. α -L-arabinofuranosidase (*abfB*) (GH54) and *endo*-1,4- β -D-glucanase (eglA) (GH12) from A. vadensis were successfully expressed using the gpdA promoter from A. vadensis. Both enzymes were produced extracellularly in A. vadensis as soluble proteins and successfully purified by affinity chromatography, giving a maximum yield of 30 mg/L for AbfB and 25 mg/L for EglA. It should be noted that the A. vadensis strain used in these experiments was near wild type and had not undergone the extensive strain improvement strategies that have resulted in the commercial A. niger and T. reesei strains that secrete much higher levels of enzyme, suggesting room for improvement. With an initial expression yield of ~ 30 mg/L, A. vadensis shows positive signs of becoming an industrially significant contender but considerable improvements will be required to rival the secretion abilities of commercial production strains such as A. niger, A. oryzae and T. reesei. Strategies for improving recombinant protein production in fungi, including the use of strong promoters and effective secretion signals or gene fusions to genes associated with well-expressed and secreted proteins have been implemented successfully to other systems in the past [36, 45]. Other methods, such as UV-mediated mutagenesis have been successfully applied to Aspergillus strains to generate ultra-high producing mutants [23]. Successes have also been obtained using a bioprocessing approach of optimizing fungal morphology, mycelia immobilization and culture conditions [2, 7].

To date, the use of His-tag affinity chromatography in fungal systems has not been practical due, in part to the difficulties experienced with degradation of the target protein and/or Histidine residues by extracellular proteases [41], especially as was found to be the case with *A. niger* (unpublished data). Purification of recombinant enzymes from *A. vadensis* through His-tag affinity chromatography have worked well thus far (**Chapter 5**; a further eight *A. vadensis* proteins were also produced and purified in this way (unpublished data)), suggesting its potential as a versatile host in the fundamental research of proteins as well as for industrial enzyme production.

AbfB from *A. vadensis* shared characteristics with the previously reported *A. niger* AbfB [38]. However, comparative analysis of EglA from *A. vadensis* to the orthologous EglA from *A. niger* demonstrated significant variations in biochemical characteristics, despite these organisms being taxonomically very close. In particular, the difference in temperature optima and stability for these orthologous enzymes is noteworthy and would directly impact the applications of these enzymes. Screening of orthologs from related fungi may therefore be worthwhile for many industrial applications and suggests that it is not always necessary to go to distantly related species or enzyme classifications to get significant changes in biochemical properties.

Optimisation of native enzyme production in Aspergillus

Inulin is found widely distributed in nature as a storage polysaccharide and consists of a linear polymer of β -1,2-linked D-fructose molecules which can be hydrolyzed by *endo-/exo*-inulinases and fructofuranosidase (invertase) to give D-fructose and fructooligosaccharides. Fructose and fructooligosaccharides have gained considerable interest recently as important ingredients in the food and pharmaceutical industry due to their high sweetening capacity combined with their many functional and nutritional properties. Conventional methods of fructose production have proven to be both costly and inefficient with attention now being directed towards the use of microbial inulinases as a more promising approach for obtaining optimum D-fructose yields.

In **Chapter 6** an evolutionary screening method was used to improve the inulin degradation potential of *Aspergillus oryzae* through the upregulation of *exo*-inulinase. As an organism with no predicted *endo*-inulinase function, improved inulin degradation would be largely dependent on the overproduction of this enzyme. Subsequent generation growth of *Aspergillus oryzae* (Rib40) on inulin for 9 weeks successfully resulted in *exo*-inulinase overproducing mutants. This study demonstrates the genomic flexibility of fungi and their potential for improved enzyme production. The approach used to improve *exo*-inulinase production can be applied for many other enzymes and is mainly dependent on the design of a good screen to push the strains in the right direction. The result that the evolved strains are genetically stable demonstrates that the improvements are genetic rather than pleiotropic. The approach introduced in this study is therefore an attractive alternative to GMO methods, in particular with respect to use in food applications.

Conclusion

The aim of this PhD project was to examine and optimise the plant polysaccharide degradation abilities of *Aspergillus*. It revealed the highly diverse enzymatic strategies for the degradation of plant biomass used by closely related fungi that appeared to have equal efficiency. This demonstrated that it is not always necessary to go to distantly related species or enzyme classifications to get significant changes in biochemical properties between the enzymes produced by these fungi. Therefore, the screening of orthologs from related fungi and/or the identification of enzyme sets employed by different fungi can be used to design better and combined commercial enzyme cocktails for many industrial applications, such as the bio-fuel industry.

