1 2	Characterization of a cellobiohydrolase (MoCel6A) in Magnaporthe oryzae
3	Takahashi et al., Supplementary Materials
4	
5	Content:
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7	Fig. S1-S3
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9	Figure Legends
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11	Fig. S1. Rice leaves infected with <i>M. oryzae</i> . Rice leaves were sprayed with a
12	suspension of <i>M. oryzae</i> spores and harvested 1-5 days after inoculation. The
13	leaves were used to prepare cDNA by reverse-transcription.
14	
15	Fig. S2. Preparation of recombinant proteins prepared in <i>M. oryzae</i> and <i>A.</i>
16	oryzae.
17	<i>M. oryzae</i> and <i>A. oryzae</i> transformants overexpressing either MoCel6A,
18	MoCel7A or TrCel6A were cultured in YG (0.5% yeast extract and 2% glucose)
19	medium for 4 days at 25 $^\circ\text{C}$ with gentle rotation at 120 rpm. Culture medium was
20	desalted, concentrated and applied to a His-binding resin, and then bound
21	protein was eluted with elution buffer (50 mM sodium phosphate, pH 7.0, 50 mM
22	NaCl, 200 mM imidazol). Crude and eluted fractions were electrophoresed on an

23 SDS-acrylamide gel and then stained with Coomassie Brilliant Blue R-250.

Lanes 1, 3, 5 and 7, 5 μ g of the crude fraction; lane 2, 1 μ g of the eluted

25 MoCel6A expressed in *M. oryzae*; lane 4, 1 µg of the eluted MoCel7A expressed

in *M. oryzae*; lane 6, 5 μg of the eluted MoCel6A expressed in *A. oryzae*; lane 8,

27 5 µg of the eluted TrCel6A expressed in *A. oryzae*.

28

Fig. S3. Amino acid alignment of fungal GH-6 family cellobiohydrolases.

30 Amino acid sequences of cellobiohydrolases from *M. oryzae* (MoCel6A,

31 MoCel6B and MoCel6C), *T. reesei* (AF302657), *T. viride* (AY368688),

32 A.fumigatus (XP_748511), A. oryzae (XM_001825308), H. insolens (AB048710),

33 *C. thermophilum* (DQ020255) and *C. globosum* (XM_001226028) were aligned

34 using Clustal W and standard conditions. A putative secretion signal sequence is

underlined and a putative cellulose binding domain is in a box. Asterisks

36 represent highly conserved amino acids that are proposed to be associated with

37 substrate binding according to the previous reports (41-43). Closed circles

represent highly conserved amino acids in fungal cellobiohydrolases that are

absent in MoCel6A.

