

**Figure S1. (A) The conservation pattern of the BcStc3 sequence calculated by Con Surf server.** Amino acid residues are colored turquoise to bordeaux according to the conservation scores. Green boxes indicate the two conserved magnesium binding motifs. Orange box denotes conserved effector triad. Blue box indicates conserved R and Y residues involved in substrate binding. Red box indicates H1 $\alpha$ -loop ending at the conserved asparagine (N329) residue. **(B) Position-specific evolutionary conservation scores of each residue of BcStc3.** Scores were calculated with AL2CO (<http://prodata.swmed.edu/al2co>) for an alignment of 160 BcStc3-like proteins downloaded from NCBI and aligned with Clustal Omega (<https://www.ebi.ac.uk/services>). Conservation indexes were averaged in windows of seven residues to smooth the graph. The conserved residues of the canonical motifs of the class I terpenoid cyclases are indicated and colored according to the boxes in (A).

1  
MNLV MKANGN  
e e e e b b e e e e

11  
GSLKNVPADL  
e e b e e b e e e b

21  
DNFTNGKNAD  
e e b e e e e e e b

31  
TICPIRYIYD  
e b b b b b b b b e

41  
YLPQKPKWLQ  
b b e e e e e b b e

51  
AQQVTLNDLL  
b e e b b e e e e b

61  
QLQSV EIQGN  
e b e e e e e e e e

71  
TRKNRIAIYP  
e e e e b b e b b b  
f s

81  
GAAHLPVHTG  
e e e e b b b e e b  
f f

91  
LGNFRQSKHW  
b e e b e e e e e b  
f f f s

101  
KANERATREL  
e b b e e b b e e e

111  
LELFAQDQHC  
b e b b e e e e e b  
f

121  
KDAMLTNGQS  
e e b b e e e e e b

131  
MATLAKRQLR  
b b b b b e e e e b  
s

141  
SPVLDTYSRF  
e e e e e b b e e b  
s

151  
SIYMFADANE  
