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Phosphoproteome profiles of the phytopathogenic fungi [i]Alternaria brassicicola[/i] and [i]Botrytis cinerea[/i] during exponential growth in axenic cultures

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1 **Dataset Brief**

2 **Phosphoproteome profiles of the phytopathogenic fungi *Alternaria***
3 ***brassicicola* and *Botrytis cinerea* during exponential growth in**
4 **axenic cultures**

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20

21 Key-words: gray mold, black spot, fungal disease, post-translational modifications, protein
22 phosphorylation, signal-transduction

24 *Abstract*

25 This study describes the gel-free phosphoproteomic analysis the phytopathogenic fungi
26 *Alternaria brassicicola* and *Botrytis cinerea* grown *in vitro* under non-limiting conditions.
27 Using a combination of strong cation exchange and immobilized metal affinity
28 chromatography (IMAC) prior to LC-MS, we identified over 1350 phosphopeptides per
29 fungus representing over 800 phosphoproteins. The preferred phosphorylation sites were
30 found on serine (>80%) and threonine (>15%), whereas phosphorylated tyrosine residues
31 were found at less than 1% in *A. brassicicola* and at a slightly higher ratio in *B. cinerea*
32 (1.5%). Biological processes represented principally among the phosphoproteins were those
33 involved in response and transduction of *stimuli* as well as in regulation of cellular and
34 metabolic processes. Most known elements of signal transduction were found in the datasets
35 of both fungi. This study also revealed unexpected phosphorylation sites in histidine-kinases,
36 a category overrepresented in filamentous ascomycetes compared to yeast.

37 The data have been deposited to the ProteomeXchange database with identifier PXD000817.

38

39 The phytopathogenic fungi *Botrytis cinerea* (Bc) and *Alternaria brassicicola* (Ab) belong to
40 two distantly related taxa (*i.e.*, Leotiomyces and Dothideomyces) within the Ascomycetes.
41 They provoke, respectively, gray mold on over 200 dicotyledonous plant hosts and black spot
42 on major *Brassica* crop species. These fungi are ubiquitous and use similar necrotrophic
43 strategies to interact with their host plants.

44 The genome sequences of both fungal species are available [1, 2] and genomic tools to
45 investigate infectious strategies are being developed and used including transcriptomics,
46 proteomics, yeast-one-hybrid and others [3-5]. One bottleneck of proteomic studies is the
47 identification of post-translational modifications that are involved in the regulation of protein-
48 activity. Especially phosphorylation and dephosphorylation are commonly used regulatory

49 events involved in signal-transduction particularly important for pathogenicity, development
50 and adaptation to changing environmental conditions including fungicides [reviewed in 6].
51 Only few papers report on global identification of phosphoproteins in non-model fungi [7, 8],
52 whereas numerous studies describe the development of phosphoproteomics in several yeast
53 species [reviewed in 9, 10], but also in plants [11]. In this work we established a gel-free
54 phosphoproteomics protocol for the above cited fungi grown under non-limiting *in vitro*
55 conditions leading to a first set of roughly 1350 phosphopeptides per fungus representing over
56 800 phosphoproteins each.

57

58 *Characterization of the Ab and Bc phosphoproteomes*

59 Spores of the *B. cinerea* reference strain B05.10 were inoculated at 2×10^5 spores mL⁻¹ in
60 liquid YSS medium (2 g L⁻¹ KH₂PO₄, 1.5 g L⁻¹ K₂HPO₄, 1 g L⁻¹ (NH₄)₂SO₄ x 7H₂O, 2 g L⁻¹
61 yeast extract, 10 g L⁻¹ D-glucose). Spores of *A. brassicicola* strain Abra43 were inoculated at
62 2×10^5 spores mL⁻¹ in PDB (potato dextrose broth) medium. Four independent cultures of 150
63 mL were grown for 22 h at 23°C in the dark on a rotary shaker at 150 rpm. The mycelium was
64 harvested by filtration and immediately frozen in liquid nitrogen prior to freeze-drying. To
65 simplify the lecture of this paper data derived from *B. cinerea* will be referred to as Bc and
66 those from *Alternaria brassicicola* as Ab in the forthcoming sections.

