

Supplemental Material:

Contribution of Peroxisomes to Secondary Metabolism and Pathogenicity in the Fungal Plant Pathogen *Alternaria alternata*

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TABLE S1. Oligonucleotide primers used in this study

Primer	Sequence (5' to 3') ^a
AKT1-f	ATCCT <u>GATCA</u> TGGTTTCGCAGCTCCCTG
AKT1-r	TGC <u>CTGATCA</u> CTATATCTTGCTCGACAGCC
AKT2-f	TTA <u>AGGATC</u> CTGAT <u>GCAGCAGCCC</u> CATTGG
AKT2-r	GCC <u>CGAATT</u> CTTAGAGTTCGACTGCAGAT
AKT3-f	GCC <u>CTTGATCA</u> TGCTTAACCGGATTCTTTA
AKT3-r	TGC <u>CGAATT</u> CTCAGAGCTTGGCTTGGAAAG
AKT1-r2	TC <u>CTGATCA</u> CTACGACAGCCTGCCATTGACTT
AKT2-r2	GCC <u>CGAATT</u> CTTACTGCAGATATTCATCCAAG
AKT3-r2	AAC <u>CGAATT</u> CTCACTTGGAAAGCCAGGCCAACTA
PEX6-f	GCCAAAGGGCATGAAGAACCG
PEX6-r	AGGTACAGCATCTTGTGAA
PEX6-6	GTCTTGACCTGCCCTCCTAT
PEX6-9	TCTGTAGCGAAATGGTCG
AaPEX6-f	<i>GAAGTTATCAGTCGAAGAACGCGTCTGTACGTGAGT</i>
AaPEX6-r	<i>ATGGTCTAGAAAGCTAGACTTGAGCGCGATATCAC</i>

^a The initiation and termination codons are indicated as bold letters. Restriction enzyme sites added are underlined: TGATCA, *BclI*; GGATCC, *BamHI*; GAATTC, *EcoRI*; CTGCAG, *PstI*; CTCGAG, *XbaI*. AaPEX6-f and AaPEX6-r have 15 bases (italicized) identical to sequences at the ends of the linearized plasmid pDNR-CMV. The linearized plasmid and the 6.5-kb AaPEX6 fragment were subjected to the In-Fusion cloning reaction.

