

Colhig3 1 MILP~~HA~~AVRSLQALS~~LN~~AA~~RQ~~PLQSS~~SI~~QCPIVLDGRIPQNF~~TL~~QVFD~~TN~~ESPFNPGFTK
Colinc3 1 MMVPHMAVRSLQALS~~LN~~SALQSLQ-SSVQCPIVLDGRIPQNF~~S~~LQVFD~~TN~~ESPFNPGFTK
Coltof3 1 MVLPHAAVRSLQALS~~LN~~SALQSLQ-LPVQCPIVLDGRIPQNF~~S~~LQVFD~~TN~~DSPFNPGFTK
Colfio3 1 MILP~~HA~~AVRSLQALS~~LN~~APRASFD-SPVKCPVVDGRIPQNF~~TL~~QVFD~~TN~~YASPFNPGYTK
Colnym3 1 MILP~~HA~~AVRSLQALS~~LN~~APRASLD-SPVKCPVVDGRIPQNF~~TL~~QVFD~~TN~~YASPFNPGYTK
Colsim3 1 MILP~~HA~~AVRSLQALS~~LN~~APRASLD-SPIKCPVVDGRIPQNF~~TL~~QVFD~~TN~~YD~~SP~~FNPGYTK
Colsal3 1 MILP~~HA~~AVRSLQALALNVP~~PR~~ASLD-SPVKCPVVDGRIPQNF~~TL~~EVFD~~TN~~STSPFNPGFTK
Colchl3 1 MILPHTAVRSLQALS~~LN~~APFQSFQ-SPVRCPVVDGRIPQNF~~TL~~EVFD~~TN~~ASPFNPGFTK
Colglo3 1 MILPHLAVRAL-S-----ASVQCPIVLDGRIPQNF~~TL~~ENFD~~TN~~VSPNPGFTK
Colorb3 1 MILPQVAARALLGLS-----SAVKCPIVLDGRIPQNF~~TL~~DEFD~~TN~~ASPNPGFTK

Colhig3 61 GPAPWSEILLFPAGVTPSRFDV~~V~~SEP---TATSEFKPLEVTIDDKSIFD-PSGNNPQLGFR
Colinc3 60 GPAPWSDILLFPNTTPSRFDVSN-----STGNFKPVEVTINDQSI~~FD~~-PSGNNPQVIGFR
Coltof3 60 GPAPWSEILLFPATTPSRFDVSS-----STGDSKPEVTINDQSI~~FD~~-PSGNNPQVIGFR
Colfio3 60 GPAKWSEILLFPVPTPSRFDASN-----ATSEFKPLEVTINDQSI~~FN~~-PSGNSPQLGFR
Colnym3 60 GPAKWSEILLFPVPTPSRFDTSN-----ATGNFKPLEVTINDQSI~~FN~~-PSGNSPQLGFR
Colsim3 60 GPAKWSEILLFPVPTPSRFDTSN-----ATGNFKPLEVTINDQSI~~FN~~-PSGNSPQLGFR
Colsal3 60 GPAPWSEILLFPVPTPSRFDASN-----ATGNFKPLEVTINDQSI~~FN~~-PSGNSPQLGFR
Colchl3 60 GPALWSEILLFPNISTTPSRFDVSS-----QTGDFKPLEVTINDQSI~~FN~~PPGNNPQLGFR
Colglo3 48 GSDPWSKIIILFPVAAPSRFDAAASANATS~~SG~~SGSEFKPLEVTINDRSI~~FN~~PPGKNPQLGFR
Colorb3 51 GPD~~P~~WSKILLFPVVAQT~~SR~~FDVAGR----SPANTKPLEVTINDSI~~FN~~PPGKNPQLGFR