67

68 Total extraction of proteins was performed on 100 mg of mycelium using phenol according to
69 a protocol adapted from [12]. Pellets of proteins were suspended in 6 M urea, 2 M thio-urea,
70 2% (w/v) CHAPS, 30 mM Tris-HCl pH8.8. Protein content was determined using the 2D
71 Quant-kit (GE Healthcare, Velizy-Villacoublay, France) with BSA as standard.

72 For each biological replicate, 2 mg of proteins were reduced with 10 mM DTT and alkylated
73 with 40 mM iodoacetamide.

74 Protein digestion was performed with modified trypsin (Promega, Madison, WI, USA) at an
75 enzyme/substrate ratio of 1:33 (w/w) by overnight incubation at 37 °C, and stopped by adding
76 1% formic acid (v/v).

77 Prior to the enrichment of phosphopeptides using immobilized metal ion affinity
78 chromatography (IMAC) as described in [13], peptides were labeled using stable isotope as
79 described by Boersema et al. [14] and separated in 10 fractions using strong cation exchange
80 chromatography (SCX) (Zorbax 300, 5 µm, 2.1 mm id, 150 mm length).

81 Each enriched phosphopeptide fraction was separated on a Nano 2DLC-Ultra system
82 (Eksigent) using a C18 column and analyzed on line with a LTQ XL ion trap (Thermo
83 Electron, Courtaboeuf, France) using a nano-electrospray interface. Peptide ions were
84 analyzed using Xcalibur 2.07 with the following data-dependent acquisition steps: (1) full MS
85 scan in enhanced scan rate mode (mass-to-charge ratio (m/z) 350 to 1400, profil mode), (2)
86 MS/MS on the two highest ions detected in full scan (qz = 0.25, activation time = 30 ms, and
87 collision energy = 35%, centroid mode), (3) MS3 was performed when neutral loss of H₃PO₄
88 was detected in the MS2 spectra. Dynamic exclusion was set to 45 sec.

89 Identification of proteins and phosphorylation sites were performed using X!Tandem Cyclone
90 (<http://www.thegpm.org/TANDEM/2010.12.01>). Enzymatic cleavage was described to be due
91 to trypsin digestion with one possible miss cleavage. Cys carboxyamidomethylation was set
92 as static modifications while Met oxidation and phosphorylation of tyrosine, serine and
93 threonine residues were set as variable modifications. Precursor mass and fragment mass
94 tolerance were 2.0 and 0.5 Da, respectively. Identifications were performed using the *Botrytis*
95 *cinerea* genome databases (Broad Institute of Harvard at MIT, <http://www.broadinstitute.org>,
96 16448 entries, INRA URGI <http://urgi.versailles.inra.fr/Species/Botrytis>, 16360 entries) and
97 the *Alternaria brassicicola* genome database (2010, DOE Joint Genome Institute,
98 <http://genome.jgi-psf.org/Altbr1/Altbr1.home.html>, 10685 entries) completed with a

99 homemade contaminant database. Identified proteins were filtered and grouped using the
100 X!Tandem pipeline v3.3.0 (<http://pappso.inra.fr/bioinfo/xtandempipeline/>). Data filtering was
101 achieved according to a peptide E value < 0.01. The false discovery rate (FDR) was estimated
102 < 1% for both analyses (reverse database strategy). The assignment of phosphorylation sites
103 in MS² and MS³ spectra were scored (Document S2) using the Olsen-Mann algorithm [15]
104 implemented in PhosCalc [16]. 82% of the phosphosites proposed by X!Tandem matched
105 with the first Phoscalc hit or with the second Phoscalc hit when the difference between the
106 first and second score was <10. Phosphorylation site assignment was confirmed by manual
107 inspection of the raw data for all phosphopeptides from proteins involved in signal
108 transduction (Tables 1 and 2).