b b b b b e e e e e  
s f

161  
NRIQLLAQSV  
e b b e b b b b b b  
s s

171  
ILIFIFDDMW  
b b b b b b b b b b  
s s s s

181  
ENASSKFTSQ  
e e e e e e e e e e

191  
VRDDFIGRIQ  
b e e e b e e e e e  
f s f

201  
GNVSKSIADT  
e e e e e e e e e e  
f

211  
PLQRRMHEIR  
e b e e b b e e b b  
s f s

221  
QGFLDGDKEE  
e e b e e e e e e e  
f

231  
GGNAGAEILQ  
e e e e e e e b e e  
f f

241  
ELIEFCRHEH  
e b b e b b e e e e  
f

251  
PEGFSSSVREY  
e e e e b e e e e b  
f s

261  
LLYRYDDIAN  
b b e e b b b b b b  
s f f

271  
RFTWACTKFS  
e b b b b b b b b b  
f s f

281  
LHLKVDLRSP  
b e b e b b e e e e  
s s f

291  
KLRRLLGLYL  
e b e e b b b b b e

301  
DQISIANDLA  
e e b b b b e e e e  
f f f f

311  
SYDK EAKQFL  
e b e e e e e e e e  
f f f f

321  
EGRSKEMINL  
e e e b e b b b b b  
f s f

331  
VHTIMQVNRRI  
b b b b e e b b b e  
s

341  
NGVAAAKSMA  
e e e e e b b b b b  
s f

351  
YAIQLWTENE  
b b b e e e e e e e  
f f

361  
ILEELRALSG  
b e e e b e e e e e  
f

371  
QGQLSDEEWK  
e e e b e e e e e e  
f

381  
LVDGCLVAAS  
b b e b b b b b b b

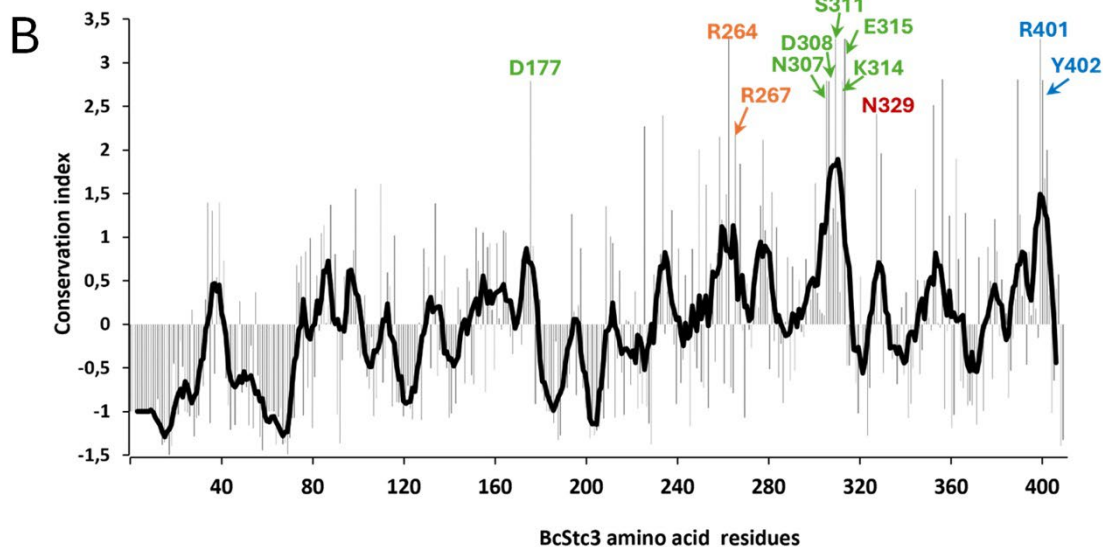
391  
GNLLTSIVIS  
f f s s

401  
RYGGEAKIA  
e e e e e e e e e e  
f f f f

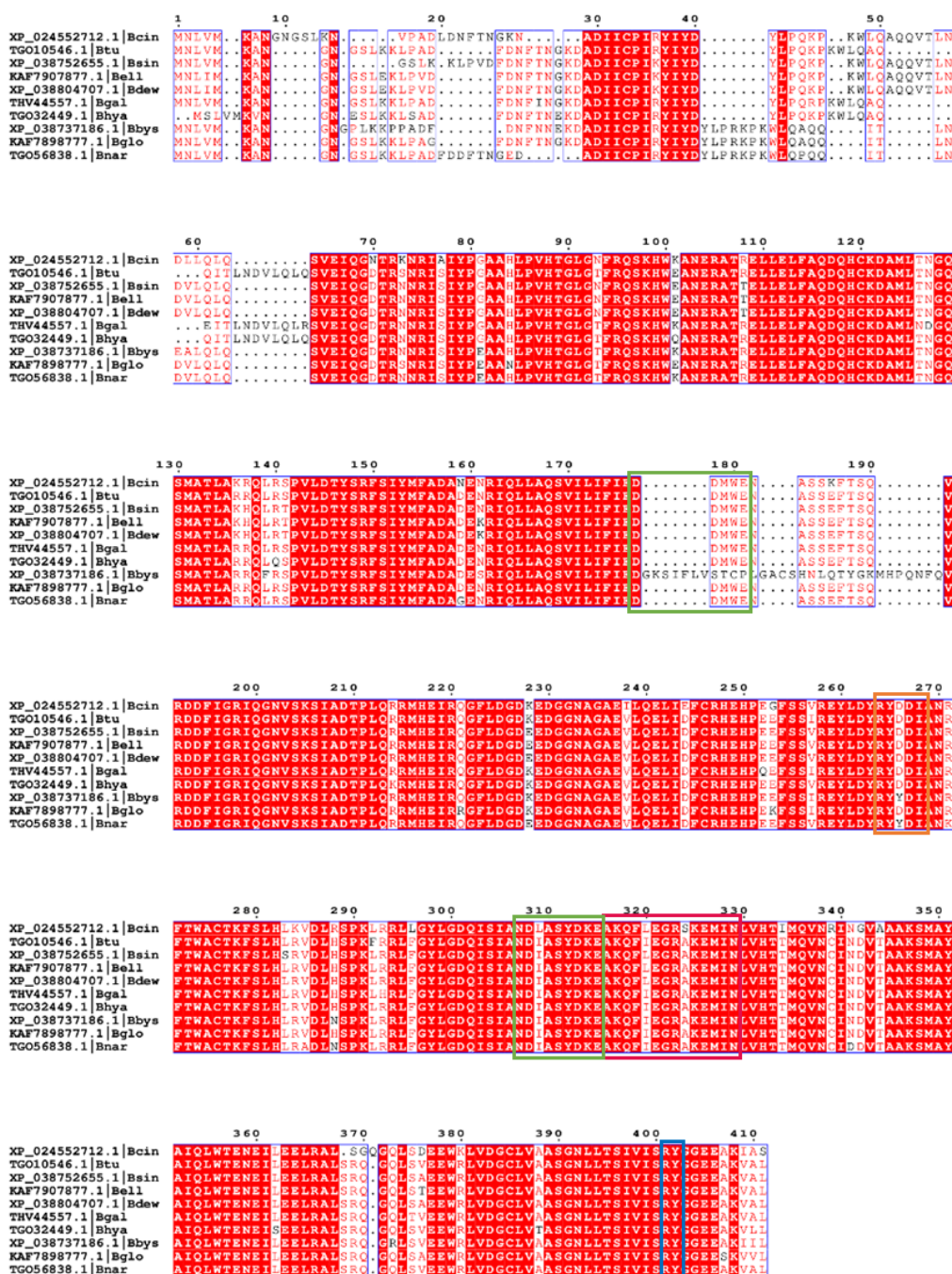
411  
S  
e

? 1 2 3 4 5 6 7 8 9  
 variable                      Average                      Conserved

- e** - An exposed residue according to the neural network algorithm.
- b** - A buried residue according to the neural network algorithm.
- f** - A predicted functional residue (highly conserved and exposed).
- s** - A predicted structural residue (highly conserved and buried).