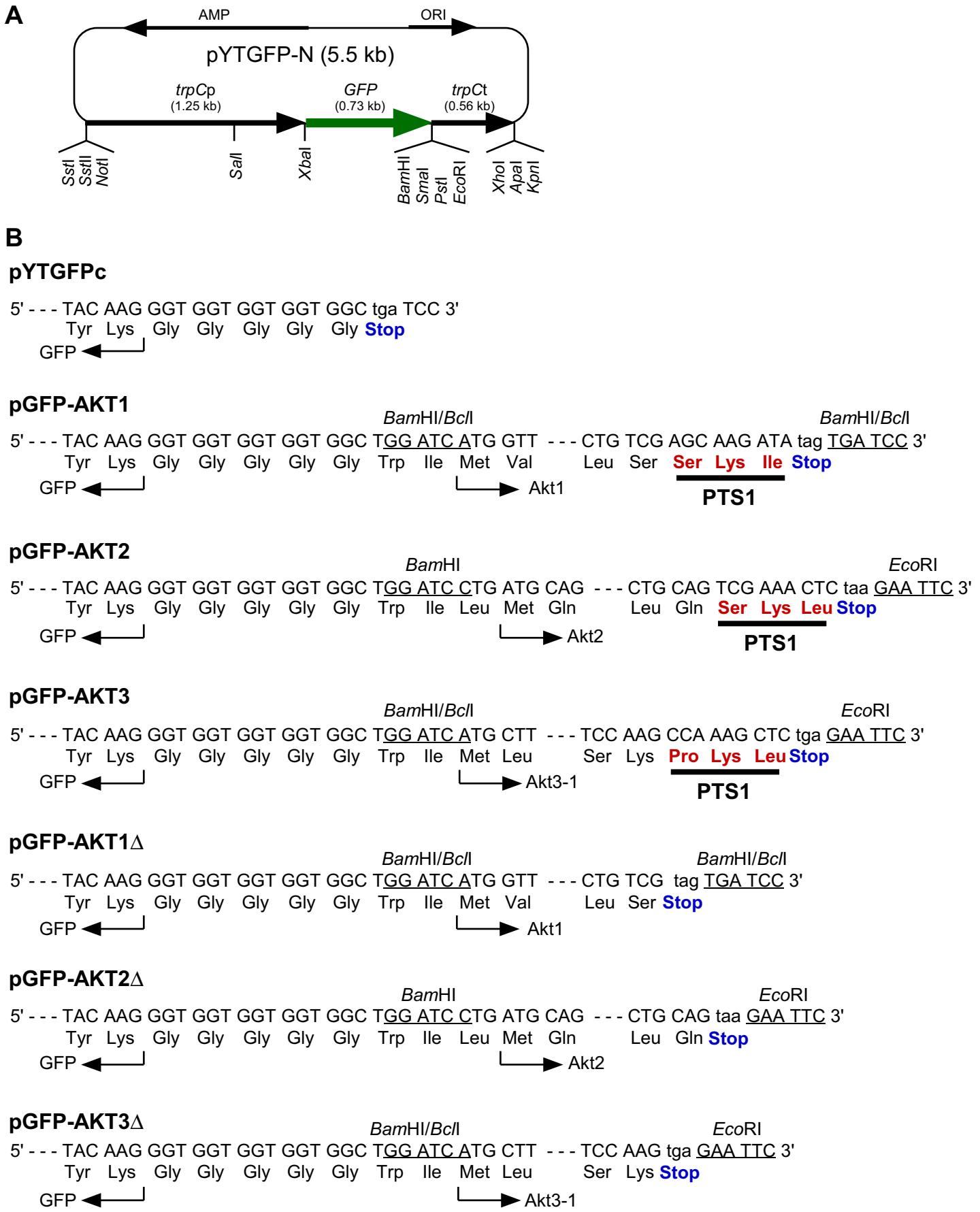


FIG. S1. GFP-fusion protein expression vectors used in this study. (A) The plasmid pYTGFP-N used for construction of GFP-fusion protein expression vectors. (B) GFP-fusion protein expression vectors. The pGFP-AKT-series vectors were constructed by inserting the *AKT1*, *AKT2*, and *AKT3-1* cDNAs downstream of *GFP* in pYTGFP-N. The pGFP-AKT Δ -series vectors contain the mutated *AKT1*, *AKT2*, and *AKT3-1* cDNAs, of which nine nucleotides encoding peroxisomal targeting signal type 1 (PTS1) sequences were eliminated.