While much research has been applied to the development of Aspergillus as hosts for recombinant protein production, selecting the most efficient strategy for the construction of a particular expression strain remains a complex task. Factors such as control mechanisms (transcription and regulation) and functional properties (posttranslational modifications and toxicity) of the target gene are required before expression strain aspects, such as constitutive vs. inducible promoters, effective secretion signals, carrier proteins and host strain physiology can be considered. In this thesis, the development of a strong constitutive promoter for expression of homologous (α -L-arabinofuranosidase and *endo*-1.4- β -D-glucanase from A. vadensis) and heterologous (α -arabinofuranosidase from *Fusarium oxysporum*) proteins on a simple carbon source such as sucrose is defined. The potential of protease deficient hosts, such as A. vadensis, is examined and emphasized with successful and efficient production and purification of recombinant enzymes facilitating many downstream processes in industrial enzyme production. Finally, the over-production of native fungal enzymes through generation screening methods offer an alternative to genetic approaches for the optimisation of the plant biomass degrading potential of fungi, such as Aspergillus.

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Nederlandse samenvatting

Planten biomassa is de meest abundante en bruikbare koolstof bron voor de meeste schimmels op aarde. Schimmels breken de polysacchariden in dit substraat af door enzymen uit te scheiden die mono- en oligosacchariden vrijmaken. Deze worden vervolgens opgenomen en gebruikt dor de schimmel cel. Aspergilli zijn schimmels die in veel verschillende biotopen voorkomen en zij produceren een uitgebreide set enzymen die een breed scala aan polysacchariden kunnen afbreken. In de afgelopen twee decennia is veel onderzoek verricht om *Aspergillus* te ontwikkelen als een gastheer voor de productie van recombinante enzymen. Meer recent is dit onderzoek, in het bijzonder gericht op de afbraak van planten biomassa, in een versnelling gekomen door de sterk toenemende behoefte aan brandstof en chemicaliën gemaakt uit planten biomassa.

In **hoofdstuk 2** zijn acht *Aspergillus* species vergeleken met betrekking tot hun genomisch potentieel voor afbraak van planten biomassa. Ondanks dat dit potentieel vergelijkbaar was in de species, de resultaten lieten zien dat zelfs nauw verwante species hiervoor sterk verschillende strategieën gebruiken. De variatie in de CAZy genen in de genomen was relatief klein en hiërarchische clustering hiervan volgde de taxonomische relaties van de species. In tegenstelling hiermee werd een hoge variatie geobserveerd in de enzymen die door de species geproduceerd werden. The verschillen met betrekking tot pectinolytische genen in de genomen konden deels verklaard worden door de pH voorkeur van de gerelateerde enzymen. Echter, de verschillend werden vooral toegewezen aan regulatoire verschillen, wat suggereert dat de functie van een regulator of de set van genen die deze controleert verschilt in de species.

In **hoofdstuk 3** werden de moleculaire en fysiologische eigenschappen van *Aspergillus vadensis* in vergelijking met andere zwarte Aspergilli bestudeerd. *A. vadensis*, een recentelijk geïdentificeerde species van de zwarte Aspergilli, is een interessante kandidaat als producent van heterologe eiwitten, aangezien deze species het medium niet verzuurd en zeer lage niveaus van extracellulaire proteases heeft. Met uitzondering van de groei op maltose en zetmeel werden geen significante groeiverschillen gevonden met alle andere species. Groei op het eiwit caseine was slecht voor alle species met uitzondering van *Aspergillus niger*. Genoom analyse toonde aan dat de *prtT* en *amyR* regulatoire genen, alsmede de *agdA/aglU* en *amyA* genen, geconserveerd waren in alle zwarte Aspergilli. Op basis van expressie data werd duidelijk dat het fenotype van *A. vadensis* op maltose, zetmeel en eiwit hoogstwaarschijnlijk veroorzaakt is door lage expressie van de regulator genen *amyR* en *prtT*. Dit onderstreept de unieke aanpassingen in nauw verwante *Aspergillus* species met betrekking tot efficiënte afbraak van planten biomassa.

In **hoofdstuk 4** zijn zes nieuwe constitutieve promotoren van *A. niger* (pefla, ptktA, pefl β , ptal1, pcetA and ppgkA) en vijf van *A. vadensis* (pefla, prps31, pgpdA, pubil and poliC) getest voor enzym productie in *A. vadensis* waarbij een gen coderend voor een gesecreteerd arabinofuranosidase van *Fusarium oxysporum* als reporter is gebruikt. Op basis van ABF activiteit bleek dat 9 van de 11 promotoren betere productie gaven in vergelijking met de veel gebruikte gpdA promoter van Aspergillus. *nidulans*. Analyse van het copy-nummer van de transformanten had een significant effect op de bepaalde sterkte van de promotoren, waarbij slechts drie promotoren van *A. niger* (pefla > ptal > ppgkA) en drie van *A. vadensis* (pefla > poliC > prps31) tot hogere ABF activiteit leiden dan gpdA van *A. nidulans*.