109 Identified proteins, phosphopeptides and their corresponding spectra were deposited online
110 using PROTIcDb database [17] at the following URL: <http://moulon.inra.fr/protic/necrophos>
111 using the following login: “necrophos” and password (required during the review process
112 only): “review”. The data have also been deposited to the ProteomeXchange Consortium
113 database (<http://www.proteomexchange.org>) via the PRIDE partner repository [18] with the
114 dataset identifier PXD000817 and DOI 10.6019/PXD000817 at the following URL:
115 <http://tinyurl.com/oorc5n9>, username: reviewer90580@ebi.ac.uk and password: tkRgUihL.

116 This experimental procedure lead to the identification of 1361 (Bc) and 1379 (Ab)
117 phosphopeptides representing 818 (Bc) and 820 (Ab) phosphoproteins respectively
118 (Document S1). Among the 1439 detected phosphosites (p-sites) in the Bc extracts, 82.7%
119 were phosphoserine, 15.8% phosphothreonine, and 1.5% phosphotyrosine. The ratio found
120 among the 1442 Ab phosphosites was slightly but significantly different (Chi²-test p-value
121 0.0037, df=2) with 80.4% serine, 18.7% threonine and only 0.9% tyrosine residues
122 phosphorylated. The experimental procedure did not allow the identification of the acid labile
123 phosphorylation of histidine or aspartate residues, because the buffers used for IMAC and

124 SCX chromatography were at pH3. Specific experimental conditions would have been
125 necessary to identify these phosphorylation events. Most proteins contained a single
126 phosphosite.

127

128 *Gene-ontologies in the phosphoproteomes*

129 We then applied the gene-ontology annotations developed for both fungi in their respective
130 genome projects to the phosphoproteomic data in order to identify the biological processes
131 affected by protein phosphorylation. Statistical analyses conducted on the GOEAST web-
132 server [<http://omicslab.genetics.ac.cn/GOEAST/>; 19] highlighted those GO categories over-
133 represented among the phosphoproteome in comparison to the genome content (Document
134 S3). Fig. 1 shows the categories of biological processes (A) and molecular functions (B)
135 enriched in both phosphoproteomes according to the GOEAST-analysis. As expected
136 regulation of signaling, of cellular processes, response to stimuli and cell communication
137 figure among these, but also metabolic processes and in *B. cinerea*, the category of
138 developmental processes seems enriched in phosphoproteins.

139 Regarding the molecular functions identified in our screen, those dealing with nucleoside- or
140 nucleotide binding build common categories for both fungi. The comparison of the enriched
141 GO-categories between both fungi (Multi-GOEAST), pinpoints to a specific enrichment in the
142 categories of oxidoreductase and protein kinase regulatory activity in *A. brassicicola*.

143

144 *Signal-transduction proteins*

145 We then selectively screened for signal-transduction proteins. Tables 1 and 2 summarize the
146 hits for both fungi. Briefly, the principal protein kinases were found among the
147 phosphoproteins in both fungi, such as the cAMP dependent protein kinases PKA (catalytic
148 and regulatory sub-units respectively), the Ca²⁺/calmoduline dependent protein kinases, the

149 mitogen-activated protein kinases (MAPKs) Hog1-like and Slt2-like as well as the
150 corresponding MAPK-kinases. The third MAPK (Fus-like) and its kinase were identified only
151 in the *B. cinerea* data set. As additional ST proteins, we identified regulators of G-proteins
152 and small GTPases, but also protein phosphatases. As mentioned above, we did not expect
153 histidine-kinases (HKs) in our data sets with the exception of potentially phosphorylated
154 serine, threonine or tyrosine residues in conserved signaling domains (e.g. PAS, GAF) typical
155 for some classes of HKs [20]. Intriguingly, we found three and two phosphorylated HKs in *A.*
156 *brassicicola* and *B. cinerea* respectively, of the classes V, VIII, XI (Ab), I and III (Bc)
157 respectively. AB08544.1 (class V) had five phosphorylated residues (serine and tyrosine),
158 AB05093.1 (class VIII) two and the other HKs one phospho-site respectively. None of these
159 residues affected conserved domains, and we were unable to identify common sequence
160 motifs between the phosphorylation sites (data not shown). It will therefore be challenging to
161 characterize the role of these phosphorylations in HK functions. A good candidate would be
162 the class III HK Bos1 of *B. cinerea* whose involvement in adaptation to hyper-osmotic
163 conditions and to some fungicides is known. The same issue with the other HKs seems more
164 difficult as their functions are still unknown.

