Supplementary document

**A multifunctional GH39 glycoside hydrolase from the anaerobic gut fungus *Orpinomyces* sp. strain C1A**

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**Table S1. Properties of enzymes with β-xylosidase activity.** Table is organized by origin, fungal, then bacterial, then plant. All values listed are from enzymatic activities against *p*-nitrophenyl-β-D-xylopyranoside, PNPX. For the “Known GH39-Family Enzyme?” column, a “+” indicates that the enzyme listed is known to be a member of the GH39-family, whereas a “-“ indicates that it either is not a GH39-family enzyme, or its classification is unknown. “-“, not reported in the study. Anaerobic fungi are highlighted.

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| **Origin** | **Protein Name (Annotation)** | **Known GH39-family Enzyme?** | **Specific Activity**  **(U/mg)** | **Kinetics**  **Km**  **(mM)** | **Kinetics**  **Vmax**  **(U/mg)** | **Optimal pH** | **pH Stability** | **Optimal Temp. (°C)** | **Temp. Stability (°C)** | **Other Activities** | **Ref.** |
| *Orpinomyces* sp. strain C1A | Bgxg1 | **+** | 11.5 | 0.00485 | 127 | 6 | 4-12 | 39 | 4-70 | β-glucosidase  β-galactosidase  Xylanase | This  Study |
| *Acremonium persicinum* | β-glucosidase | - | 0.002 | - | - | 5.5 | - | - | - | β-glucosidase | (Pitson et al. 1997) |
| *Aspergillus awamori* | β-xylosidase | - | 34.1 | 0.003 | 476 | 6.5 | - | 70 | to 70 | Xylanase | (Kormelink et al. 1993) |
| *Aspergillus carnonarious* | β-xylosidase | - | 3.29 | 0.198 | 3.64 | 4 | 3.5-6.5 | 60 | to 50 | Arabinosidase | (Kiss & Kiss 2000) |
| *Aspergillus fumigatus* | β-xylosidase | - | 27.5 | 2 | - | 4.5 | 2-8 | 75 | to 65 | β-glucosidase | (Kitpreechavanich et al. 1986) |
|  | β-Glucosidase | - | 1.03 | - | - | 4.5 | 2-8 | 65 | to 60 | β-glucosidase | (Kitpreechavanich et al. 1986) |
| *Aspergillus niger* | β-xylosidase | - | 5.2 | 0.22 | - | 6.7-7 | - | 42 | to 46 | β-glucosidase | (John et al. 1979) |
|  | β-xylosidase 1 | - | 60.2 | - | - | - | - | - | - | β-glucosidase  Arabinosidase | (vanPeij et al. 1997) |
|  | β-xylosidase 2 | - | 60.9 | - | - | - | - | - | - | β-glucosidase  Arabinosidase | (vanPeij et al. 1997) |
| *Aspergillus nidulans* | β-xylosidase | - | 1.2 | 1.1 | 25.6 | 5 | 4-6 | 50 | to 45 | None | (Kumar & Ramon 1996) |
| *Aspergillus phoenicis* | β-xylosidase | - | 821 | 2.36 | - | 4-4.5 | 4-6 | 75 | to 60 | None | (Rizzatti et al. 2001) |
| *Aspergillus pulverulentus* | β-xylI | - | 32 | - | - | 2.5-3.5 | 1.5-6.5 | 60 | to 50 | None | (Sulistyo et al. 1995) |
|  | β-xylII | - | 12 | - | - | 4-5 | 1.5-6.5 | 60 | to 50 | None | (Sulistyo et al. 1995) |
| *Aureobasidium* sp. | β-xylosidase | - | 626 | 2 | 940 | 3.5 | 3.5-9 | 80 | to 80 | β-glucosidase | (Hayashi et al. 2001) |
| *Aureobasidium pullulans* | β-xylosidase | - | 7.3 | - | - | 4.5 | 2-9.5 | 80 | to 70 | None | (Dobberstein & Emeis 1991) |
| *Chaetomonium cellulolyticum* | Xylanase 3 | - | 0.03 | - | - | 6-7 | - | 50 | - | Xylanase  Arabinosidase  Endoglucanase | (Baraznenok et al. 1999) |
| *Fusarium oxysporum* | Xylanase | - | 0.5 | - | - | 7.4 | 5.8-8.2 | 50 | to 50 | Xylanase  Endoglucanase  Exoglucanase | (Christakopoulos et al. 1996) |
| *Fusarium proliferatum* | β-xylosidase | - | 53 | 0.77 | - | 4.5 | - | 60 | - | None | (Saha 2003) |
| *Fusarium vercillioides* | β-xylosidase | - | 57 | 0.85 | - | 4.5 | - | 65 | - | None | (Saha 2001) |
| *Humicola grisea* | β-glucosidase 1 | - | 4.38 | - | - | 4.5 | 4-12 | 60-65 | 30-60 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-glucosidase 2 | - | 0.07 | - | - | 6-8 | 4-12 | 60-65 | 30-50 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-glucosidase 3 | - | 0.08 | - | - | 6-8 | 4-12 | 60-65 | 30-50 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-glucosidase 4 | - | 0.21 | - | - | 6-7 | 4-12 | 50-55 | 30-50 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-glucosidase 5 | - | 0.36 | - | - | 6 | 4-12 | 60 | 30-55 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-glucosidase 6 | - | 0.17 | - | - | 6-7 | 4-12 | 65-70 | 30-65 | β-glucosidase  β-galactosidase  Cellobiohydrolase | (Takashima et al. 1996) |
|  | β-xylosidase | - | 19.6 | 1.37 | 13 | 6.5 | 4-9 | 55 | to 50 | None | (Iembo et al. 2006) |