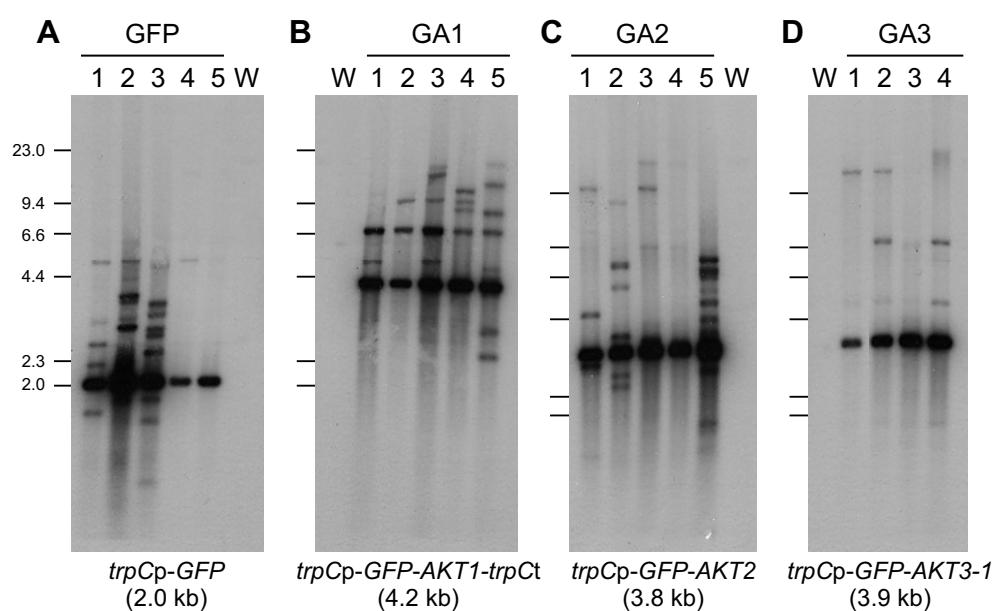


FIG. S2. DNA gel blot analysis of transformants. Total DNA was digested with *NotI-EcoRI* (A, C and D) or *NotI-KpnI* (B) and fractionated in 0.8% agarose gels. The blots were probed with the *GFP* sequence. Sizes (in kilobases) of marker DNA fragments (*HindIII*-digested λ DNA) are indicated on the left. W, wild-type strain 15A; GFP, pYTEGFPc transformants; GA1, pGFP-AKT1 transformants; GA2, pGFP-AKT2 transformants; GA3, pGFP-AKT3 transformants.

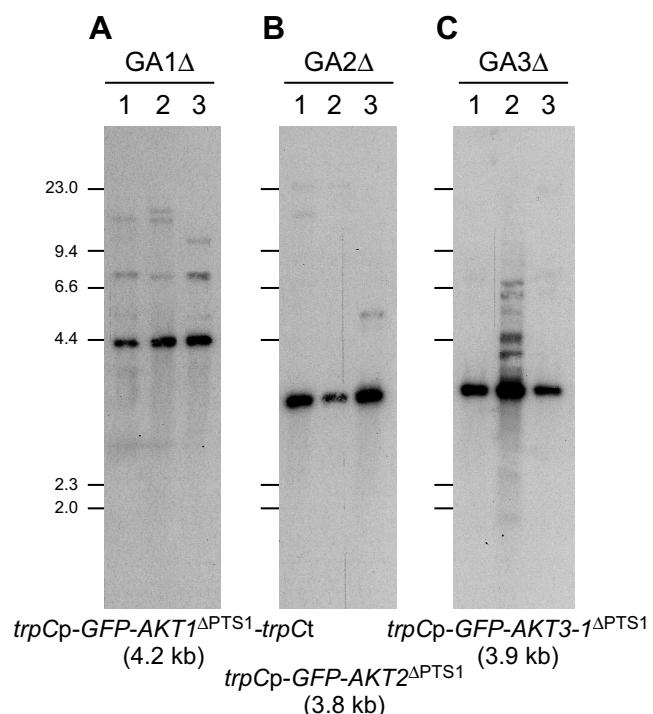


FIG. S3. DNA gel blot analysis of transformants. Total DNA was digested with *NotI-KpnI* (A) or *NotI-EcoRI* (B and C) and fractionated in 0.8% agarose gels. The blots were probed with the *GFP* sequence. Sizes (in kilobases) of marker DNA fragments (*HindIII*-digested λ DNA) are indicated on the left. GA1 Δ , pGFP-AKT1 Δ transformants; GA2 Δ , pGFP-AKT2 Δ transformants; GA3 Δ , pGFP-AKT3 Δ transformants.