165

166 *Concluding remarks*

167 This work is, to our knowledge, the first description of a phosphoproteomic dataset of two
168 important necrotrophic ascomycetes based on a gel-free analysis protocol that was proven to
169 work in different organisms [8, 11, 21]. With 1638 phosphoproteins identified in both fungi,
170 this data covers 7.7 % of the 10.688 predicted Ab proteins and 7.8 % of the predicted 10.427
171 Bc B05.10 proteins [22], a starting point for the functional analysis of protein-
172 phosphorylation sites. With this limited data sets, we found some interesting features, such as
173 previously unknown phosphorylation sites in histidine-kinases.

174 Additional growth conditions shall now be tested to identify i/ additional phosphoproteins and
175 phosphosites in order to complete the Bc and Ab phosphoproteome datasets, and ii/ to
176 compare phosphoproteomic profiles between growth conditions using dimethylformamide
177 labeling and quantitative phosphoproteome comparisons. In particular, this type of approach
178 may be applied to study the impact of external stress conditions on complex protein
179 phosphorylation profiles.

180

181

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185 The authors have declared no conflict of interest.

186

187 **References**

188 [1] Amselem, J., Cuomo, C. A., van Kan, J. A. L., Viaud, M., *et al.*, Genomic Analysis of the
189 Necrotrophic Fungal Pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet*
190 2011, 7, e1002230.

191 [2] Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., *et al.*, Diverse Lifestyles and
192 Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen *Dothideomycetes*
193 Fungi. *PLoS Pathog* 2012, 8, e1003037.

194 [3] Gonzalez-Fernandez, R., Jorrin-Novo, J. V., Contribution of proteomics to the study of
195 plant pathogenic fungi. *J Proteome Res* 2012, 11, 3-16.

196 [4] Leroch, M., Kleber, A., Silva, E., Coenen, T., *et al.*, Transcriptome profiling of *Botrytis*
197 *cinerea* conidial germination reveals upregulation of infection-related genes during the
198 prepenetration stage. *Eukaryot Cell* 2013, 12, 614-626.

199 [5] Simon, A., Dalmais, B., Morgant, G., Viaud, M., Screening of a *Botrytis cinerea* one-
200 hybrid library reveals a Cys2His2 transcription factor involved in the regulation of secondary
201 metabolism gene clusters. *Fungal Genet Biol* 2013, 52, 9-19.

202 [6] Hamel, L. P., Nicole, M. C., Duplessis, S., Ellis, B. E., Mitogen-activated protein kinase
203 signaling in plant-interacting fungi: distinct messages from conserved messengers. *Plant Cell*
204 2012, 24, 1327-1351.

205 [7] Rampitsch, C., Subramaniam, R., Djuric-Ciganovic, S., Bykova, N. V., The
206 phosphoproteome of *Fusarium graminearum* at the onset of nitrogen starvation.
207 *PROTEOMICS* 2010, *10*, 124-140.

208 [8] Rampitsch, C., Tinker, N. A., Subramaniam, R., Barkow-Oesterreicher, S., Laczko, E.,
209 Phosphoproteome profile of *Fusarium graminearum* grown in vitro under nonlimiting
210 conditions. *PROTEOMICS* 2012, *12*, 1002-1005.

211 [9] Beltrao, P., Trinidad, J. C., Fiedler, D., Roguev, A., *et al.*, Evolution of
212 Phosphoregulation: Comparison of Phosphorylation Patterns across Yeast Species. *PLoS*
213 *biology* 2009, *7*, e1000134.

214 [10] Amoutzias, G. D., He, Y., Lilley, K. S., Van de Peer, Y., Oliver, S. G., Evaluation and
215 Properties of the Budding Yeast Phosphoproteome. *Molecular & Cellular Proteomics* 2012,
216 *11*.