| *Neocallimastix frontalis* | β-xylosidase | - | 4.3 | 0.33 | - | 6.5 | - | 35 | - | Xylanase | (Hebraud & Fevre 1990) |
|  | β-xylosidase | - | 0.9 | 2.98 | 0.27 | 6.4 | - | 37 | to 40 | β-glucosidase  Arabinosidase | (Garcia-Campayo & Wood 1993) |
|  | β-xylosidase | - | 16 | 0.34 | - | 6.5 | 5-8 | 35 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Neocallimastix patriciarum* | β-xylosidase I | - | 30.4 | 0.59 | 38 | 6 | 5-8 | 50 | 45-60 | Arabinosidase | (Zhu et al. 1994) |
|  | β-xylosidase II | - | 8.7 | 0.13 | 8.9 | 6 | 5-7 | 40 | 25-45 | None | (Zhu et al. 1994) |
| *Neurospora crassa* | β-xylosidase | - | 0.26 | 0.047 | - | 4.5-5 | - | 55 | - | Cellobiohydrolase | (Deshpande et al. 1986) |
| *Penicillium wortmanni* | Xylosidase 1 | - | 22 | 4.2 | 42.6 | 3-4.5 | - | 55-65 | - | None | (Matsuo et al. 1987) |
|  | Xylosidase 2 | - | 40 | 2.3 | 73.2 | 3-4.5 | - | 55-65 | - | None | (Matsuo et al. 1987) |
|  | Xylosidase 3 | - | 61.5 | 1.0 | 88.2 | 3-4.5 | - | 55-65 | - | None | (Matsuo et al. 1987) |
|  | Xylosidase 4 | - | 30.5 | 3.3 | 106.6 | 3-4.5 | - | 55-65 | - | None | (Matsuo et al. 1987) |
| *Pichia pastoris* X-33 | EXG1 | - | 11.6 | - | - | - | - | - | - | β-glucosidase  β-galactosidase | (Xu et al. 2006) |
| *Piromyces communis* | β-xylosidase | - | 28 | 0.4 | - | 6.0 | 5-8 | 39 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Sphaeromonas communis* | β-xylosidase | - | 27 | 0.36 | - | 6.5 | 5-8 | 39 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Talaomyces emersonii* | Xyl I | - | 3.0 | 0.13 | - | 2.5 | - | 60 | - | None | (Tuohy et al. 1993) |
|  | Xyl II | - | 92.9 | 32.9 | - | 4.2 | - | 78 | - | None | (Tuohy et al. 1993) |
|  | Xyl III | - | 0.2 | 1.4 | - | 3.5 | - | 67 | - | None | (Tuohy et al. 1993) |
| *Trichoderma harzianum* | β-xylosidase | - | 3.42 | 0.103 | - | 4-4.5 | - | 60 | - | β-glucosidase  β-galactosidase  Arabinosidase | (Ximenes et al. 1996) |
| *Trichoderma reesei* | CBHII | - | 0.00001 | - | - | - | - | - | - | β-glucosidase  Mannanase  Xylanase  Cellobiohydrolase  Exoglucanase | (Bailey et al. 1993) |
|  | EGI | - | 0.00001 | - | - | - | - | - | - | β-glucosidase  Mannanase  Xylanase  Cellobiohydrolase  Exoglucanase | (Bailey et al. 1993) |
|  | XYL 9 | - | 0.00002 | - | - | - | - | - | - | β-glucosidase  Mannanase  Xylanase | (Bailey et al. 1993) |
|  | XYL 5.5 | - | 0.00005 | - | - | - | - | - | - | β-glucosidase  Mannanase  Xylanase | (Bailey et al. 1993) |
|  | β-xylosidase | - | 470 | 0.08 | - | 4 | 3-7 | 60 | to 55 | Arabinosidase | (Poutanen & Puls 1988) |
| *Trichoderma viride* | β-xylosidase | - | 10.8 | - | - | 3.5 | 4-5 | 55 | to 65 | None | (Matsuo & Yasui 1984) |
| *Bacillus halodurans* C-125 | BH1068 | + | - | 30.1 | - | 6.5 | 5-7 | 47 | to 45 | None | (Wagschal et al. 2008) |
| *Bacillus stearothermophilus* | β-xylosidase | - | 34.2 | 0.0012 | - | 6 | 6-8 | 70 | to 60 | None | (Nanmori et al. 1990) |
| *Bacillus thermantarcticus* | β-xylosidase | - | 160 | 0.5 | - | 6.0 | - | 70 | to 60 | None | (Lama et al. 2004) |
| *Butyrivibrio fibrisolvens* | xylB | - | 8.9 | - | - | - | - | - | - | Arabinosidase | (Utt et al. 1991) |
| *Caulobacter crescentus* | CcXynB2 | **+** | 215 | 9.3 | 402 | 6 | 4.5-7.5 | 55 | to 50 | None | (Correa et al. 2012) |
| *Clostridium stercorarium* | β-glucosidase | - | 5.7 | - | - | 5.5 | 5-7 | 65 | to 75 | β-glucosidase  Cellobiohydrolase  Exoglucanase | (Bronnenmeier & Staudenbauer 1988) |
|  | xylA | - | 3.5 | 2.5 | 5.9 | 7 | 5-10 | 65 | - | Arabinosidase | (Sakka et al. 1993) |
| *Geobacillus stearothermophilus* | β-xylosidase | **+** | 133 | 2.38 | 147 | 6.5 | 5.5-7 | 70 | 50-70 | None | (Bhalla et al. 2014; Czjzek et al. 2005) |
| *Ruminococcus flavefaciens* | Exo A | - | 0.01 | - | - | 5 | 4.5-5.5 | - | - | Cellobiohydrolase  Exoglucanase | (Gardner et al. 1987) |
| *Thermoanaerobacterium*  *saccharolyticum* | β-xylosidase | **+** | 53.8 | 28 | 276 | 6 | 6-6.5 | 65 | to 67 | None | (Shao et al. 2011) |
| *Thermoanaerobacter* sp. | β-xylosidase | - | 66 | 0.038 | 183 | 5.9 | 5-8.5 | 93 | to 86 | Arabinosidase | (Shao & Wiegel 1992) |
| *Hordeum vulgare* | β-xylosidase | - | 6.06 | - | - | - | - | - | - | Arabinosidase | (Lee et al. 2003) |