**Figure S2. Alignment of BcStc3 and homologous protein sequences of other *Botrytis* species.** Protein sequences were selected after running similarity search by BlastP using BcStc3 as query sequence. Multiple alignments of protein sequences were carried out with Clustal-Omega and were visualized using ESPrpt 3.0. Green boxes indicate the two conserved magnesium binding motifs. Orange box denotes conserved effector triad. Blue box indicates conserved R and Y residues involved in substrate binding. Red box indicates H1α-loop ending at the conserved asparagine (N329) residue. Bcin, *Botrytis cinerea*; Btu, *Botrytis tulipae*; Bsin, *Botrytis sinoallii*; Bell, *Botrytis elliptica*; Bdew, *Botrytis deweyae*; Bgal, *Botrytis galanthina*; Bhya, *Botrytis hyacinthi*; Bbys, *Botrytis byssoidea*; Bglo, *Botryotinia globosa*; Bnar, *Botryotinia narcissicola*. NCBI accession number of each sequence is shown.



**Figure S3. Alignment of BcStc3 and homologous protein sequences of other fungi species.** It was selected at least one protein from each of the fungal taxonomic orders in which homologous proteins to BcStc3 had been identified among the first 55 sequences retrieved from the BlastP search using BcStc3 as the query sequence. Multiple alignments of protein sequences were carried out with Clustal-Omega and were visualized using ESPript 3.0. Green boxes indicate the two conserved magnesium binding motifs. Orange box denotes conserved effector triad. Blue box indicates conserved R and Y residues involved in substrate binding. Red box indicates H1 $\alpha$ -loop ending at the conserved asparagine (N329) residue. Bot\_cin, *Botrytis cinerea*; Rutst\_sp, *Rutstroemia sp* (Leotiomyces, Helotiales); Pyc\_pra, *Pycnora praestabilis* (Candelariomyces); Sei\_lac, *Seiophora lacunosa* (Lecanoromyces); Sti\_urc, *Stictis urceolata* (Lecanoromyces); Exo\_mes, *Exophiala m4esophile* (Eurotiomyces, Chaetothryales); Cir\_nov, *Cirrosporium novae-zelandiae* (Xylobotryomyces); Alt\_pan, *Alternaria panax* (Dothideomyces, Pleorales); Neo\_par, *Neofusicoccum parvum* (Dothideomyces, Botryosphaerales); Dia\_amy, *Diaporthe amygdali* (Sordariomyces, Draprhales); Dot\_sym, *Dothidotthia symphoricarpi* (Dothideomyces, Pleorales); Coll\_tam, *Colletotrichum tamarilloi* (Sordariomyces, Glomerellales); Micr\_niv, *Microdochium nivale* (Sordariomyces, Xylariales); Gal\_mar, *Galerina marginata* (Basidiomycota, Agaricomycetes). NCBI accession number of each sequence is shown.



XP\_024552712.1\_Bot\_cin .....MNLVMMKANGNGSLKNVPADL  
PQE05643.1\_Rutst .....MTNMNDVTYNTGKGRHKSILQHT  
KAI19816264.1\_Pyc\_pra .....MIDVTKEIQI  
KAI1482135.1\_Sel\_lac .....MKC .....NDHVHT  
MCJ1446520.1\_Stl\_urc .....PEADEYGPQA  
RVX66215.1\_Exo\_mes .....AVVNGSRNRPAPAPETDRHNLHL  
KAI19735008.1\_Cir\_nov .....MATNGRISSHFELK...QL  
KAG9195858.1\_Alt\_pan .....MTTSTPKFADV  
EOD45492.1\_Neo\_par .....MAGQNL...QL  
XP\_056580837.1\_Pen\_con .....MLPPPTVESVN  
KAI1247300.1\_Eut\_lat .....MPSQFLKYADV  
XP\_052986662.1\_Dia\_amy .....MPSSELTLSFVN  
XP\_033521855.1\_Dot\_sym .....MPTSKLTSSSVH  
XP\_060379205.1\_Coll\_tam .....MPSKLSVSSVN  
KAI1323763.1\_Micr\_niv .....MEASQL...QL  
KDR73888.1\_Gal\_mar .....MDM