AaPex6	-----MDAQMGVVHAKKRRRRRLDRPPIASARLILDDRIKE-EVGVLSEDLFMDLPGGSRSRASAANGQITPDSEHRWQHVAITPWLPSSIVASOMTPUTILPVRPADKTEGNTARPSS	: 112
MoPex6	-----MDPAQCTRKRFRRRRQQDPIASARLALDDHVR-DVGLSLEDLFADLFPHLS-----	: 99
CoPex6	-----MTTSELVP PPPPARSPFTRRRRQDKPALSARLVLDDHEINGDVGVLSEDLFIDLFPHLN-----	: 111
PcPex6	MD FEQYQGSSQQPRQRRAKGKRLLMKPIAARLALPQLRG-KVGILSEDILANDLHQOQALQ-----	: 113
ScPex6	-----MKASLTFSLSGLYAPCSISRDIVLEYGDKKAECHYGTIRLPQYGP-----	: 83
AaPex6	LQIPASSLALQSFSHILONIAAPNKTQRD-TPIEVRIDDVWPVGLDTWVNLDTEAELKLDEWHAKFGGGGTTQMGCAASKSRLEANGTKGKAVDLAQEEWVRQAVREALQLPTWLH	: 231
MoPex6	ASA-----LALQIIFATSLQWAPSLSKLSHSRSRGVILRVDLALASLDLSDTVFVSLDGEELAKRLEHGEWVHREHF-----IGKCSATPP-----	: 197
CoPex6	S-----LSQLSFATILOQVAPSLSKLSHSRSRGIEWQILDVVVALSSTDYVSEELDRKPLQEGCITFDRPMKGKKAQPOD-----	: 210
PcPex6	VLFPEFADSLSOPFLQALGRKDSSRMSLQAHRSEWIRLDLTDIETVTEHRLFNMHDWOTKFGGGFTNAQGPNGLGKGTGKSVEAKYSKRAAADAEORLTAAREALGAQRIWH	: 233
ScPex6	-----FCYFEPILDNWVWPVLDSTYFLIMEQLYSKLMDLP-----	: 128
AaPex6	TGDLPLPKLPAHPITHVPPFPKISASEPVSQGLLPDTRIVLQSHRSSSKQAVLFPVKQPATITNGFGDDDETDDTSNDIFYSAEADNSRNAPDDEESTDNFESELESADGLLSE	: 351
MoPex6	SGDLFLPLPPHPITHVPPNPCKVTLCEPVAQGILSPNTKIIIVSSGR-----SPLGNRDRGSRQSSRMLDGVAEADED-TANDAFSAAEFYKTEASETADESAAITSASETELSSGDIDDL	: 314
CoPex6	SGCDLFLPLPPHPITHVPPNPCKVTLCEPVAQGILSPNTKIIIVLMLRGR-----VHAARGQSAAPAIQPMESLNGWPEDEDDTTANDQFYSAAEDRYKTDAAITMDTWIPTEEEDLSGWDHDDL	: 328
PcPex6	TGDVFLPLPLPSHPITYAPPBPAPRISFCFEPWSQGLLMSITKIVLWQAR-----POCIRAAQTMPSPRSALIKQVAEDEADDTSNEOFYSAEADKPGESCTEMEVTSAAPESE-TECSAGSMSDS	: 349
ScPex6	KYNINSMETVWHSRDLTSLCQLDNCPSPFPQGLWDFETQLILWMD-----	: 203
AaPex6	S----DDIISLMAPMLPPQSSGVLSFSITATPPRPNWFR-INGIATPGSWYSSFTATTARGPTSKSFKQAGLLSVPPDIWHPKPGESEDDEFEARVFDINTLWVKGCFSGDUWKLEGTE	: 466
MoPex6	SDDSDDMMISLQTPMLPSNLSAQSGLTAQACTPMTIGRKRTNGATPGSVFSSFTATTARPDRPRGLFLKAQGLWPKIPPELLHPKFSAEDDEAHYIWVWSHSLRIGCFSGDUWRVEASA	: 434
CoPex6	SDDSDDMMISLQAPDLPITIASGVSITMOPCIPMTIGRKRTNGIATPASVFSNFTATTARPDRPRGLFLKAQGLRPLIDLLHPKFAPEDDEEARIFWIDASLISKIGCFSGDUWRVEEAE	: 448