**Table S2. Properties of enzymes with β-glucosidase activity.** Table is organized by origin, fungal, then bacterial, then plant. All values listed are from enzymatic activities against *p*-nitrophenyl-β-D-glucopyranoside, PNPG. “-“, not reported in the study. Rows of anaerobic fungi are highlighted.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Origin** | **Protein Name (Annotation)** | **Specific Activity**  **(U/mg)** | **Kinetics**  **Km**  **(mM)** | **Kinetics**  **Vmax**  **(U/mg)** | **Optimal pH** | **pH Stability** | **Optimal Temp. (°C)** | **Temp. Stability (°C)** | **Other Activities** | **Ref.** |
| *Orpinomyces* sp. strain C1A | Bgxg1 | 73.4 | 0.000013 | 769 | 6 | 4-12 | 39 | 4-70 | β-xylosidase  β-galactosidase  Xylanase | This  Study |
| *Acremonium persicinum* | β-glucosidase | 0.0183 | 0.3 | - | 5.5 | - | - | - | Cellobiohydrolase | (Pitson et al. 1997) |
| *Aspergillus fumigatus* | β-xylosidase | 0.76 | - | - | 4.5 | 2-8 | 75 | to 65 | β-xylosidase | (Kitpreechavanich et al. 1986) |
|  | β-Glucosidase | 1.03 | - | - | 4.5 | 2-8 | 65 | to 60 | β-xylosidase | (Kitpreechavanich et al. 1986) |
| *Aspergillus nidulans* | B-Gluco-I | 4.52 | 0.24 | 8 | 5 | - | 65 | - | None | (Bagga et al. 1990) |
|  | B-Gluco-II | 0.55 | 0.12 | 0.7 | 6 | - | 35 | - | None | (Bagga et al. 1990) |
| *Aspergillus niger* | β-xylosidase | 0.15 | - | - | 6.7-7 | - | 42 | to 46 | β-xylosidase | (John et al. 1979) |
|  | β-xylosidase 1 | 0.2 | - | - | - | - | - | - | β-xylosidase  Arabinosidase | (vanPeij et al. 1997) |
|  | β-xylosidase 2 | 0.3 | - | - | - | - | - | - | β-xylosidase  Arabinosidase | (vanPeij et al. 1997) |
| *Aureobasidium* sp. | β-xylosidase | 15.6 | - | - | 3.5 | 3.5-9 | 80 | to 80 | β-xylosidase | (Hayashi et al. 2001) |
| *Candida peltata* | β-glucosidase | 108 | 2.3 | 221 | 5 | 3.5-6 | 50 | 25-45 | Cellobiohydrolase | (Saha & Bothast 1996) |
| *Chrysosporium lucknowense* | EG60 | 0.12 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Endoglucanase | (Bukhtojarov et al. 2004) |
|  | CBH1 | 0.02 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Cellobiohydrolase | (Bukhtojarov et al. 2004) |
| *Fusarium oxysporum* | Xylanase | 0.9 | - | - | 7.4 | 5.8-8.2 | 50 | to 50 | Xylanase  Endoglucanase  Exoglucanase | (Christakopoulos et al. 1996) |
| *Fusarium solani* | Cellulase | 4.65 | - | - | 5 | 5.7 | 65 | to 65 | Cellobiohydrolase  Endoglucanase  Exoglucanase | (Wood 1971) |
| *Humicola grisea* | Exoglucanase 1 | 1.14 | - | - | 6-8 | 4-14 | 55-70 | 30-75 | Cellobiohydrolase  Endoglucanase  Exoglucanase | (Takashima et al. 1996) |
|  | β-glucosidase 1 | 85.7 | 0.16 | 64 | 4.5 | 4-12 | 60-65 | 30-60 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 2 | 12.5 | 0.25 | 21 | 6-8 | 4-12 | 60-65 | 30-50 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 3 | 12.6 | 0.22 | 23 | 6-8 | 4-12 | 60-65 | 30-50 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 4 | 8.34 | 0.34 | 8.7 | 6-7 | 4-12 | 50-55 | 30-50 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 5 | 17.9 | 0.56 | 18 | 6 | 4-12 | 60 | 30-55 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 6 | 33.4 | 0.12 | 40 | 6-7 | 4-12 | 65-70 | 30-65 | Cellobiohydrolase  β-galactosidase  β-xylosidase | (Takashima et al. 1996) |
|  | BGL I | 26.1 | 0.34 | 25 | 6 | 6-11 | 55 | to 50 | Cellobiohydrolase  β-galactosidase | (Takashima et al. 1999) |
| *Neocallimastix frontalis* | β-xylosidase | 0.2 | 2.98 | 0.27 | 6.4 | - | 37 | to 40 | β-xylosidase  Arabinosidase | (Garcia-Campayo & Wood 1993) |
|  | β-glucosidase | 9.0 | 0.031 | - | - | - | - | - | β-galactosidase  Cellobiohydrolas | (Wilson et al. 1994) |
|  | β-glucosidase | 15 | 0.55 | - | 6 | 5-8 | 50 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Orpinomyces* sp. strain PC-2 | β-glucosidase | 33.5 | 0.39 | 47.5 | 6.2 | 5-8 | 50 | to 65 | Cellobiohydrolase | (Chen et al. 1994) |
| *Paecilomyces thermophila* | β-glucosidase | 97.2 | 0.27 | 780 | 6.2 | 5-8.5 | 75 | 65-70 | Exoglucanase | (Yang et al. 2008) |
| *Penicillium funiculosum* | Cellulase | - | 0.77 | - | 5 | - | 50 | to 60 | Cellobiohydrolase  Exoglucanase | (Wood & Mccrae 1982a) |