Colhig3 117 RAGLLLGNGSDASNQGVMTFHWSSRQDPARKLNLTHEYLTSF~~HET~~NDSGGAQFSLQVGTGTP
Colinc3 114 RAGLLLGNGSDASNQGVMTFHWSSMQDPTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Coltof3 114 RAGLLLGNGSDASNQGVKTFHWSSRQDSTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Colfio3 114 RAGLLLGNGSDASNQGVMTFHWSSMQDSTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Colnym3 114 RAGLLLGNGSDASNQGVMTFHWSSMQDSTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Colsim3 114 RAGLLLGNGSDASNQGVMTFHWSSMQDSTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Colsal3 114 RAGLLLGNGSDASNQGVMTFHWSSMQDSTRKLNLT~~HE~~YLVSF~~HET~~NDSGGAQFALQIGTGP
Colchl3 115 RAGLLLGNGSDASNEGVMTFHWSSRQDSARKLNLT~~HE~~YLVAF~~HET~~NDSGGAQFALQIGTGP
Colglo3 108 RAGLLLGNGSDASNQGVMTFHWSSAMQDSARKLNLT~~HE~~YLVAF~~HET~~NDSGGAQFALQVGLP
Colorb3 107 RAGLLLGNGSDAANE~~GA~~VTIHWSSRQDPARLNL~~HE~~YLVAF~~HET~~NDSGGAQFTLQIGLP

Colhig3 177 LGQDGAAGKPAASDWKMLNRN~~SV~~VFSTPLKFD~~AW~~QNEAVTLDV~~PK~~NTMQVYFSEGN~~D~~PL
Colinc3 174 LGQNGTAGRPSPTDWKMLDRN~~SV~~VFSTPLKFD~~AW~~QNEAVTLDV~~PK~~NTMQVFFSEGN~~D~~PL
Coltof3 174 LGQNSTAGRPSLSDWKMLDRN~~SV~~VFSTPLKFD~~AW~~QNEAVTLDV~~PK~~NTMQVFFSEGN~~E~~PL
Colfio3 174 LGQNSTAGKPLSDDWKMLDRN~~SV~~VFSTALKFDVWQNEAVTLDV~~PK~~NTIQVFFSEGN~~D~~PL
Colnym3 174 LGQNSTAGKPLSDDWKMLDRN~~SV~~VFSTALKFDVWQNEAVTLDV~~PK~~NTIQVFFSEGN~~D~~PL
Colsim3 174 LGQNSTV~~G~~KPLSDDWKMLDRN~~SV~~VFSTALKFDVWQNEAVTLDV~~PK~~NTIQVFFSE~~N~~DPL
Colsal3 174 LGQNSTTGGPSSADWKMLDRN~~SV~~VFSTALKFDVWQNEAVTLDV~~PK~~NTIQVFFSEGN~~D~~PL
Colchl3 175 LGQNSTLSGEASSDWKMLDRN~~SV~~VFSTPLKFD~~AW~~QNEAVTLDI~~TP~~NTLQVYFSEGD~~D~~PL
Colglo3 168 LGQNGT--EPPKENWKMLDRN~~SV~~VFSTPLKFD~~AW~~QNEAVTLDV~~PK~~NTMQVFFSEGN~~E~~PL
Colorb3 167 LGQNK~~T~~--EPPAANWKMLNRN~~SV~~VFTPLQ~~EG~~KWQNEAVTLDV~~PK~~NTMQVYFSEGN~~E~~PL

Colhig3 237 KPVTEILPNDNSGGGALQIGLLKKPTETKT~~V~~VFDGFQESNLNEGQILGSI~~F~~VEDSANGCV
Colinc3 234 KPVTEVLPNDNSGGGALQIGLLKKPTETKT~~V~~VFDGFQESNLNEGQILGGIFVENSANGCI
Coltof3 234 KPVTEVLPNDNSGGGVLOIGLLKKPTETKT~~V~~VFDGFQESNLNEGQILGGIFVEDSANGCV
Colfio3 234 KSVTEVLPNNNAGGGALQIGLLKKPTETKT~~V~~VNDGFQESNLNEGQILGGIFVENSANGCV
Colnym3 234 KSVTEVLPNNNAGGGALQIGLLKKPTETKT~~V~~VNDGFQENLNNEGQILGGIFVENSANGCV
Colsim3 234 KSVTEVLPNNNAGGGALQIGLLKKPTETKT~~V~~VNDGFQESNLNEGQILGGIFVENSANGCV
Colsal3 234 KSVTEVLPNNNAGGGALQIGLLKKPTETKT~~V~~VNDGFQESNLNEGQILGGIFVENSANGCI
Colchl3 235 KPVTEVLPNNNAGGGALQIGLLKKPTETKT~~V~~VNDGFQENLNNEGQILGGIFVENSANGCV
Colglo3 226 KAVTKILPNNNAGGGALQIGLLKKPTETKT~~V~~VANDGFQESNLNEGQILGGIFVENSANGCI
Colorb3 225 SAVTGVLPNNNAGGGALQIGLLKKPTETKT~~V~~VFDGFQESNLNEGQILGGIFVENSANGCV