PcPex6	SDDSDDMMISLQAPDLPQPSGVQDSSLTSATPRAGGR-SDGIEHTPGSWASNTSAWPRPGGGKFQKWEGLLQQWPNEWLHPKPERDDWDSDWVFDISTLAKIGCFSGDUWRVEIAAE	: 468
ScPex6	S-----IDLESLPCTISRDLLRPAEHINDDNNSIYAFIDIAETLRLDVIESGSFITWSNMG-----	: 257
AaPex6	APSSHPLUGLCAFGRAD-EEENTWURPWERVFGLPENNMSKQWARYPVQKTSLDLRSSESGFSQPA33LHAYLSPILLAMICSPSLLRIPPLTPSQS-SGLPRSAIQKPKPLNSSLPPSKA	: 584
MoPex6	APPANGFPPALGSGIFPDAQEWPURPARVFGLPEGYSNMRPWSRIPSSKHRGRSLSFESQVOKSCLPQASAPILLAMIDLSPSLRLLPLKRTIP-HHNGQPSQ-----ATAIVQPYYAR	: 550
CoPex6	EPPANGFCAFGLGSGFTSLESTEWSNURPWRVYGLPEGYSRWPVIRPSSKHRGRSLSFESQVOKSCLPQKPTSPATYISPWLAMLESPSYRLSPKIRGTI-YQCKGTLPK-----FTSASRPPYAR	: 563
PcPex6	EPQLNNFASLKFGSMNDSPDSDGMWRPKWIFGSLCPSPKPRYAIN--HSGERRPSISQRPPTRITPSVFPPLLLGMIEMPKYLRISMTFAFPNGSSKPGILQHMKMTAAKMPPIAK	: 585
ScPex6	-CVRLKVLKFVLLPNG-----FKKRIIIVAPPKIIASFPDCSVVIIISKNIGHT-----DIPIAN	: 310
AaPex6	EVTLLKVSTPLSSDRLLQPSLFAALEDHFEQKRRIVKSGDLIGVSIDESLGRAVFLQTTEDVGNDELLARAKPTASDESTSVSSRNPKWWSUFKIGWSSPHTSHGEEWVLIWGGAVT	: 704
MoPex6	GWWWWQWRTPLTSSKDVVEAVIAGLKWHFEEKKLRLVIKAQDLDIAVPIDTQGLGTIRETSNGEGSAIDNLSAESSMSGTAASRRLKYEDEVAUFWKVSHVQTQKODAAD-QDEDLWGSAAAC	: 669
CoPex6	DITIOWHRSPTAERAYOSAVLGLLKRYPQKIRLWRTGLDIAVPIDTQGLGTIRETSNGEGSAIDNLSAESSMSGTAASRRLKYEDEVAUFWKVSHVQTQKODAAD-QDEDLWGSAAAC	: 674
PcPex6	EVTLLKVSTPLSMDRWLQPALFAGLKLQYFESRRLILKSGDLVGJISVDEGLGRAVFTSG-TGSCDSASQEDEDITIRLQGQANATNAQTKICWAVFWGQWAPTIVEELFTGEDQWGGWAV	: 704
ScPex6	QWF13FWGGGUQSQKCFQNIILITLKKFSESESKRILCQMDLIPFQSSMADLNIAEENDS3DDEDELQGYKKMS-----LVWFWTSAELDCFSKDNHSFIIIDPNRTKL	: 416
AaPex6	VDAASTKMEMMGSEQCKTPAPISNAWQYQYLGAQKRAPWSSS-QKTMALDELPKB--YISSLRRRLREIMSVATSPrAIHLGLPPIAWLITSTQRCIGKSTWATKACEDLGHLTFSIDADDI	: 821
MoPex6	VEITIIMAHFESSEGMVNGRWPWDKSMWPPYYGLVLPPIPNNPGSDSLPVAICEPARK--HISALLRRRMPSSLLAAATSPrAIHLKAPAVAILWSTORNIGKATLATDTCRDIGLHTFTIMAYDI	: 787
CoPex6	IDSSSEVAMHGCGCATSRTPATKASTWPPYYGLVWKPMEKNCASALMWPQDQR--FWSPLRRRLRELLAAATSPrAIHLKMPVAILLTSTERNIGKATLASeACSDIGHTYDAYIDI	: 792