| *Phanerochaete chrysosporium* | β-glucosidase | - | 0.096 | - | 4-5.2 | 4-8 | 25 | to 45 | None | (Lymar et al. 1995) |
| *Pichia pastoris* X-33 | EXG1 | 37.5 | - | - | - | - | - | - | β-xylosidase  Exoglucanase | (Xu et al. 2006) |
| *Piromyces communis* | β-glucosidase | 20 | 0.52 | - | 6 | 5-8 | 50 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Piromyces* sp. E2 | β-glucosidase | 0.25 | 1.0 | 66.5 | 5.7-6.3 | - | 47 | to 37 | Cellobiohydrolase | (Harhangi et al. 2002) |
| *Sphaeromonas communis* | β-glucosidase | 46 | 0.51 | - | 6 | 5-8 | 50 | to 40 | None | (Hebraud & Fevre 1988a) |
| *Sporotrichum thermophila* | β-glucosidase I | 89 | 0.29 | - | 5.4 | 4-6.5 | 65 | to 65 | None | (Bhat et al. 1993) |
|  | β-glucosidase A | 0.595 | 0.5 | - | 5.6 | - | 50 | to 40 | None | (Meyer & Canevascini 1981) |
|  | β-glucosidase B | 1.295 | 0.18 | - | 6.3 | - | 50 | to 40 | None | (Meyer & Canevascini 1981) |
| *Stachybotrys* sp. | β-glucosidase p21 | 0.33 | 0.27 | 78 | 5 | 5-7 | 50 | to 60 | None | (Amouri & Gargouri 2006) |
| *Thermoascus aurantiacus* | β-glucosidase | 232 | 0.52 | 6500 | 5 | 6-8 | 70 | to 75 | None | (Tong et al. 1980) |
|  | β-glucosidase | 0.3 | 0.11 | - | 4.5 | 4.4-5.2 | 80 | 70-90 | Cellobiohydrolase | (Parry et al. 2001) |
| *Trichoderma harzianum* | BGL | 0.35 | - | - | - | - | 28 | - | None | (Ahmed et al. 2009) |
|  | β-xylosidase | 0.28 | - | - | 4-4.5 | - | 60 | - | β-xylosidase  β-galactosidase  Arabinosidase | (Ximenes et al. 1996) |
| *Trichoderma koningii* | β-glucosidase | 16 | 0.37 | - | 4-5 | 4-6 | 45 | to 65 | Cellobiohydrolase | (Wood & Mccrae 1982b) |
|  | β-glucosidase II | 14 | 0.85 | - | 4-5 | 4-6 | 45 | to 65 | Cellobiohydrolase | (Wood & Mccrae 1982b) |
| *Trichoderma reesei* | BGL2 | 23.9 | 2.22 | 40 | 6 | 6-11 | 45 | to 45 | Cellobiohydrolase  β-galactosidase | (Takashima et al. 1999) |
|  | BGLI | 767.7 | - | - | 4 | 5-9 | 70 | to 55 | Cellobiohydrolase | (Takashima et al. 1998) |
|  | CBH I | 5.5 | - | - | - | - | - | - | Cellobiohydrolase  Endoglucanase | (Woodward et al. 1994) |
|  | CBH II | 33 | - | - | - | - | - | - | Cellobiohydrolase  Endoglucanase | (Woodward et al. 1994) |
|  | CBH III | 0.00001 | - | - | - | - | - | - | Cellobiohydrolase  Xylanase  Mannanase  β-xylosidase  Exoglucanase | (Bailey et al. 1993) |
|  | EG I | 0.00005 | - | - | - | - | - | - | Cellobiohydrolase  Xylanase  Mannanase  β-xylosidase  Exoglucanase | (Bailey et al. 1993) |
|  | XYL 9 | 0.00001 | - | - | - | - | - | - | Xylanase  Mannanase  β-xylosidase | (Bailey et al. 1993) |
|  | XYL 5.5 | 0.000001 | - | - | - | - | - | - | Xylanase  Mannanase  β-xylosidase | (Bailey et al. 1993) |
| *Trichoderma viride* T100-14 | β-glucosidase | 5.6 | - | - | - | - | - | - | Cellobiohydrolase | (Zhou et al. 2008) |
| *Bacillus* sp. KSM-S237 | Cellulase | 5.4 | - | - | 8.6-9 | 5-11 | 45 | to 60 | Endoglucanase  Cellobiohydrolase  Exoglucanase  Xylanase | (Hakamada et al. 1997) |
| *Bacillus pumilus* EB3 | Cellulase | 0.038 | - | - | 6 | 5-9 | 60 | 30-70 | Exoglucanase  Endoglucanase | (Ariffin et al. 2006) |
| *Bacillus sphaericus* JS1 | Cellulase | 3.07 | - | - | 8 | 7-10.5 | 60 | to 70 | Endoglucanase | (Singh et al. 2004) |
| *Cellulomonas fimi* | CenA | 1.6 | - | - | - | - | - | - | Exoglucanase  Endoglucanase  Cellobiohydrolase | (Tomme et al. 1996) |
|  | Exg | 0.9 | - | - | 6 | 5-9 | - | 60-75 | Xylanase  Endoglucanase  Exoglucanase  Cellobiohydrolase | (Curry et al. 1988) |
| *Clostridium stercorarium* | β-glucosidase | 31.6 | 0.8 | - | 5.5 | 5-7 | 65 | to 75 | Cellobiohydrolase | (Bronnenmeier & Staudenbauer 1988) |
| *Clostridium thermocellum* | β-glucosidase | 127 | - | - | 6-6.5 | 5.5-7.5 | 60 | to 68.5 | Cellobiohydrolase | (Ait et al. 1982) |
| *Thermotoga neapolitana* | CelA | 0.01 | - | - | 6 | - | 95 | to 95 | Endoglucanase  Cellobiohydrolase | (Bok et al. 1998) |
|  | CelB | 0.18 | - | - | 6-6.6 | - | 106 | to 106 | Endoglucanase  Cellobiohydrolase | (Bok et al. 1998) |
| *Panesthia cribrata* | GD1 | 9 | 10.6 | - | - | - | - | - | Endoglucanase | (Scrivener & Slaytor 1994) |
|  | GD2 | 3 | 13.8 | - | - | - | - | - | Endoglucanase | (Scrivener & Slaytor 1994) |