XP\_024552712.1\_Bot\_cin DNF TNGKNADITCPTRVYDYLPQKPKWLQAQQ.....VTLND...LLQL...QSV.EIQGNTRKNRIATVFGA  
PQE05643.1\_Rutst PQE05643.1\_Rutst VSLDD...VLEL...DFT.DIOGSTKENGVISFGA  
KAI19816264.1\_Pyc\_pra NKLAAGADLIDLCPIRYVYDYLPFGTTFLKHP.....VLPERSFINTMKE...DPT.LSQDNTKVHSIALYFEE  
KAI1482135.1\_Sel\_lac SAUNGSADGNTLCPIHYVYDYLPFTTAWTLPRP.....VSLSSIWPNDDA...WPALPYAEAGKNSFGIHAHA  
MCJ1446520.1\_Stl\_urc ENTENRSDDEVVFCPIKRYDYDYLPKQGTAGWFKPRQ.....LGIPASIRQQLDA...QPL.EIQLHTRMNSLTQNSD  
RVX66215.1\_Exo\_mes DALILHDDRSHLFFPIFCYDYDYLPKQGTPIAST.....TEF.....DS...PFHVIEKNRGAHSIALYFPLW  
KAI19735008.1\_Cir\_nov SKLADPENEGSVCPVIFSWDYDLPMSFSLITQD...WTFENP...DQNR.....T.DDKTYKNAPFIYFKA  
KAG9195858.1\_Alt\_pan SKLAAPEDAPWLCPIVRFSDYDLDHATSEHNPNAPLQYSLELP...SPDPGSGGVKLSSTFKQVITSYNNAGFIYFSE  
EOD45492.1\_Neo\_par DKLAAPENVADVIFVFSWNTIPIRLPSPFIVAH...WSFELP...DANKR.....EAVDSFRMSFIFYFKA  
XP\_056580837.1\_Pen\_con AALAPPKNRDRSRVRYVSLNLEIPKLPQOLEYTVVPGKRS...VKSQRHETFTNAFTLDVFA  
KAI1247300.1\_Eut\_lat YNLVAQENAPWLPVRFAYDLDQARSKYLVAPPLYVAPESL...SLSDPPGDKCAIGVLEGGGRTISGDDAGFIYFAR  
XP\_052986662.1\_Dia\_amy SHIASQDNCKDVSRISSYSDYDLDKDVSAAYLTCKPPESGYRR...SSRSSTPS...TDESETIVDVNGFVSYPRE  
XP\_033521855.1\_Dot\_sym SKICAPENVEELSAISYAYNWLVDAPTYLSTPPPAITEYOELVOPGTPSSN...GKQKTGDINAFVDFPRA  
XP\_060379205.1\_Coll\_tam GNICAPENLQDVSOIAYAYNWLHDAPYLDLTSLPLAGYER...QOCTPSTD...GEDEPADVNGFIYFKA  
KAI1323763.1\_Micr\_niv GEITAAEELKNVPIVIFSWNYMRLPAFLAAKD...WNFENPVS.DR...AADPFGNSFAFLFVR  
KDR73888.1\_Gal\_mar KKLNSPENDSSLWTFYNYDYMGSCPPDSKFSS...TAQM...PATVNANVDLAS.LDRLEETNPTIKNSFOLRVE

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PQE05643.1\_Rutst ANIPVHTGLGTBRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMAALARRLOQSSVDTYSRFSIYMFADANEDR  
KAI19816264.1\_Pyc\_pra ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
KAI1482135.1\_Sel\_lac ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
MCJ1446520.1\_Stl\_urc ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
RVX66215.1\_Exo\_mes ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
KAI19735008.1\_Cir\_nov ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
KAG9195858.1\_Alt\_pan ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
EOD45492.1\_Neo\_par ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
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KAI1323763.1\_Micr\_niv ANPLHTELEKLRQSKHKWKAERATRELELEFAOQHCCKDAMLTNGOSMATLAEQOLNSSVLDYSRFSIYMAPADEKRR  
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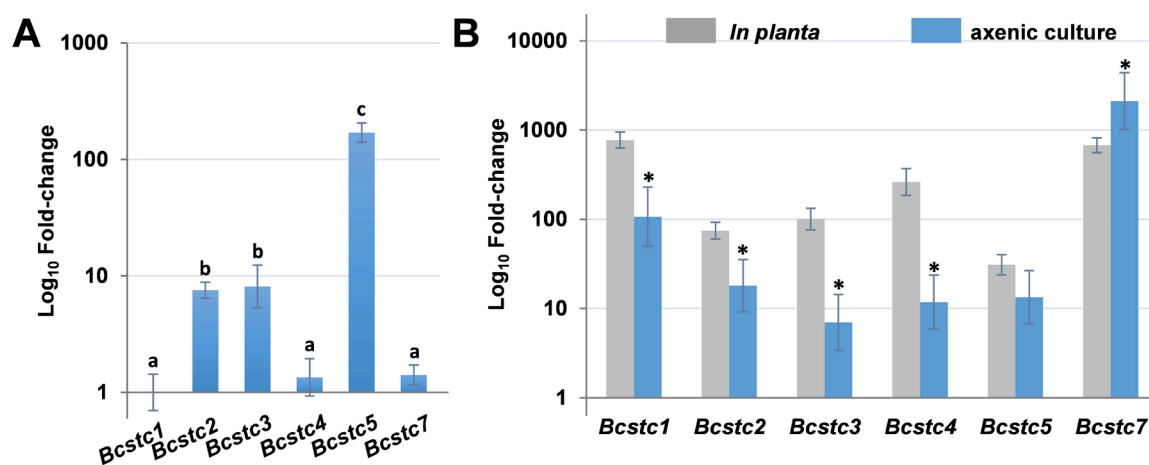
XP\_024552712.1\_Bot\_cin IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
PQE05643.1\_Rutst IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAI19816264.1\_Pyc\_pra IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAI1482135.1\_Sel\_lac IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
MCJ1446520.1\_Stl\_urc IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
RVX66215.1\_Exo\_mes IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAI19735008.1\_Cir\_nov IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAG9195858.1\_Alt\_pan IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
EOD45492.1\_Neo\_par IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
XP\_056580837.1\_Pen\_con IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAI1247300.1\_Eut\_lat IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
XP\_052986662.1\_Dia\_amy IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
XP\_033521855.1\_Dot\_sym IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
XP\_060379205.1\_Coll\_tam IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KAI1323763.1\_Micr\_niv IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG  
KDR73888.1\_Gal\_mar IQLLAQSIVLIFIEED...MWNASSKFTS...QVRDDFIG