PcPex6	IDDPATIEMVQAGSDWSRVPWCWLGNQMEYOLGVKTIIPKTVHDAPTPHGWADPQSVIPLQOPIRDLMSAATSPRAIQLGKWPVILLRSQQRHIGKATWATRACSDIGHTFIDAYIDI	: 824
ScPex6	ITTMNIDMRRPLPLSRSNLNQRYYQHAEFTFYDLDLHIFPYVRQLVNILETSFNCSQ-----RQITLNAS-----VLLHSTINWQGKAMWRFASKEYLGIHLLIEDCLSL	: 512
AaPex6	VTEGGAGGGDWKTEGFLKARAERALTCGAETTALLWVHDALIADRMTALKIELADSR-----WLIATITTEIDKVPEGIRGLFTHEIEMTAPDEGEREGLIRSI	: 921
MoPex6	IMEGGCCGSDWVKTDCILKARAERALSCGPFCAALLIRHTEALTADRMTASLRSILSMDP-----WLIATITTEIDKVPEGIRGLFTHEIEMTAPDEGEREGLIRSI	: 887
CoPex6	LSEACTGGGDWVKTCHLRTDSERAMSCGPDTTALLIKEWALTEALTRDMVSTMKEILODTR-----WLIATITTEIDKVPEGIRGLFTHEIEMTAPDEGEREGLIRSI	: 892
PcPex6	ITEGGAGGGDWKTEAFLKARAERALFHGCANCTALLIRHIEVLTADRLVIAAMSIDLILMDA-----WLIATITTEIDKVPEGIRGLFTHEIEMTAPDEGEREGLIRQMA	: 924
ScPex6	TENSRQLDSTSIIIGHTRAKCENVLPY-ASPAVIFLAELDSILLDNIAQDPEAIKLQKSINFEMSKLDDFTFKPGTTFVGSVNNIDWPSSEFRSHIRFEILVVPSEAFQRLRFQWY	: 631
AaPex6	WDDAGIRLSPFWDLIAWAKTAALVAGDLWDVWVDRALVAKQORLEALAWSASKSLTP-PDTIITRDEELACCHFSNSLTKADLDTAVDAARKNFADAIGAPKIPMVGMSDVGGLTHWKDA	: 1040
MoPex6	WDQRGIMLDPCWDLGWALKTAALWAGDLWDVWVDRALVAKQRLRLEQISSKTG-----QAVTVDLQVAGGAMARCVTKGDFDVAEEAARKNFAGAIGAPKIPNVTDWVGGLNNVKDA	: 1007
CoPex6	WEDRGINLDPEDWLDLGIALKTAALVAGDLWDVWVDRALVAKQRLRLEQISSKTG-----QAVTVDLQVAGGAMARCVTKGDFDVAEEAARKNFAGAIGAPKIPNVTDWVGGLNNVKDA	: 1005
PcPex6	WTGIRLISADWDLCSIALKTAALVAGDLWDVWVRAAGARTARLESLAEASKWISGS-----EVFVRDWLLAGGDCARGVTKADFDAEEAARKNFADSIGAPKIPNVTDWVGGLNNVKDA	: 1041
ScPex6	LSSHELMRDWQKQVPSVSYMDNISFSSLSSYSSAGLTPLDIPIKSISETARMTIATARFYQE-----SKRCGULPQSILITQEDLSKATSKARNEFWSVSIGAPQIPWVTDIDIGTIDFWGCE	: 743
Walker A		
AaPex6	WMETIQLPLPRLPELFAKGKMKRSGILFYGPGTGKTLIILAKIAATEFSLNFFSVKGPELLNMNYIGESEANVRRVFQKARDARPCAVFFDELDSSVAPKRGNGQDGGVMDRIVSQLAELDG	: 1160
MoPex6	WMETIQLPLPRLPELFAKGKMKRSGILFYGPGTGKTLIILAKIAATEFSLNFFSVKGPELLNMNYIGESEANVRRVFQKARDARPCVFFFDELDSSVAPKRGNGQDGGVMDRIVSQLAELDG	: 1127
CoPex6	WETIQLQLPRLPELFAKGKMKRSGILFYGPGTGKTLIILAKIAATEFSLNFFSVKGPELLNMNYIGESEANVRRVFQKARDARPCVFFFDELDSSVAPKRGNGQDGGVMDRIVSQLAELDG	: 1125