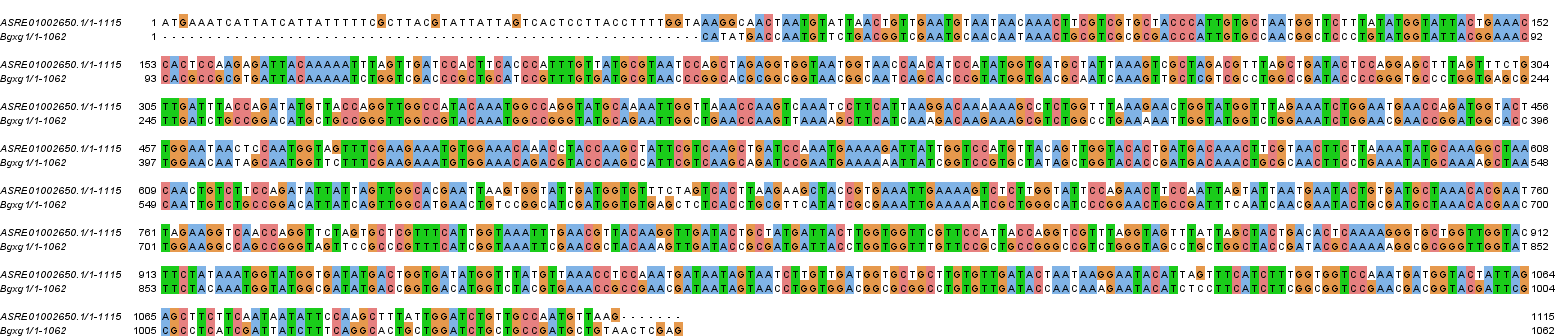
**Table S3. Properties of enzymes with β-galactosidase activity.** Table is organized by origin, fungal, then bacterial, then plant. All values listed are from enzymatic activities against *p*-nitrophenyl-β-D-galactopyranoside, PNPGal. “-“, not reported in the study. Rows of anaerobic fungi are highlighted.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Origin** | **Protein Name (Annotation)** | **Specific Activity**  **(U/mg)** | **Kinetics**  **Km**  **(mM)** | **Kinetics**  **Vmax**  **(U/mg)** | **Optimal pH** | **pH Stability** | **Optimal Temp. (°C)** | **Temp. Stability (°C)** | **Other Activities** | **Ref.** |
| *Orpinomyces* sp. strain C1A | Bgxg1 | 54.6 | 0.000214 | 769 | 6 | 4-12 | 39 | 4-70 | β-glucosidase  β-xylosidase  Xylanase | This  Study |
| *Aspergillus aculeatus* | β-galactosidase | 24 | 3.28 | 284 | 5.4 | - | 55-60 | - | None | (van Casteren et al. 2000) |
| *Aspergillus niger* | β-galactosidase | 33.3 | 1.3 | 51.2 | 4 | 3.5-4.5 | 60-65 | to 50 | None | (Manzanares et al. 1998) |
| *Humicola grisea* | β-glucosidase 1 | 0.15 | - | - | 4.5 | 4-12 | 60-65 | 30-60 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 2 | 0.03 | - | - | 6-8 | 4-12 | 60-65 | 30-50 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 3 | 0.04 | - | - | 6-8 | 4-12 | 60-65 | 30-50 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 4 | 2.70 | - | - | 6-7 | 4-12 | 50-55 | 30-50 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 5 | 0.03 | - | - | 6 | 4-12 | 60 | 30-55 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | β-glucosidase 6 | 0.08 | - | - | 6-7 | 4-12 | 65-70 | 30-65 | Cellobiohydrolase  β-glucosidase  β-xylosidase | (Takashima et al. 1996) |
|  | BGL1 | 11.2 | 1.82 | 21.1 | 6 | 6-11 | 55 | to 50 | Cellobiohydrolase  β-glucosidase | (Takashima et al. 1999) |
| *Hypocrea jecorina* | β-galactosidase | 0.828 | 0.36 | 144.6 | 5 | 3-6 | 60 | to 65 | None | (Gamauf et al. 2007) |
| *Kluyveromyces fragilis* | β-galactosidase | 2.5 | - | - | 6.5-6.8 | 6-7 | 40 | to 40 | None | (Ladero et al. 2002) |
| *Neocallimastix frontalis* | β-glucosidase | 0.1 | - | - | - | - | - | - | β-glucosidase  Cellobiohydrolase | (Wilson et al. 1994) |
|  | β-galactosidase | 3 | 1.89 | - | 6 | 5-7 | 50 | to 40 | None | (Hebraud & Fevre 1988b) |
| *Pichia pastoris* X-33 | EXG1 | 0.232 | - | - | - | - | - | - | β-glucosidase  β-xylosidase | (Xu et al. 2006) |  |
| *Piromyces communis* | β-galactosidase | 2 | 1.24 | - | 6 | 5-7 | 50 | to 40 | None | (Hebraud & Fevre 1988b) |  |
| *Sphaeromonas communis* | β-galactosidase | 3.7 | 1.2 | - | 6 | 5-7 | 50 | to 40 | None | (Hebraud & Fevre 1988b) |  |
| *Sterigmatomyces elviae* | β-galactosidase | 20.2 | 9.5 | 96 | 4.5-5 | 2.5-7 | 85 | to 80 | None | (Onishi & Tanaka 1995) |  |
| *Thermus* sp. | β-galactosidase | 5.4 | - | - | 5 | 5-11 | 90 | to 80 | None | (Ladero et al. 2002) |  |
| *Trichoderma harzianum* | β-xylosidase | 1.54 | - | - | 4-4.5 | - | 60 | - | β-xylosidase  β-glucosidase  Arabinosidase | (Ximenes et al. 1996) |
| *Trichoderma reesei* | BGL2 | 1.72 | 20 | 20.8 | 6 | 6-11 | 45 | to 45 | Cellobiohydrolase  β-glucosidase | (Takashima et al. 1999) |
| *Alicyclobacillus acidocaldarius* | β-galactosidase | 229 | 6 | - | 5.8 | 5-10.5 | 70 | to 70 | None | (Yuan et al. 2008) |
| *Bifidobacterium adolescentis* | β-galactosidase | 526 | 60 | 1129 | 6 | - | 50 | to 40 | None | (Hinz et al. 2004) |
| *Streptococcosu lactis* | β-galactosidase | 0.75 | - | - | - | - | - | - | None | (Citti et al. 1965) |
| *Thermus aquaticus* | β-galactosidase | 1750 | 0.002 | - | 5 | - | 80 | to 70 | None | (Ulrich et al. 1972) |