XP\_024552712.1\_Bot\_cin RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
PQE05643.1\_Rutst RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
KAI19816264.1\_Pyc\_pra RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
KAI1482135.1\_Sel\_lac RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
MCJ1446520.1\_Stl\_urc RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
RVX66215.1\_Exo\_mes RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
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KAG9195858.1\_Alt\_pan RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
EOD45492.1\_Neo\_par RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
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XP\_060379205.1\_Coll\_tam RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT  
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KDR73888.1\_Gal\_mar RQGNVSK...SIADTFLRRMHEIRQGLFDCQKEDGNAEILQELIEFERBHF...EGFSVREYLDVYDDIANRFT

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PQE05643.1\_Rutst WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
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KAI1482135.1\_Sel\_lac WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
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RVX66215.1\_Exo\_mes WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
KAI19735008.1\_Cir\_nov WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
KAG9195858.1\_Alt\_pan WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
EOD45492.1\_Neo\_par WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
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XP\_033521855.1\_Dot\_sym WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
XP\_060379205.1\_Coll\_tam WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
KAI1323763.1\_Micr\_niv WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI  
KDR73888.1\_Gal\_mar WACTKESLHLLKVDLRSPKRLRLGLYDGOISINDLASDYREKAKQFLEGRSKEMINJVHTIMQVNNRITGVAAAKSMAIAI

XP\_024552712.1\_Bot\_cin QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
PQE05643.1\_Rutst QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAI19816264.1\_Pyc\_pra QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAI1482135.1\_Sel\_lac QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
MCJ1446520.1\_Stl\_urc QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
RVX66215.1\_Exo\_mes QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAI19735008.1\_Cir\_nov QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAG9195858.1\_Alt\_pan QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
EOD45492.1\_Neo\_par QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
XP\_056580837.1\_Pen\_con QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAI1247300.1\_Eut\_lat QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
XP\_052986662.1\_Dia\_amy QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
XP\_033521855.1\_Dot\_sym QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
XP\_060379205.1\_Coll\_tam QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KAI1323763.1\_Micr\_niv QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS  
KDR73888.1\_Gal\_mar QWTNEILEELRAISGQGOISDEWKLVDGCLVAASGNLLTSIVISRYGGEAEKATAS

**Figure S4. Gene expression of the *Bstc* gene family in the *B. cinerea* B05.10 strain. (A)** Relative expression levels in non-germinated conidia. mRNA levels were quantified by qRT-PCR, normalized to *actA* and *B-tub* mRNA levels, and expressed as a fold change over *Bcstc1*. **(B)** Relative expression levels in axenic culture and *in planta*. mRNA levels were quantified by qRT-PCR in mycelium grown in YGG medium (axenic culture) and in leaves of *N. tabaccum* infected with a conidial suspension (*in planta*). Values were normalized to *actA* and *B-tub* mRNA levels and expressed as a fold change over mRNA levels of each gene in non-germinated conidia. Error bars show standard deviation of three biological replicates (n = 3). Different letters above the bars in (A) mean significant differences between n fold changes of the six genes ( $p$ -value<0.05). \* in (B) means significant differences between mRNA levels in axenic and in planta and axenic culture for each gene ( $p$ -value<0.05).



**Figure S5. Virulence assays.** Different hosts were inoculated with 5  $\mu$ L droplets containing 2500 conidia of the indicated strain in TGGK solution, incubated in the dark at 20°C under conditions of high humidity and the degree of damage was estimated. **(A)** The damage on inoculated tomato fruits was estimated using a qualitative scale ranging from 1 (very good appearance) to 4 (bad appearance) and results are presented as the percentage of fruits in each disease grade to the total number of infected fruits at 2, 3 and 4 days post-inoculation. **(B)** A qualitative scale ranging from 1 (very good) to 4 (bad appearance) was used to estimate the damage on inoculated grapefruits and results are presented as the percentage of fruits in each disease grade to the total number of infected fruits at 2-, 3- and 4-days post-inoculation. **(C)** Representative images of the lesions produced by each fungal strain on gerbera and **(D)** detached tobacco leaves 4 days after inoculation.