Colhig3 297 SL
Colinc3 294 SL
Coltof3 294 SL
Colfio3 294 SL
Colnym3 294 SL
Colsim3 294 SL

Colsa13	294	SL
Colch13	295	SL
Colglo3	286	SL
Colorb3	285	SL

Sordariomycetes

Hypocreomycetidae - Glomerellales (Colletotrichum but not Verticillium)

Colch13 = OLN81946.1 hypothetical protein CCHL11_08641 [Colletotrichum chlorophyti]
 Colfio3 = EXF72940.1 hypothetical protein CFIO01_04646 [Colletotrichum fioriniae PJ7]
 Colglo3 = KAF3801482.1 hypothetical protein GCG54_00014696 [Colletotrichum gloeosporioides]
 Colletotrichum graminicola - no orthologue
 Colhig3 = CCF43865.1 hypothetical protein CH063_03177 [Colletotrichum higginsianum]
 which is equivalent to CH063_00647 described by Anasontzis et al. (2019)
 Colinc3 = KZL79966.1 hypothetical protein CI238_09667 [Colletotrichum incanum]
 Colnym3 = KXH53449.1 hypothetical protein CNYM01_01194 [Colletotrichum nymphaeae SA-01]
 Colorb3 = TDZ24818.1 hypothetical protein Cob_v002377 [Colletotrichum orbiculare MAFF 240422]
 Colsal3 = KXH68549.1 hypothetical protein CSAL01_02206 [Colletotrichum salicis]
 Colsim3 = KXH27864.1 hypothetical protein CSIM01_06783 [Colletotrichum simmondsii]
 Coltof3 = KZL72700.1 hypothetical protein CT0861_06503 [Colletotrichum tofieldiae]

Hypocreomycetidae - Hypocreales - Clavicipitaceae - no orthologues

Hypocreomycetidae - Hypocreales - Cordycipitaceae - no orthologues

Hypocreomycetidae - Hypocreales - Hypocreaceae - no orthologues

Hypocreomycetidae - Hypocreales - Nectriaceae - no orthologues

Hypocreomycetidae - Hypocreales - Ophiocordycipitaceae - no orthologues

Hypocreomycetidae - Hypocreales - Stachybotryaceae - no orthologues

Hypocreomycetidae - Microascales - no orthologues

Sordariomycetidae - Coniochaetales - no orthologues

Sordariomycetidae - Togniales - no orthologues

Sordariomycetidae - Diaporthales - no orthologues

Sordariomycetidae - Magnaporthales - no orthologues

Sordariomycetidae - Ophiostomataceae - no orthologues

Sordariomycetidae - Sordariales - Sordariales incertae sedis - no orthologues

Sordariomycetidae - Sordariales - Chaetomiaceae - no orthologues

Sordariomycetidae - Sordariales - Sordariaceae - no orthologues

Xylariomycetidae - Xylariales - no orthologues

Supplementary Figure S8. ChGluc131B alignment. Multiple protein-sequence alignment of ChGluc131B orthologues in the Fol25 clade of Sordariomycete GH131 (glycosyl-hydrolase 131) sequences. All sequences shown were the highest blastP matches to ChGluc131B (= Colhig3 highlighted in green in the alignment) obtained for each genome-sequenced species represented. Sequences were aligned using ClustalW at <https://www.ebi.ac.uk/Tools/msa/mafft/>. Amino-acid identities were highlighted in black and similarities in grey using BoxShade at https://embnet.vital-it.ch/software/BOX_form.html. Conserved cysteines have been highlighted manually in blue, residues conserved with the catalytic residues of PaGluc131A and CcGH131A in red and non-conserved residues in teal. None of these sequences were found to have predicted signal peptide sequences when analysed using SignalP 5.0 (<http://www.cbs.dtu.dk/services/SignalP/>) or SignalP 4.1 (<http://www.cbs.dtu.dk/services/SignalP-4.1/>) but Colorb3 and Colsal3 had predicted signal peptide sequences when analysed using SignalP 3.0 (<http://www.cbs.dtu.dk/services/SignalP-3.0/>) and all were predicted to be secreted proteins when analysed using Secretome P 2.0 (<http://www.cbs.dtu.dk/services/SecretomeP/>).