**Table S4. Properties of enzymes with xylanase activity.** Table is organized by origin, fungal, then bacterial, then plant. All values listed are from enzymatic activities against xylan. “-“, not reported in the study. Rows of anaerobic fungi are highlighted.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Origin** | **Protein Name (Annotation)** | **Specific Activity**  **(U/mg)** | **Kinetics**  **Km**  **(mg/mL)** | **Kinetics**  **Vmax**  **(U/mg)** | **Optimal pH** | **pH Stability** | **Optimal Temp. (°C)** | **Temp. Stability (°C)** | **Other Activities** | **Ref.** |
| *Orpinomyces* sp. strain C1A | Bgxg1 | 10.8 | 0.038 | 25.6 | 6 | 4-12 | 39 | 4-70 | β-glucosidase  β-xylosidase  β-galactosidase | This  Study |
| *Acrophialophora nainiana* | Xyn III | 31.25 | 4.37 | 0.24 | 6.5 | - | 50 | to 55 | None | (Cardoso & Ferreira 2003) |
|  | Xyn II | 33.2 | 40.9 | - | 7 | - | 55 | to 55 | None | (Salles et al. 2000) |
|  | Xyn I | 13.6 | 0.731 | - | 6 | - | 50-60 | to 50 | None | (Ximenes et al. 1999) |
| *Aspergillus awamori* | β-xylosidase | 1.7 | - | - | 6.5 | - | 70 | to 70 | β-xylosidase | (Kormelink et al. 1993) |
| *Aspergillus niger* | Xylanase I | 18.9 | - | - | 6-6.5 | - | 65-80 | to 80 | Exoglucanase | (John et al. 1979) |
|  | Xylanase IA | 35.2 | - | - | 5.5-6 | - | 65-80 | to 80 | Exoglucanase | (John et al. 1979) |
|  | Xylanase II | 24.5 | - | - | 4-4.5 | - | 65-80 | to 80 | None | (John et al. 1979) |
|  | Xylanase IIA | 48.0 | - | - | 4 | - | 65-80 | to 80 | None | (John et al. 1979) |
| *Chaetomonium cellulolyticum* | Xylanase 1 | 22 | - | - | 6-7 | - | 50 | - | Endoglucanase  Exoglucanase | (Baraznenok et al. 1999) |
|  | Xylanase 2 | 18 | - | - | 6-7 | - | 50 | - | Endoglucanase  Exoglucanase | (Baraznenok et al. 1999) |
|  | Xylanase 3 | 11 | - | - | 6-7 | - | 50 | - | Endoglucanase  Arabinosidase | (Baraznenok et al. 1999) |
| *Chrysosporium lucknowense* | EG28 | 0.2 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Endoglucanase  Exoglucanase | (Bukhtojarov et al. 2004) |
|  | EG44 | 0.07 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Endoglucanase  Exoglucanase | (Bukhtojarov et al. 2004) |
|  | EG47 | 0.08 | - | - | 4.5-6 | 5-8.5 | 60-70 | 60-75 | Endoglucanase  Exoglucanase | (Bukhtojarov et al. 2004) |
|  | EG51 | 0.18 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Endoglucanase  Exoglucanase | (Bukhtojarov et al. 2004) |  |
|  | CBHII | 1.4 | - | - | 4.5-6 | 5-7 | 60-70 | 60-75 | Cellobiohydrolase  Exoglucanase | (Bukhtojarov et al. 2004) |  |
| *Fusarium oxysporum* | Xylanase | 5.2 | 3.8 | - | 7.4 | 5.8-8.2 | 50 | to 50 | Endoglucanase  Exoglucanase | (Christakopoulos et al. 1996) |  |
| *Gloephyllum trabeum* | Cel5A | 0.77 | - | - | - | - | - | - | Endoglucanase  Exoglucanase | (Cohen et al. 2005) |  |
|  | Xyn10A | 390 | - | - | - | - | - | - | Endoglucanase  Exoglucanase | (Cohen et al. 2005) |  |
|  | Cel12A | 0.88 | - | - | - | - | - | - | Endoglucanase  Exoglucanase | (Cohen et al. 2005) |
| *Humicola grisea* | X1 | - | 7.233 |  | 5 |  | 50 | to 50 | None | (Lucena-Neto & Ferreira 2004) |
|  | X2 | 10.23 | 10.87 | - | 4.5-6.5 | - | 55-60 | to 60 | None | (Lucena-Neto & Ferreira 2004) |
|  | Xylanase | 169.4 | 3.3 | 229 | 5.5 | - | 70 | to 50 | None | (Monti et al. 1991) |
| *Neocallimastix frontalis* | Xylanase | 0.36 | 1.13 | - | 6 | 3-8 | 50 | to 40 | None | (Hebraud & Fevre 1988b) |
| *Orpinomyces joyonii* | Cellulase | 5.2 | - | - | 5.5 | 5-7.5 | 40 | to 50 | Exoglucanase  Endoglucanase  Cellobiohydrolase | (Ye et al. 2001) |
| *Paecilomyces thermophila* | Xylanase | 2063 | 2 | 2344 | 7 | 6-10 | 75-80 | to 75 | None | (Li et al. 2006) |
| *Penicillium brasilianum* | XYL | 279 | - | - | - | - | - | - | None | (Jorgensen et al. 2003) |