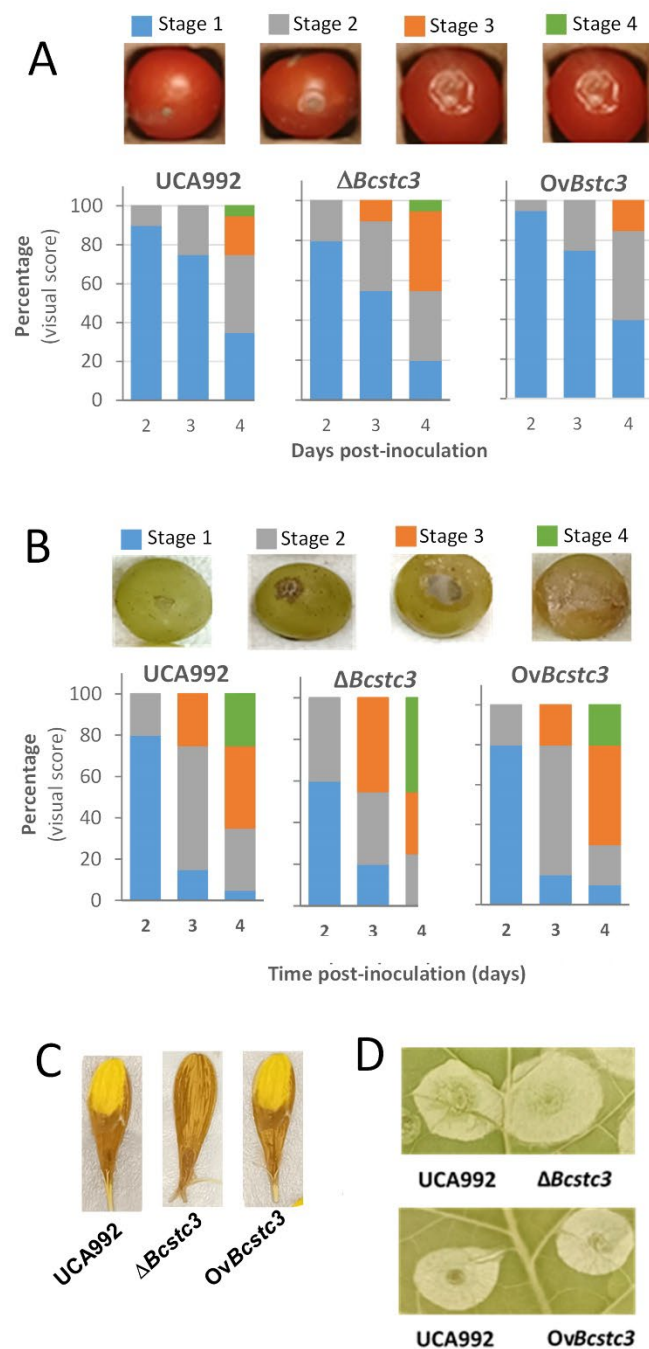
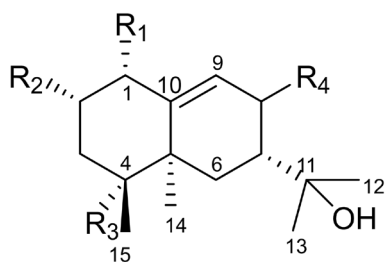
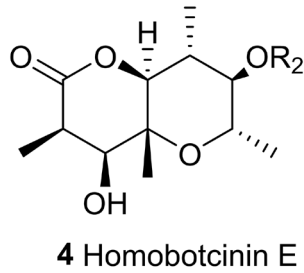
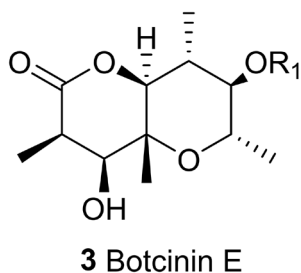
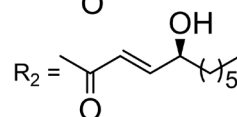
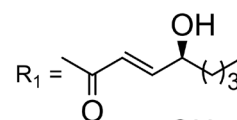
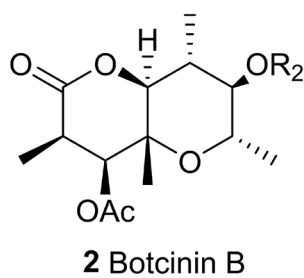
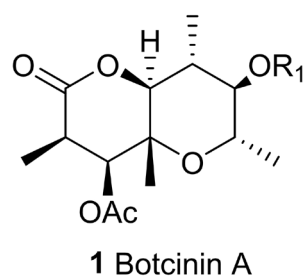
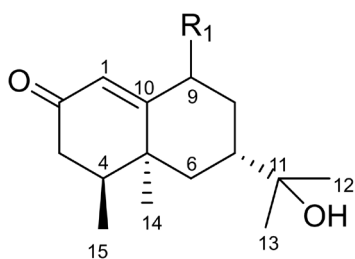


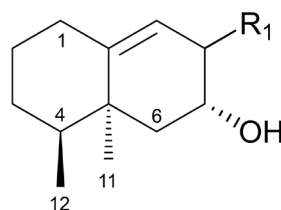
Figure S6. Secondary metabolites isolated from the fermentation of the  $\Delta Bcstc3$  or  $OvBcstc3$  strains.