 $\mathbf{2}$

40 Fig. S1



41 Fig. S2



42 Fig. S3

MoCel6A <i>T. ressei</i> (AF302657) <i>T. viride</i> (AY368688) <i>A. fuigatus</i> (XP_748511) <i>A. oryzae</i> (XM_001825308) <i>H. Insolens</i> (AB048710) <i>C. thermophilum</i> (DQ020255) <i>C. globosum</i> (XM_001226028)	MASKLFLAAALLQ-GALSSPLAVEERQACAAQWGQCGGQDYTGPTCCQSGSTCV MIVGILTT-LATLATLAASVPLEERQACSSVWGQCGGQNWSGPTCCASGSTCY MIVGILTT-LATLAASVPLEERQACSSVWGQCGGQNWSGPTCCASGSTCY MATRPVEPSTFNCMISCRNYSASWHHLTDSSFQLHTQVTDRVLASSIALTLLPAVQAQ MAK-FFLTAAFAA-AALAAPV-VEERQNCAPTWGQCGGIGFNGPTCCQSGSTCVK MAKQLLLTAALAA-TSLAAPL-LEERQSCSSVWGQCGGINYNGPTCQSGSTCYK MAKLFLAAALAA-TALAAPV-VEERQNCATLWGQCGGNGWNGATCCASGSTCK
MoCel6A	SNOWYSQCLPGSSNP-TTTSRTSISSSSSTSRTSSSTSRPPSSVPTTPISVPPTITTPT
<i>T. reesei</i> (AF302657)	SNDYYSQCLPG-A-A-SSSSS-T-RAAST-IS-RV-S-PTTSRSSSATPPPGSI-T-T
<i>T. viride</i> (AY368688)	SNDYYSQCLPG-A-A-SSSSS-I-RAAST-IS-RV-S-PTTSRSSSATPPPGSI-T-T
<i>A. fuigatus</i> (XP_748511)	QTWGQCGGQGWSGPTSCVAGAACSTLNPYYAQCIPGATATSTTLTTTAATTSQTITK
<i>A. oryzae</i> (XM_001825308)	TVPSPEFCTPTHHLQSSVNSKLSYLFIMHTINWQALVALSPLLFSAATALPQASVTPSPS
<i>H. Insolens</i> (AB048710)	QNDWYSQCLPG-S-Q-VTTTST-ISTSSSSTIS-RATSTTRTGGVTSITTAPTR-I-V-T
<i>C. thermophilum</i> (DQ020255)	LNDWYSQCLPGGA-Q-PGTTST-IARTTSTSIT-STSSVRPTTSNTPVTAPPT-T-T
<i>C. globosum</i> (XM_001226028)	QNDWYSQCLPGGAVTTPGTTTKPISTSITSISSSTSTSQGGGVSSSISSPPVVINPPT
MoCel6A	TTPTGGSGPGTTASFTGNPFAGVNLFPNKFYSSEVHTLAIPSLT-GSLVAKASAVAQVPS
<i>T. reesei</i> (AF302657)	RVPPVGSGTATYSGNPFVGVTPWANAYYASEVSSLAIPSLT-GAMATAAAAVAKVPS
<i>T. viride</i> (AY368688)	RVPPVGSGTATYSGNPFVGVTPWANAYYASEVSSLAIPSLT-GAMATAAAAVAKVPS
<i>A. fuigatus</i> (XP_748511)	PTTTGPTTSAPITVTASGNPFSGYQUYANPYYSEVHTLAMPSLP-SSLQPKASAVAKVPS
<i>A. oryzae</i> (XM_001825308)	SSVPASSGPAPITATAGGNPEGYQUYANPYYSEVHTLAMPSLP-SSLQPKASAVAEVPS
<i>H. Insolens</i> (AB048710)	-IP-GGATTASYNGNPFEGVQLWANNYYRSEVHTLAIPQITDPALRAAASAVAEVPS
<i>C. thermophilum</i> (DQ020255)	SIP-GGASSTASYNGNPFSGVQLWANTYYSEVHTLAIPSLS-PELAAKAAKVAEVPS
<i>C. globosum</i> (XM_001226028)	SIP-GGASSTASYNGNPFSGVQLWANTYYSEVHTLAIPSLS-PELAAKAAKVAEVPS
MoCel6A T. reesei (AF302657) T. viride (AY368688) A. fuigatus (XP_748511) A. oryzae (XM_001825308) H. Insolens (AB048710) C. thermophilum (DQ020255) C. globosum (XM_001226028)	FOWLDIAAKWETLMPGALADWRAANAAGGNYAAQUVVYDLPDRDCAAAASNGEFSIAD FMWLDT-LDKTPLMEQTLADIRTANKNGGNYAGQFVVYDLPDRDCAALASNGEYSIAD FMWLDT-LDKTPLMEQTLADIRTANKNGGN-YAGQFVVYDLPDRDCAALASNGEYSIAD FVWLDYAAKVPTM-GTYLADIQAKNKAGANPPTAGTFVVYDLPDRDCAALASNGEYSIAN FHWLDTTOKVPQM-GEFLEDIKTKNAAGANPPTAGTFVVYDLPDRDCAALASNGEFISD FQWLDRNVTVDTLLVETLSETRAANQAGANPPYAAQTVVYDLPDRDCAAALASNGEWAIAN FQWLDRNVTVDTLLVETLSETRAANQAGANPPYAAQTVVYDLPDRDCAAAASNGEWAIAN FQWLDRNVTVDTLLSGTLAETRAANQAGANPPYAAQTVVYDLPDRDCAAAASNGEWSIAN YQWMDRNVTVDTLFSGTLAQTRAANQAGASPPYAGTFVVYDLPDRDCAAAASNGEWSIAN
MoCel6A	GGVVKYKAYIDAIRKQLLAYSDVRTILVIEPDSLANMVTNMGVPKCAGAKDAYLECTIYA
<i>T. reesei</i> (AF302657)	GGVAKYKNYIDTIRQIVVEYSDIRTLLVIEPDSLANLVTNLGTPKCANAQSAYLECINYA
<i>T. viride</i> (AY368688)	GGVAKYKNYIDTIRQIVVEYSDIRTLLVIEPDSLANLVTNLGTPKCANAPSAYLECINYA
<i>A. fuigatus</i> (XP_748511)	NGVANYKAYIDAIRAQLVKYSDYHTTLVIEPDSLANLVTNLNVAKCANAQSAYLECINYA