217 [11] Bonhomme, L., Valot, B., Tardieu, F., Zivy, M., Phosphoproteome Dynamics Upon
218 Changes in Plant Water Status Reveal Early Events Associated With Rapid Growth
219 Adjustment in Maize Leaves. *Molecular & Cellular Proteomics* 2012, *11*, 957-972.

220 [12] Bestel-Corre, G., Dumas-Gaudot, E., Poinot, V., Dieu, M., *et al.*, Proteome analysis and
221 identification of symbiosis-related proteins from *Medicago truncatula* Gaertn. by two-
222 dimensional electrophoresis and mass spectrometry. *Electrophoresis* 2002, *23*, 122-137.

223 [13] Nuhse, T. S., Bottrill, A. R., Jones, A. M., Peck, S. C., Quantitative phosphoproteomic
224 analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune
225 responses. *Plant J* 2007, *51*, 931-940.

226 [14] Boersema, P. J., Raijmakers, R., Lemeer, S., Mohammed, S., Heck, A. J., Multiplex
227 peptide stable isotope dimethyl labeling for quantitative proteomics. *Nat Protoc* 2009, *4*, 484-
228 494.

- 229 [15] Olsen, J. V., Blagoev, B., Gnad, F., Macek, B., *et al.*, Global, in vivo, and site-specific
230 phosphorylation dynamics in signaling networks. *Cell* 2006, *127*, 635-648.
- 231 [16] MacLean, D., Burrell, M. A., Studholme, D. J., Jones, A. M., PhosCalc: a tool for
232 evaluating the sites of peptide phosphorylation from mass spectrometer data. *BMC research*
233 *notes* 2008, *1*, 30.
- 234 [17] Langella, O., Valot, B., Jacob, D., Balliau, T., *et al.*, Management and dissemination of
235 MS proteomic data with PROTeoCdb: example of a quantitative comparison between methods
236 of protein extraction. *PROTEOMICS* 2013, *13*, 1457-1466.
- 237 [18] Vizcaino, J. A., Cote, R. G., Csordas, A., Dianes, J. A., *et al.*, The PRoteomics
238 IDentifications (PRIDE) database and associated tools: status in 2013. *Nucleic Acids Res*
239 2013, *41*, D1063-1069.
- 240 [19] Zheng, Q., Wang, X. J., GOEAST: a web-based software toolkit for Gene Ontology
241 enrichment analysis. *Nucleic Acids Res* 2008, *36*, W358-363.
- 242 [20] Catlett, N. L., Yoder, O. C., Turgeon, B. G., Whole-genome analysis of two-component
243 signal transduction genes in fungal pathogens. *Eukaryot Cell* 2003, *2*, 1151-1161.
- 244 [21] Villen, J., Gygi, S. P., The SCX/IMAC enrichment approach for global phosphorylation
245 analysis by mass spectrometry. *Nat Protoc* 2008, *3*, 1630-1638.
- 246 [22] Staats, M., van Kan, J. A. L., Genome Update of *Botrytis cinerea* Strains B05.10 and T4.
247 *Eukaryotic Cell* 2012, *11*, 1413-1414.

248

249 **Legends to figures**

250 Fig. 1: GO categories (A, biological processes, B, molecular functions) enriched in the *B.*
251 *cinerea* and *A. brassicicola* phosphoproteomic datasets according to a GOEAST analysis [19]
252 performed on the Ab and Bc datasets (phosphoproteomes vs. genomic data). The y-scale

253 indicates the percentage of phosphorylated proteins among all annotated proteins in the
254 corresponding GO-category. The GO annotation levels are indicated between the brackets.

255

256 Supplemental Data

257 Document S1: Phosphopeptides and protein IDs identified from *A. brassicicola* and *B.*
258 *cinerea* mycelia.

259 Document S2: PhosCalc phosphorylation score of MS2 and MS3.

260 Document S3: Statistics of GO-annotations in the phosphoproteomic data.

261

Table 1: Signal transduction proteins identified among the *A. brassicicola* phosphoproteins. The annotations were selected from the genome data (<http://genome.jgi-psf.org/Altbr1/Altbr1.home.html>, [2]) and names of genes indicated if known.