- |           |            |  |
|-----------|------------|--|
| <b>5</b>  | $R_1 = OH$ | $R_2 = R_3 = R_4 = H$                  |
| <b>6</b>  | $R_1 = O$  | $R_2 = R_3 = R_4 = H$                  |
| <b>7</b>  | $R_1 = H$  | $R_2 = OH$ $R_3 = R_4 = H$             |
| <b>8</b>  | $R_1 = H$  | $R_2 = H$ $R_3 = OH$ $R_4 = O$         |
| <b>9</b>  | $R_1 = H$  | $R_2 = OH$ $R_3 = H$ $R_4 = \alpha OH$ |
| <b>10</b> | $R_1 = OH$ | $R_2 = OH$ $R_3 = R_4 = H$             |
| <b>11</b> | $R_1 = H$  | $R_2 = OH$ $R_3 = H$ $R_4 = \beta OH$  |
| <b>12</b> | $R_1 = H$  | $R_2 = H$ $R_3 = OH$ $R_4 = \beta OH$  |
| <b>13</b> | $R_1 = O$  | $R_2 = R_3 = H$ $R_4 = \beta OH$       |
| <b>14</b> | $R_1 = H$  | $R_2 = H$ $R_3 = OH$ $R_4 = H$         |



- 15**  $R_1 = O$   
**16**  $R_1 = H$



- 17**  $R_1 = \beta OH$   
**18**  $R_1 = \alpha OH$



**Table S1.** Oligonucleotides used in this work.

Primer	Sequence (5' → 3')	Features	Used for	References
<b>Hph-F</b>	GTCGGAGACAGAAGATGATATTGAAGGAGC	<i>PtrpC</i>	Cloning – amplification of <i>PtrpC::hph</i> for $\Delta bcstc3$ construct	[1]
<b>Hph-R</b>	GTTGGAGATTTTCAGTAACGTTAAGTGGAT	<i>Hph</i>	Cloning – amplification of <i>PtrpC::hph</i> for $\Delta bcstc3$ construct	[1]
<b>TrpC-P2</b>	CCTCCACTAGCTCCAGCCAAGCCC	<i>PtrpC</i>	Diagnostic PCR – homologous integration at <i>bcstc3</i> -5'	[1]
<b>TrpC-T</b>	GGAATAGAGTAGATGCCGACCGG	<i>TtrpC</i>	Diagnostic PCR – homologous integration at <i>bcstc3</i> -3'	[1]
<b>Bcstc3-5F</b>	GTAACGCCAGGGTTTCCAGTCACGACGTT TGCTGCCCTTGACGGA	pRS426-5F – <i>bcstc3</i> -5'	Cloning KO construct– amplification of 5' flank for $\Delta bcstc3$ construct	This study
<b>Bcstc3-5R</b>	ATCCACTTAACGTTACTGAAATCTCAA CCCAAGCCGTTAATGATTCG	<i>Hph</i> – <i>bcstc3</i> -5'	Cloning – amplification of 5' flank for $\Delta bcstc3$ construct	This study
<b>Bcstc3-3R</b>	GCGGATAACAATTTACACAGGAAACAGCAT CTGGCGCAATAAGGAGTG	pRS426-3R – <i>bcstc3</i> -3'	Cloning – amplification of 3' flank for $\Delta bcstc3$ construct	This study
<b>Bcstc3-3F</b>	CTCCTTAATATCATCTTCTGTCTCCGACCTAC TGAAATGACTTCTTGACAC	<i>PtrpC</i> – <i>bcstc3</i> -3'	Cloning – amplification of 3' flank for $\Delta bcstc3$ construct	This study
<b>Bcstc3-hi5F</b>	GGAGGACGCATATAGATCCA	<i>Bcstc3</i> -5'	Diagnostic PCR – homologous integration at <i>bcstc3</i> -5'	This study
<b>Bcstc3-hi3R</b>	GTGGAGGAATCTGGACTTGA	<i>Bcstc3</i> -3'	Diagnostic PCR – homologous integration at <i>bcstc3</i> -3'	This study
<b>Bcstc3-WT-F</b>	CTTGCCAAACGACAGCTCCG	<i>Bcstc3</i> ORF	Diagnostic PCR – detection of <i>bcstc3</i> alleles	This study
<b>Bcstc3-WT-R</b>	CCCGTACAGATGAAATCCC	<i>Bcstc3</i> ORF	Diagnostic PCR – detection of <i>bcstc3</i> alleles	This study
<b>PactA-amp-F</b>	GGGGTTGATAAATTAAGACGTTAAGTCTAT TACCGTATTCATTGG	PactA – <i>bcstc3</i> -5'	Cloning – amplification of PactA for overexpression construct and Diagnostic PCR – homologous integration at <i>bcniaD</i> -3'	This study
<b>PactA-PtrpC-R</b>	GCCCCAAAAATGCTCCTCAATGTCACTAGTT GTGCGTCCTCTTCTGCCTACCCA	PtrpC – PactA	Cloning – amplification of PactA for overexpression construct	[1]