In **hoofdstuk 5** is de succesvolle expressie van genen coderend voor α -Larabinofuranosidase (*abfB*, GH54) en *endo*-1,4- β -D-glucanase (*eglA*, GH12) beschreven. Beide enzymen werden extracellulair als oplosbare eiwitten geproduceerd en succesvol gezuiverd via affiniteit chromatografie, met een opbrengst van 30 mg/L voor AbfB en 25 mg/L voor EglA. AbfB was functioneel vergelijkbaar met de eerder beschreven AbfB van *A. niger*. Echter, vergelijking van *A. vadensis* en *A. niger* EglA toonde significante verschillen aan in hun biochemische eigenschappen, ondanks de nauwe verwantschap van deze twee species. Verschillen werden vooral gevonden in temperatuur optima en stabiliteit wat direct relevant is voor de toepassing van deze enzymen. Dit suggereert dat de vergelijking van orthologen uit verwante schimmels tot relevante data kan leiden voor industriële applicaties en dat het niet altijd nodig is om naar onverwante species te gaan voor enzymen met afwijkende eigenschappen.

In **hoofdstuk 6** is een evolutionaire screening methode gebruikt om het inuline afbraak potentieel van *A. oryzae* door de verhoging van *exo*-inulinase activiteit. Aangezien dit organisme geen *endo*-inulinases lijkt te produceren, zou verbeterde afbraak van inuline vooral afhankelijk zijn van overproductie van *exo*-inulinase. Groei van *Aspergillus oryzae* (RIB40) via herhaaldelijke aanenten op inuline gedurende 9 weken resulteerde in *exo*-inulinase overproducerende mutanten. Deze studie toont de genomische flexibiliteit van schimmels aan en het potentieel hiervan voor verbeterde enzym productie. Aangezien de geëvolueerde stammen stabiel zijn is het waarschijnlijk dat de oorzaak van de verhoogde productie genetisch is en niet pleiotropisch. Dit laat zien dat de aanpak van deze studie een aantrekkelijk alternatief is voor GMO methoden, vooral met betrekking tot gebruik in levensmiddelen toepassingen.

Curriculum Vitae

Helena Marie Culleton was born on April the 3rd, 1986 in Wexford, Ireland. She followed her second education in St. Mary's College, Arklow and graduated in 2004 with an honours Leaving Certificate. In September of the same year she began her study in Genetics and Cell Biology at Dublin City University where she did her first internship in molecular biology at Megazyme International Ireland in Bray, Co. Wicklow under the supervision of Dr. R.M. Lloyd and Dr. S.J. Charnock. As part of her final year, she did her second internship in the National Institute for Cellular Biotechnology (NICB), Dublin under the supervision of Prof. C.E. Loscher. Helena obtained her honours BSc degree in 2008. In June of the same year she began employment as a Molecular Biologist/Biochemist with Megazyme International Ireland and began her PhD in Fungal Physiology in conjunction with Utrecht University, The Netherlands and CBS-KNAW Fungal Biodiversity Centre, The Netherlands under the supervision of Prof. Dr. ir. R.P. de Vries and Dr. V.A. McKie.

List of Publications

Culleton, H.M., McKie, V.A. and de Vries, R.P. 2013. Physiological and molecular aspects of degradation of plant polysaccharides by fungi: What have we learned from Aspergillus? Biotechnology Journal. **8**: 884-894.

Culleton, H.M., Bouzid. O., McKie, V.A. and de Vries, R.P. 2014. New promoters to improve heterologous protein production in *Aspergillus vadensis*. Curr. Biotechnol. **3**: 1-8.

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Abstracts and Presentations

Bouzid, O., **Culleton, H.M.,** McKie, V.A., Mc Cleary, B.V. and de Vries, R.P. – The 9th International *Aspergillus* meeting (ASPERFEST9). March 29-30, 2012, Marburg, Germany. (Poster presentation).

Benoit, I., **Culleton, H.M.,** Wiebenga, A., Coutinho, P.M., Brouwer, C.P.J.M., McKie, V.A., McCleary, B.V., Henrissat, B. and de Vries, R.P. – The 11th European Conference on Fungal Genetics (ECFG11). March 30-April 2, 2012, Marburg, Germany. (Poster presentation).

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