ID	Name	Function	Phospho-site position	Phospho-peptides
AB03810.1		PQ-loop	130, 152/153:	RD(pS)SNIGLPGSHR, A(s)(s)LPTIPDVGDGASEWVR
AB03884.1		Regulator of G protein (RGS)	373	FIGTTANETQSDSD(pS)IHEFSK
AB08967.1		Phosducin	37	HGVIPEKPP(pS)PTPMIQEALEQAR
AB06955.1		PKA regulatory subunit	52/53, 71	TEYLLSQQH(s)(s)PQGR, AENTFPGSNPFST(pS)PSSSGGFTR
AB06955.1		PKA regulatory subunit	99, 163, 164, 166, 175	LEEEENDTMTSP(pT)ASTFAAVDFGR, (p)T(p)SV(p)SAESLNPAS(p)SAADNFTPPFHQK
AB08717.1		PKA catalytic subunit	25, 311	DLDSP(pT)SPIKDSNSPITPTTSR, EVPDITW(pT)LCGTPDYLAPEVVASK
AB09880.1		PKA catalytic subunit	245/246/247	E(t)(y)(t)LCGTPEYLAPEVIR
AB01474.1	AbSch9	Sch9-like protein kinase	179/180/181/182, 289, 291	K(s)(s)(s)(t)VNPSAPVSTCQVSDPR, (p)TE(p)SQMGTPMAIPMK
AB01474.1	AbSch9	Sch9-like protein kinase	572/574	ANLTENAT(t)N(t)FCGTTEYLAPEVLLDEQGYTK
AB08261.1		CLK1-like protein kinase	229, 1069	RN(pT)LPVLAAEDHTSK, RA(pS)NAPIDPSEELNLTR
AB00575.1		Hrk1-like protein kinase	113, 184	SSIPGSPASAMH(pS)PPLTPAGTSSR, IAPQ(pS)PRPSSDLSGIVQPHPK
AB00575.1		Hrk1-like protein kinase	378	SGIM(pT)PPITNGAMSVPFADHGLQSK
AB01755.1		Yak1-like protein kinase	36, 476, 665/666, 668	QNSVPLQ(pS)PSGTASR, QTVYT(pY)IQSR, SG(s)(s)R(pS)PAPGVQEQQR
AB04026.1		Psk1/2-like protein kinase	110, 328	AA(pS)LNIAATPPVSR, TL(pT)DVAPLTIQSQLHDALAPYR
AB07904.1		RhoGAP protein	175	LVEDLEEDKLAQDPQSEAAQ(pS)PK
AB07854.1		RhoGAP protein	108, 182, 580	SGAS(pT)PDLGRPVR, SK(pS)IKEGESSGSR, GNN(pS)PQVQSSK
AB07854.1		RhoGAP protein	616, 657/658, 699	NGRA(pT)PPNPSLVSESPSNPVLR,GGGGGGGGGGGGGGYGERE(t)(s)PER, APPSVSTEASSIDLDNA(pT)PTQAQHSR
Ab03936.1		RhoGAP protein	16	SS(pS)LSAVPPPQHSSNYSPDLAK
AB04861.1		RhoGEF protein	843, 920/927/931	SNSN(pS)PNNPPPR, NM(s)PPLGAD(s)NIP(t)QLK
AB01313.1		RhoGEF protein	86/88/89, 283, 490	QNDELFIGANSSPQLSQGPM(s)P(t)(t)GGYGGSYGYQR, YTSMSGNALPPTPEVALLHG(pS)PQR, VD(pT)FGTGGLEEPSAR
Ab08544.1	AbHhk5	Histidine kinase - group V	115, 121, 127, 207, 219, 393	(pT)APSNA(pS)VAALG(pS)R, WA(pS)AFHMPFTSLSP(pT)VAAGQPR, QKSN(pT)SPDTPAPLGRPR
AB05093.1	AbHhk8	Histidine kinase - group VIII	150, 265, 1263	SAISVDPTP(pT)PK, ADRAP(pS)IGGASAR, (pS)LEHISSQSVVPPHKR
AB01584.1		Histidine kinase - group XI	742	RTM(pT)GEEAMQTIK
AB09877/8.1	AbSsk1	Response regulator	301/302/306	(s)(s)SSL(s)PTTAANPEPNGVKR
AB03493.1		Response regulator	622/625/626, 667	RAD(s)PA(t)(s)DVGLSR, SMVPPPS(pS)PLR
AB08549.1	AbSkn7	Response regulator	273	MNGTLT(pT)PPPDFTR
AB09970.1	AbSte11	MAPKKK	208, 212, 219, 442	QIMNSDLGSYAYGAMPP(p)SRPG(p)SPLVDQE(p)TR, LTGESLAAVSYPL(pS)PTSAR
AB05686.1	AbHog1	MAPK	171, 173	IQDPQM(pT)G(pY)VSTR
AB01438.1	AbPbs2	MAPKK	24, 29, 125, 137	SP(pS)PAPS(pS)PTENASSPDLLSAERPPPR, GPAPQ(pS)PAPSPDNRNPM(pS)PGGNPGGIPGNFR
AB01438.1	AbPbs2	MAPKK	194, 197, 331, 334	FQGALPGGL(pS)GS(pS)PTAGGPAPPAGRK, NFDEEA(p)SPT(p)SAKPAAGTIVMAMK
AB07135.1	AbSlr2	MAPK	98	GFSMDPEENAGYM(pT)EYVATR
AB04139.1	AbMkk1	MAPKK	64, 106	LSVV(pT)PMGSNNAPHEGR, SG(pS)FGEVQANGSASSYHAALGFPLQK
AB04139.1	AbMkk1	MAPKK	132, 136, 139, 143, 154/155	A(p)TDPI(p)SAI(p)SQGG(p)SEGAPSMER, AN(s)(s)QPLPDLDAVEK