<b>TtrpC-Bcstc3-F</b>	TGACATGGAGCTATTAATCACTAAGCGGC GCTGGTACTTCTTTAAGCTTAGT	TtrpC – <i>bcstc3</i>	Cloning – amplification of <i>bcstc3</i> locus for overexpression construct	This study
<b>PactA-Bcstc3-R</b>	GACTTAACGTCCTTAATTATCAACCCGAAC TTCCAAATGAATACGGTAATAG	PactA – <i>bcstc3</i>	Cloning – amplification of <i>bcstc3</i> locus for overexpression construct and Diagnostic PCR – homologous integration at <i>bcniaD</i> -5'	This study
<b>BcniaD-5F</b>	GTGAATGGGATTCAATGTTTATTC	<i>BcniaD</i> -5'	Cloning – amplification of overexpression fragment	[1]
<b>BcniaD-3R</b>	GCATTGGATTAATAATTGTTGCTAAGC	<i>BcniaD</i> -3'	Cloning – amplification of overexpression fragment	[1]
<b>BcniaD-hi5F</b>	CGCATATCAGCATATCGAGATGTCC	<i>BcniaD</i> locus	Diagnostic PCR – homologous integration at <i>bcniaD</i> -5'	[1]
<b>BcniaD-hi3R</b>	GAGTACCCATCCGATGGAGTGTG	<i>BcniaD</i> locus	Diagnostic PCR – homologous integration at <i>bcniaD</i> -3'	[1]
<b>BcniaD-WT-F</b>	GCCACAGACTCCGCCAGATTCTAATG	<i>BcniaD</i> locus	Diagnostic PCR – detection of <i>BcniaD</i> alleles	[1]
<b>BcniaD-WT-R</b>	CAACCATTTCACGTGCGACCACC	<i>BcniaD</i> locus	Diagnostic PCR – detection of <i>BcniaD</i> alleles	[1]
<b>Bcstc1fw</b>	TATGAGCAGCAACAGTACGG	<i>Bcstc1</i> -5'	mRNA level quantification of the gene <i>Bcstc1</i>	[2,3]
<b>Bcstc1rv</b>	GCTGCTTCAATTCCTGGGTG	<i>Bcstc1</i> -3'	mRNA level quantification of the gene <i>Bcstc1</i>	[2,3]
<b>Bcstc2fw</b>	CATCATCGATGATTGCGTCC	<i>Bcstc2</i> -5'	mRNA level quantification of the gene <i>Bcstc2</i>	[2,3]
<b>Bcstc2rv</b>	GAACCTTCATCAACCATCC	<i>Bcstc2</i> -3'	mRNA level quantification of the gene <i>Bcstc2</i>	[2,3]
<b>Bcstc3fw</b>	GGAAAGCAAATGAGAGGGCG	<i>Bcstc3</i> -5'	mRNA level quantification of the gene <i>Bcstc3</i>	[2,3]
<b>Bcstc3rv</b>	TGTCCATTGGTTAGCATGGC	<i>Bcstc3</i> -5'	mRNA level quantification of the gene <i>Bcstc3</i>	[2,3]
<b>Bcstc4fw</b>	ATCCAGTACCAGAGGTACCC	<i>Bcstc4</i> -5'	mRNA level quantification of the gene <i>Bcstc4</i>	[2,3]
<b>Bcstc4rv</b>	CACGCGTACGCTATTTACGC	<i>Bcstc4</i> -3'	mRNA level quantification of the gene <i>Bcstc4</i>	[2,3]
<b>Bcstc5fw</b>	GCACGAGTTGTTAAGAAGG	<i>Bcstc5</i> -5'	mRNA level quantification of the gene <i>Bcstc5</i>	[2,3]
<b>Bcstc5rv</b>	CCATCTGAATTGCCTTCTGC	<i>Bcstc5</i> -3'	mRNA level quantification of the gene <i>Bcstc5</i>	[2,3]
<b>Bcstc7fw</b>	TGTATGAGGTGCTGCACTGG	<i>Bcstc7</i> -5'	mRNA level quantification of the gene <i>Bcstc7</i>	[2,3]
<b>Bcstc7rv</b>	GACTTCATTCTGATGACGCG	<i>Bcstc7</i> -3'	mRNA level quantification of the gene <i>Bcstc7</i>	[2,3]
<b>BcactAfw</b>	TTTGAGACCTTCAACGCCCC	<i>BcactA</i> -5'	mRNA level quantification of the gene <i>BcactA</i>	[2,3]
<b>BcactArv</b>	ACGTGAGTAACCTCGTCACC	<i>BcactA</i> -3'	mRNA level quantification of the gene <i>BcactA</i>	[2,3]
<b>Bctubfw</b>	TCCTTCGGTCAACTTCCG	<i>Bctub</i> -5'	mRNA level quantification of the gene <i>Bctub</i>	[2,3]
<b>Bctubrv</b>	CACCCTCAGTGTTAATGACCC	<i>Bctub</i> -3'	mRNA level quantification of the gene <i>Bctub</i>	[2,3]

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