<i>A. oryzae</i> (XM_001825308)	GGVEKYKAYIDSIRQVEKYSDTQILVIEPDSLANLVTNLNVAKCANAQDAYLECTNYA
<i>H. Insolens</i> (AB048710)	NGANNYKGYINRIREILISESDVRTILVIEPDSLANVTNNNVAKCSGAASTYKELTIYA
<i>C. thermophilum</i> (DQ020255)	NGANNYKGYINRIREILISESDVRTILVIEPDSLANVTNNNVAKCSMAASTYKELTIYA
<i>C. globosum</i> (XM_001226028)	GGAANYKAYIRAIRELLIQYSDIRTILVIEPDSLANVTNNNVAKCSMAASTYKELTIYA
MoCel6A T. reesei (AF302657) T. viride (AY368688) A. fuigatus (XP_748511) A. oryzae (XM_001825308) H. Insolens (AB048710) C. thermophilum (DQ020255) C. globosum (XM_001226028)	VKQLNLPHVAMYLDGGHAGWLGWPANLQPAADLFGKLYADAGKPSQLRGMATNVANYNAW VTQLNLPNVAMYLDAGHAGWLGWPANQDPAAQLFANVYKNASSPRALRGLATNVANYNGW VTQLNLPNVAMYLDAGHAGWLGWPANQDPAAQLFANVYKNASSPRALRGLATNVANYNGW LKQLNLPNVAMYLDAGHAGWLGWPANLGPAATLFAKVYTDAGSPAAVRGLATNVANYNAW LTQLNLPNVAMYLDAGHAGWLGWPANIGPAAELFAKIYEDAGKPRAVRGLATNVANYNAF LKQLDLPHVAMYMDAGHAGWLGWPANIOPAAELFAKIYEDAGKPRAVRGLATNVANYNAF LKQLDLPHVAMYMDAGHAGWLGWPANIOPAAELFAKIYEDAGKPRAVRGLATNVANYNAW LKQLNLPHVAMYMDAGHAGWLGWPANIOPAAELFAKIYEDAGKPRAVRGLATNVANYNAW LKQLNLPHVAMYMDAGHAGWLGWPANIOPAAELFAKIYEDAGKPRAAVRGLATNVANYNAW LKQLNLPHVAMYMDAGHAGWLGWPANIOPAAELFAKIYEDAGKPRAAVRGLATNVANYNAW
MoCel6A	DLTTAPSYTTPNPNFDEKKYISAFAPLLAAKGWSAHF-IIDQGRSGKQPTGQKEWGHWCN
<i>T. reesei</i> (AF302657)	NITSPPSYTQGNAVYNEKLYIHAIGPLLANHGWSNAFFITDQGRSGKQPTGQQWGDWCN
<i>T. viride</i> (AY368688)	NITSPPSYTQGNAVYNEKLYIHAIGPLLANHGWSNAFFITDQGRSGKQPTGQQWGDWCN
<i>A. fuigatus</i> (XP_748511)	SLSTCPSYTQGDPNCDEKKYINAMAPLLKEAGFDAHF-IMDTSRNGVQPTKONAWGDWCN
<i>A. oryzae</i> (XM_001825308)	SIDSGPSYTQGSTVCDEKKYINAMAPLLKEAGFDAHF-IMDTGRNGVQPTGQSQWGDWCN
<i>H. Insolens</i> (AB048710)	SIDSGPSYTQSTVCDEKTYINAFAPQLKSAGFDAHF-IMDTGRNGVQPTGQSQWGDWCN
<i>C. thermophilum</i> (DQ020255)	SISSPPPYTSPNPNYDEKHYIEAFRPLLEARGFPAQF-IVDTGRNGKQPTGQLEWGHWCN
<i>C. globosum</i> (XM_001226028)	SVSSAPAYTSPNPNYDEKHYIEAFAPLLTAAGFPANF-INDTGRNGKQPTGQLEWGHWCN
MoCel6A T. reesei (AF302657) T. viride (AY36868) A. fuigatus (XP_748511) A. oryzae (XM_001825308) H. Insolens (AB048710) C. thermophilum (DQ020255) C. globosum (XM_001226028)	QQGVGFGRRPSANTGSELADAFVWIKPGGECDGVSDPTAPRFDHFCGTDYGAMSDAPQAG VIGTGFGIRPSANTGDSLLDSFVWVKPGGECDGTSDSSAPRFDSHCALP-DALQPAPQAG VIGTGFGIRPSANTGDSLDSFVWVKPGGECDGTSDSSAPRFDSHCALP-DALQPAPQAG VIGTGFGVRPSINTGDPLQDAFVWIKPGGESDGTSNSTSPRYDAHCGYS-DALQPAPEAG KNTGFGVRPTTDTGDELVDAFVWVKPGGESDGTSDTSABRYDAHCGYS-DALQPAPEAG AIGTGFGVRPTANTGHQVVDAFVWVKPGGECDGTSDTTABRYDAHCGYS-DALPAPEAG VKGTGFGVRPTANTGHQVVDAFVWVKPGGESDGTSDTSABRYDAHCGYS-DALPAPEAG VKGTGFGVRPTANTGHQVVDAFVWVKPGGESDGTSDTSABRYDAHCGYS-DALTPAPEAG AVGTGFGQRPSANTGHQLVDAFVWVKPGGESDGTSDTSABRYDAHCGYS-DALTPAPEAG AVGTGFGQRPSANTGHQLVDAFVWVKPGGESDGTSDTSABRYDYHCGLS-DALTPAPEAG
MoCel6A	QWFQLYFEMLLTNANPPL-
<i>T. reesei</i> (AF302657)	AWFQAYFWQLLTNANPSFL
<i>T. viride</i> (AY368688)	AWFQAYFWQLLTNANPSFL
<i>A. fulgatus</i> (XP_748511)	TWFQAYFEQLLTNANPSF-
<i>A. oryzae</i> (XM_001825308)	TWFQAYFEQLVENANPSL-
<i>H. Insolens</i> (AB048710)	QWFQAYFEQLLWANPPF-
<i>C. thermophilum</i> (DQ020255)	QWFQAYFEQLLUMANPPF-
<i>C. globosum</i> (XM_001226028)	QWFQAYFEQLLTNANPPF-