AB02044.1	AbBck1	MAPKKK	853	LNADSQLD(pS)PGGR
AB01652.1		Ypk1/2-like protein kinase	440	DEDR(pT)NTFCGTPEYLAPELLTGAGYTK
AB04922.1		Ypk1/2-like protein kinase	414	(pS)QDGELHPTR
Ab04691.1		Pkh1/2-like protein kinase	360, 364, 541	AQ(pS)FVG(pT)AEYVSPELLTDK, LITELPPSQLDIDW(pS)PVLTK
AB07725.1		Fpk1-like protein kinase	157, 433/435/439	VA(pS)APNAQGLFSSGK, (t)N(s)FVG(t)EEYIAPEVIK
AB00828.1		Pom1-like protein kinase	526	AYE(pS)PKDPER
AB01801.1		Cdc28-like protein kinase	57/58	RL(s)(t)GDEDEETAK
Ab03742.1		Cdc15-like protein kinase	654	TQ(pS)QIEIQK
AB07167.1		Dbf 20-like protein kinase	451/453, 503, 532/534	DNAMDRPG(t)P(t)EAFMSPQQTPOGSPSK, TKPG(pS)PTLPNK, LQLGSSGGADYSAQDSPSFAPG(s)P(t)RK
AB07167.1		Dbf 20-like protein kinase	555, 874, 1061	ES(pS)YLTHAAQSR, (pS)IVGSPDYMAPEVLK, TADENGKPA(pS)PR
AB09330.1		Bur1-like protein kinase	203	EY(pT)TLVVTR
Ab03799.1		Yck1/2-like protein kinase	419, 449	AAPA(pS)IPNPQQPR, LANVLCCGA(pS)K
AB07752.1		Cbk1-like protein kinase	146	GGYGYG(pS)QGGSSGSR
AB09047.1		p21-activated serine/threonine protein kinase	65, 295/297	SV(pS)SASTVK, KD(s)Y(s)QGYGSQPSASSQQDR
AB09315.1		Protein kinase	353	KR(pS)EPFLSTYSAAK
AB09163.1		Rim11-like protein kinase	198	ILVENEPNVS(pY)ICSR
AB07449.1		Protein kinase C	601, 603, 606, 608, 659, 684, 685, 689, 693/694	GQ(p)SS(p)SGP(p)SM(p)SQR,SPPPGPPRQQ(pS)YPTSAASIDAAR, ASFSTSGSSAAP(pT)(pS)PTA(pS)SRP(s)(s)GPR
AB07449.1		Protein kinase C	698/700/701, 723, 1003/1004	(t)Q(s)(s)VAAAAAAMNK, SN(pT)DYQPQSGR, EEMWYGST(t)(s)TFCGTPEFMAPEILLDDKK
AB07962.1		Stt4-like protein kinase	296, 383, 472	EASE(pS)DDEATPVKK, LAPLQTPG(pS)PDPVR, EIAADMNNAEI(pT)PQQR
AB00947.1		Calcineurin A	425, 550	EELLEETPSSLASGPS(pS)PPLSSLDPDSTEFKR, RI(pS)MSSGSGR
AB03945.1	AbCrz1	CN-regulated TF	158/159, 255	H(t)(s)LDPSAAYGQITEWQGMAGGFGQHR, FNLNGSNAHISAGN(pS)PHISPR