| *Penicillium citrinum* | Xylanase | 360.7 | - | - | 8.5 | 4-10 | 50 | 0-60 | None | (Dutta et al. 2007) |
| *Piromyces communis* | Xylanase | 0.37 | 1 | - | 5.5 | - | 50 | - | None | (Hebraud & Fevre 1988b) |
| *Sphaeromonas communis* | Xylanase | 0.43 | 0.98 | - | 6 | - | 50 | - | None | (Hebraud & Fevre 1988b) |
| *Trichoderma harzianum* | XYL2 | - | 13.66 | - | 5 | 3.5-6.5 | 45 | to 50 | None | (Silveira et al. 1999) |
|  | Xylanase | 2400 | - | - | - | - | - | - | None | (Tan et al. 1987) |
| *Trichoderma reesei* | EG I | 0.55 | - | - | - | - | - | - | Endoglucanase  Cellobiohydrolase  β-glucosidase | (Suurnakki et al. 2000) |
|  | EG II | 0.003 | - | - | - | - | - | - | Endoglucanase  Cellobiohydrolase  β-glucosidase | (Suurnakki et al. 2000) |
|  | CBH I | 0.0003 | - | - | - | - | - | - | Endoglucanase  Cellobiohydrolase  β-glucosidase | (Suurnakki et al. 2000) |
|  | CBH II | 0.002 | - | - | - | - | - | - | Endoglucanase  Cellobiohydrolase  β-glucosidase | (Suurnakki et al. 2000) |
|  | CBH III | 0.0002 | - | - | - | - | - | - | Endoglucanase  β-glucosidase  β-xylosidase | (Bailey et al. 1993) |
|  | EG I | 0.29 | - | - | - | - | - | - | Endoglucanase  β-glucosidase  β-xylosidase | (Bailey et al. 1993) |
|  | XYL 9 | 11.33 | - | - | - | - | - | - | Endoglucanase  β-glucosidase  β-xylosidase | (Bailey et al. 1993) |
|  | XYL 5.5 | 3.855 | - | - | - | - | - | - | Endoglucanase  β-glucosidase  β-xylosidase | (Bailey et al. 1993) |
|  | EG I | 46.2 | - | - | 4 | 2-8 | 60 | to 45 | Endoglucanase  Exoglucanase  Cellobiohydrolase | (Takashima et al. 1998) |
| *Anaerocellum thermophilum* | CelA | 0.372 | - | - | 5-6 | - | 85-95 | - | Endoglucanase  Exoglucanase | (Zverlov et al. 1998) |
| *Bacillus amyoliquefaciens* | Cellulase | 22.5 | - | - | 7 | 4-9 | 50 | 40-80 | Endoglucanase  Exoglucanase  Cellobiohydrolase  β-glucosidase | (Lee et al. 2008) |
| *Bacillus circulans* | XylA | 2039.5 | 4 | 2667 | 6-6.5 | 4-8 | 75-80 | to 65 | None | (Dhillon et al. 2000) |
|  | XylB | 6423 | 25 | 200000 | 6-6.5 | 4-8 | 65-70 | to 65 | None | (Dhillon et al. 2000) |
| *Bacillus licheniformis* | Xylanase | 28.7 | 3.33 | 111 | 6-7.5 | 5-8 | 60 | to 60 | None | (Archana & Satyanarayana 2003) |
| *Bacillus stearothermophilus* | Xylanase | 122 | 3.8 | - | 7 | 5-11 | 60 | to 60 | None | (Nanmori et al. 1990) |
| *Bacillus subtilis* | Cellulase | 980 | - | - | 6 | 6-7.5 | 50-60 | to 50 | Endoglucanase | (Yin et al. 2010) |
| *Bacillus thermantarcticus* | Xylanase | 141 | 1.6 | - | 5.6 | - | 80 | to 60 | None | (Lama et al. 2004) |
| *Caldibacillus cellulovorans* | CMCase | 4.56 | - | - | 6.5-7 | - | 80 | to 70 | Endoglucanase  Exoglucanase | (Huang & Monk 2004) |
| *Cellulomonas fimi* | Exg | 37 | - | - | 6 | 5-9 | - | 60-75 | Endoglucanase  Cellobiohydrolase | (Curry et al. 1988) |
| *Clostridium stercorarium* | Avicelase II | 0.340 | - | - | 5-6 | 4.5-7 | 75 | 65-80 | Exoglucanase | (Bronnenmeier et al. 1991) |
| *Streptomyces lividans* | Xylanase | 364 | 0.78 | 850 | 6 | 5.5-7 | 60 | 0-37 | None | (Morosoli et al. 1986) |
| *Streptomyces rameus* | Xylanase | 4326 | 5.8 | - | 5.3 | 4.3-6.7 | 70 | 40-65 | None | (Li et al. 2010) |
| *Streptomyces thermoviolaceus* | STX-I | 1460 | - | - | 7 | 5-9 | 70 | to 50 | None | (Tsujibo et al. 1992) |
|  | STX-II | 1405 | - | - | 7 | 5-9 | 60 | to 60 | None | (Tsujibo et al. 1992) |
| *Thermatoga maritima* | CelA | 2.5 | - | - | 5 | 4-9 | 85-90 | to 95 | Endoglucanase  Exoglucanase  Cellobiohydrolase | (Liebl et al. 1996) |
|  | CelB | 0.008 | - | - | 7 | 5-9 | 85-90 | to 95 | Endoglucanase  Exoglucanase  Cellobiohydrolase | (Liebl et al. 1996) |
| *Thermatoga neapolitana* | CelB | 37 | - | - | 6-6.6 | - | 106 | to 106 | Endoglucanase  Exoglucanase  Cellobiohydrolase | (Bok et al. 1998) |