PcPex6	WETIQLQLPRLPELFAKGKMKRSGILFYGPGTGKTLIILAKIAATEFSLNFFSVKGPELLNMNYIGESEANVRRVFQKARDARPCVFFFDELDSSVAPKRGNGQDGGVMDRIVSQLAELDG	: 1161
ScPex6	ILDIDMPLKPELITSGMKKRSGLFYGPGTGKTLIILAKIAATEFSLNFFSVKGPELLNMNYIGESEANVRRVFQKARDARPCVFFFDELDSSVAPKRGNGQDGGVMDRIVSQLAELDG	: 863
Walker B		
AAA-protein signature		
AaPex6	MSDG---GEGFWVIGATNRPDLQDQLALRPLPGRFDKMLYLGVSDTHEKOQITILEALTRKFTLHPSVSLARWSQGLPTTYTGADMYALCSAMLKAITRSKASWDEKWAAMST---HNPI	: 1274
MoPex6	MSSGDDSGGGFWVIGATNRPDLQDQLALRPLPGRFDKMLYLGVSDTHEKOQITILEALTRKFTLHPSVSLARWAEKLPFTTYTGADFYALCSAMLKAITRQASHWDKIKAMSAE---RQEI	: 1244
CoPex6	MSSGDDSGGGFWVIGATNRPDLQDQLALRPLPGRFDKMLYLGVSDTHEKOQITILEALTRKFTLHPSVSLRHSVAQQLPFTTYTGADFYALCSAMLKAITRQAAQVDKIREEAQPRSRTGII	: 1245
PcPex6	MNGGEEMSGGGFWVIGATNRPDLQDQLALRPLPGRFDKMLYLGVSDTHEKOQITILEALTRKFTLHPSVSLRHSVAQQLPFTTYTGADFYALCSAMLKAITRQAAQVDKIREEAQPRSRTGII	: 1275
ScPex6	MSTD---ADGFWVIGATNRPDLQDQLALRPLPGRFDKMLYLGPIDTDTKQNLILEALTRKFTLHPSVSLRHSVAQQLPFTTYTGADFYALCSAMLKAITRQAAQVDKIREEAQPRSRTGII	: 977
AaPex6	TIAYFFDHATEEDIAVMVIEEDFMEAHFLVPSVSADELRLHYDRVNRQKQFEGAGKKGKREGAETKNGIAAAPLATEQAFSATTAKGRKAKPWHEDETEMVIRTQVNLQNGAGASGNGMLAGEG	: 1394
MoPex6	TIAYFFDHATEEDIAVMVIEEDFMEAHFLVPSVSADELRLHYDRVNRQKQFEGAGKKGKREGAETKNGIAAAPLATEQAFSATTAKGRKAKPWHEDETEMVIRTQVNLQNGAGASGNGMLAGEG	: 1325
CoPex6	STAYFFDHATEEDIAVMVIEEDFMEAHFLVPSVSADELRLHYDRVNRQKQFEGAGKKGKREGAETKNGIAAAPLATEQAFSATTAKGRKAKPWHEDETEMVIRTQVNLQNGAGASGNGMLAGEG	: 1339
PcPex6	STAUFFFDHATEEDIAVMVIEEDFMEAHFLVPSVSADELRLHYDRVNRQKQFEGAGKKGKREGAETKNGIAAAPLATEQAFSATTAKGRKAKPWHEDETEMVIRTQVNLQNGAGASGNGMLAGEG	: 1395
ScPex6	STERUFDKIAKTEDTKWVWQEDFLKQAPQQLTPSVSRAELMHYEAVRANFEGA-----	: 1030
AaPex6	VDISGGSAASSGGSSKGKKGKAPWHSQGSSVDAEAGCFFGDTAQDDDIYS-----	: 1444
MoPex6	GKKGKKGKIAASSSGGEEEDRQDGWNKGKKGKKGKAVAGSGTGSQGDDDEGLYD-----	: 1375 (53%)
CoPex6	GKKGKKGKIAASSSGGEEEDRQDGWNKGKKGKKGKAVAGSGTGSQGDDDEGLYD-----	: 1388 (52%)
PcPex6	STTSSEGGKGKGSVSKKGKSRGAESDGSTVDDDEMDADSKEDDEDWVVRTDHLRMPMEEVE	: 1459 (50%)
ScPex6	-----	: - (30%)