**Supplementary figures.**

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**Figure S1. Alignment of native Bgxg1 from *Orpinomyces* sp. strain C1A (ASRE01002650.1, range: 2346-3460) and codon-optimized Bgxg1 (for expression in *E. coli*).**

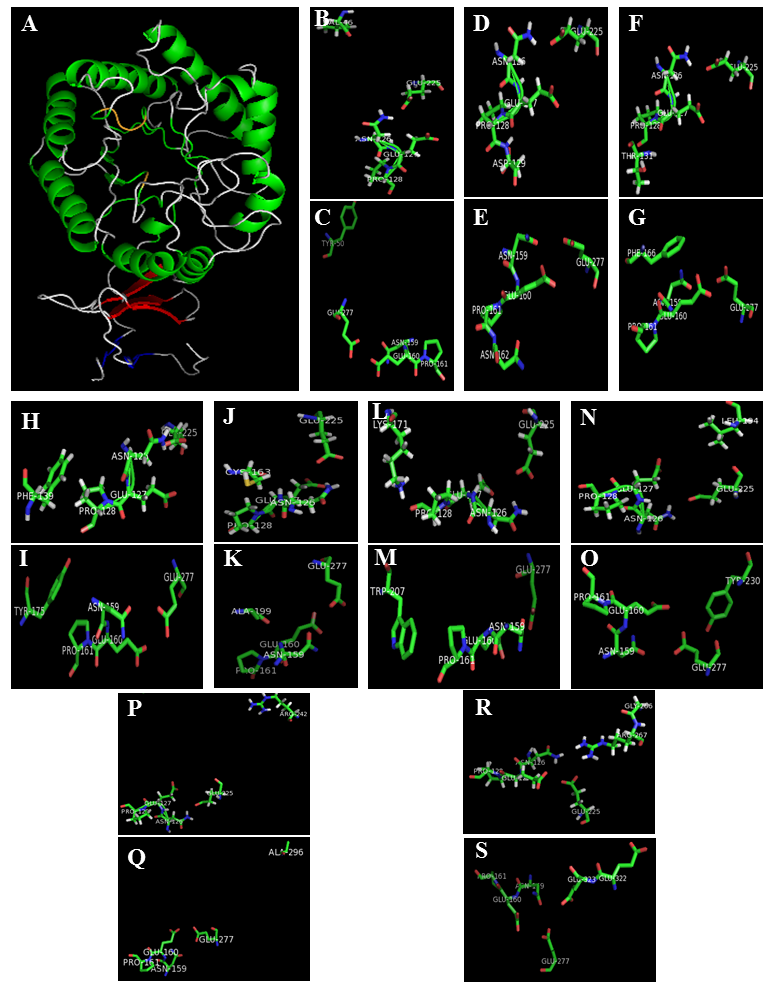
****

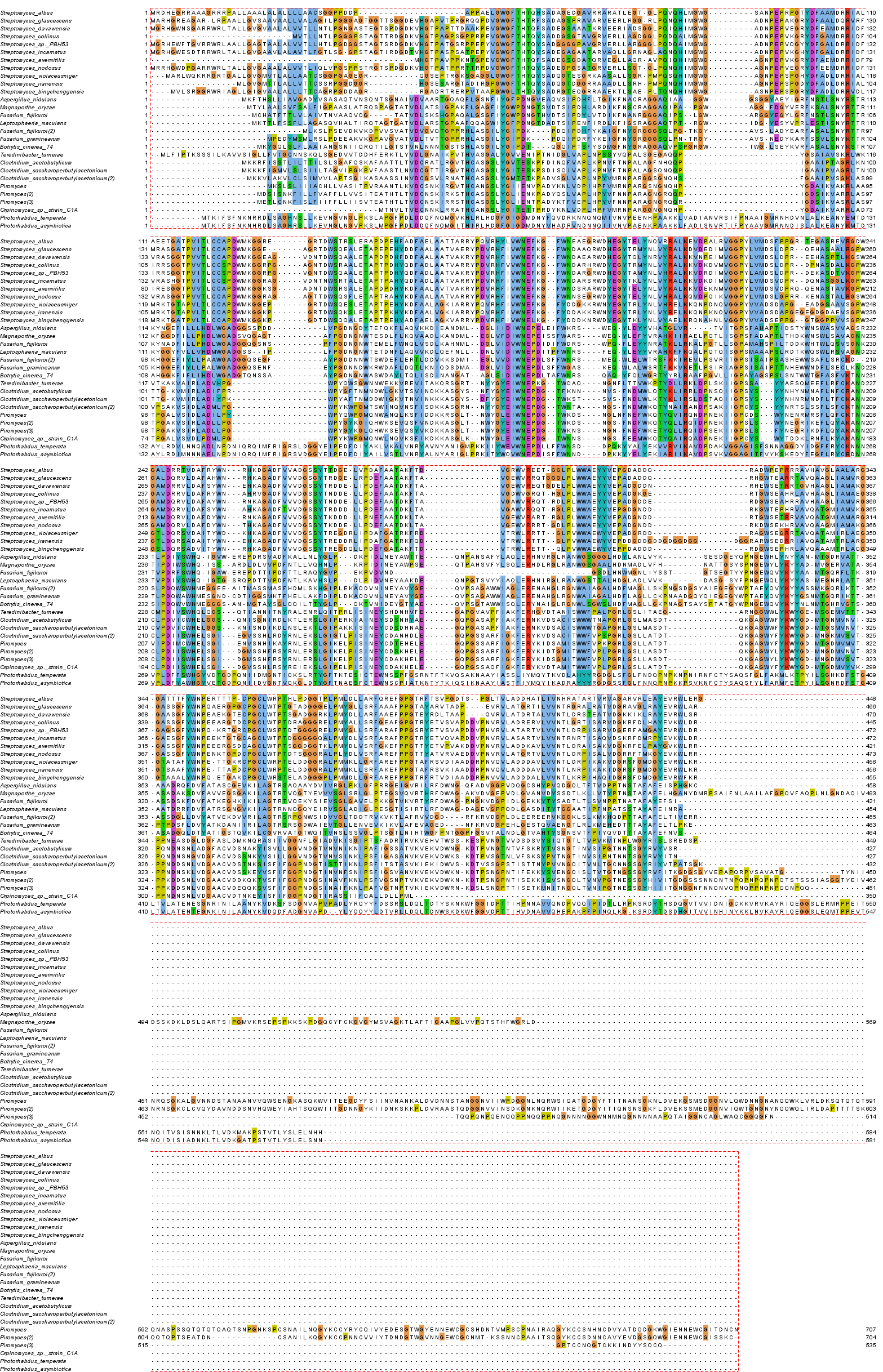
**Figure S2. SDS-PAGE analysis for Inclusion Body.** A 12.5% SDS-PAGE analysis of Inclusion Body Fraction stained with Coomassie blue. Lane A, Pre-stained Protein Ladder (Caisson Labs, Smithfield, Utah). Lane B, Inclusion Body Fraction. Note: Bgxg1 is 42.7 kDa.

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**Figure S3. SDS-PAGE analysis of Bgxg1.** A 12.5% SDS-PAGE analysis of recombinant Bgxg1 protein stained with Coomassie blue. Lane A, Pre-stained Protein Ladder (Caisson Labs, Smithfield, Utah). Lane B, Purified Bgxg1.

**Figure S4. Bgxg1 model and comparison of residues of interest. A**. Bgxg1 model obtained using I-TASSER. Residues of the α-helical domain are in blue; residues of the (α/β)8 barrel are in green; residues of the β-sandwich are in red. Active site residues (Asn126-Glu127-Pro128, Glu225) are shown in orange. **B-S** Changes in the area surrounding the active site between Bgxg1 (top panel) and 1UHV of *Thermoanaerobacter saccharolyticum* (bottom panel). **B and C**, Val46 (Bgxg1) vs Tyr50 (1UHV). **D and E,** Asp129 (Bgxg1) vs Asn162 (1UHV). **F and G,** Thr131 (Bgxg1) vs Phe166 (1UHV). **H and I,** Phe139 (Bgxg1) vs Tyr175 (1UHV). **J** **and K,** Cys163 (Bgxg1) vs Ala199 (1UHV). **L and M,** Lys171 (Bgxg1) vs Trp207 (1UHV). **N** **and O,** Leu194 (Bgxg1) vs Tyr230 (1UHV). **P and Q,** Arg242 (Bgxg1) vs Ala296 (1UHV). **S** Glu322 and Glu323 of 1UHV align with a gap in Bgxg1, but Gly266-Arg267 are predicted to occupy approximately the same position in Bgxg1 **R**. Modeling clearly suggests that these changes could putatively impact the size, charge, and/or polarity within the active site. Changes in polarity in and around the active site of a protein can directly affect the affinity of the protein for the substrate (Macgregor & Weber 1986). Changes that affect polarity include polar Tyr (1UHV, panel I) to nonpolar Phe139 (Bgxg1, panel H), as well as nonpolar Ala (1UHV, panel K) to polar, thiol-containing Cys163 (Bgxg1, panel J). Changes in charge in and around the active site can change substrate binding and intermediate stabilization (Czjzek et al. 2005). Changes that affect charge include neutral Asn (1UHV, panel E) to negative Asp129 (Bgxg1, panel D). Changes in the size of the active site can also have a direct impact on substrate binding. Combinations of the three aforementioned changes were seen. Changes in both size and polarity include: large, polar Tyr (1UHV, panel C) to small, nonpolar Val46 (Bgxg1, panel B); large, nonpolar Phe (1UHV, panel G) to small, polar Thr131 (Bgxg1, panel F); large, polar Tyr (1UHV, panel O) to small, nonpolar Leu194 (Bgxg1, panel N). A change in both charge and polarity includes nonpolar, neutral Trp (1UHV, panel M) to polar, positively Lys171 (Bgxg1, panel L). Finally, a change in charge, size, and polarity includes neutral, small, nonpolar Ala (1UHV, panel Q) to positively charged, large, and polar Arg242 (Bgxg1, panel P). Lastly, the final two changes represent deletions/gaps in the Bgxg1 sequence as opposed to negatively charged glutamic acids in the other four sequences (Table 4, panel S). However, structural modeling suggests that in lieu of the Glu322-Glu323 residues in 1UHV, Bgxg1 has Gly266 and Arg267 (panel R). This represents a significant change in charge, from negatively charged Glu to positively charged Arg and from negatively-charged Glu to neutral Gly.



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**Figure S5. Alignment of Class III enzymes (see Fig. 1).**

**Supplemental Material References**

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