FIG. S4. Similarity of AaPex6 to Pex6 proteins from other fungi. The amino acid sequence of AaPex6 of *Alternaria alternata* was aligned with those of Pex6 proteins from *Magnaporthe oryzae* (MoPex6; accession no. ABG79929), *Colletotrichum orbiculare* (CoPex6; accession no. AAK16738), *Penicillium chrysogenum* (PcPex6; accession no. AAG09749), and *Saccharomyces cerevisiae* (ScPex6; accession no. P33760-1). Amino acids that are conserved between any of two proteins are indicated as white letters on a black background. These proteins contain Walker A and Walker B motifs and AAA-protein family signature motif.

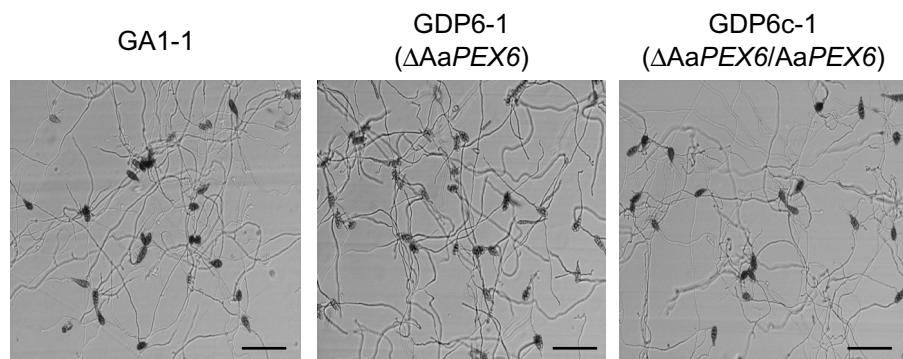


FIG. S5. Conidial germination of Δ AaPEX6 strain. Conidial suspension of each strain was incubated on glass for 24 h. GA1-1, GFP-AKT1 expressing strain made from the wilt-type strain 15A; GDP6-1, AaPEX6-disrupted transformant made from GA1-1; GDP6c-1, AaPEX6-complemented transformant made from GDP6-1. Bar=100 μ m.