AB02788.1		Mechanosensitive ion channel	76, 368	GIQ(pS)PSQTR, (pT)PGQILTEAQK
AB09176.1		Ca2+/CaM-dependent kinase	77	SEYSN(pS)EDDGSAQHR
AB00893.1		Clk1-like protein kinase	326, 443	VIWDTQTM(pT)PCGTVGTYTAPEIVKDER, EADEPTYSAYDAPPLA(pT)PAAKK
AB03068.1		Kin4 -like protein kinase	105, 151/153, 437, 438, 441	TS(pS)HRTPNASASSQPSR, (s)A(t)AIGQPPVGTSLPR, GDLMQ(pT)(pS)CG(pS)PCYAAPELVSDSLYTGR
AB03068.1		Kin4 -like protein kinase	642, 648, 832, 898	GEA(p)SPPVET(p)SPK, ESFTSTG(pS)YGGYPEEK, RF(pS)LLPSSLK
AB07973.1		Dun1-like protein kinase	32, 65/67, 637	HSRL(pS)PPPNGR, ESTGDASEGYKEG(t)Q(s)PPP GK, GDQTLY(pS)EEEDGSR
AB01426.1		Ca2+/H+ antiporter	21, 24, 353	R(p)SST(p)YGTGSDTPTQSASGTDSAR, LFFGPTTGTS(pS)LIGR
AB00595.1		Ca2+/H+ antiporter	49/51/54	TR(s)E(t)HA(t)PAIEEQR
AB00486/7.1		Ca2+ translocating ATPase	80, 81	ESRP(pT)(pS)PHNISSPVTA WNNNAGLLNVPGAR
AB08416/17.1		Ca2+ translocating ATPase	40	SS(pT)PSSTSAHSLVSAQDTAQK
AB07326.1		Protein phosphatase 6	172	SIG(pS)ASDVDSAK
AB00432.1		Protein phosphatase 4	401	RL(pS)SVLNDSFQK
AB00277/8.1		Protein phosphatase 2C	88/90	QSLHLGG(s)G(s)PTSSTSTMSEK
AB03980.1		Protein phosphatase 2C	332, 404, 418	IDD(pS)PDDIDMDLDSR, ES(pT)PGPQHESK, VQVTE(pS)PSSVQTEK
AB05290.1		Protein phosphatase 2C	392	FAASFVTD(pS)FGEER

(pS): Phosphorylated site, (p)S: Peptide found monophosphorylated at different sites, (s),(y), or (t) undistinguishable site

Table 2: Signal transduction proteins identified among the *B. cinerea* phosphoproteins. The annotations are according to the genome data [1] and names of genes indicated if known.

ID	Name	Function	Position	Peptide
BC1G_07268.1		Regulator of G protein (RGS)	426/428	TSTTTADSD(s)L(s)EYKDGIAQVR
BC1G_12682.1		Phosducin	66	SN(pS)GGLGGLSEPLR
BofuT4_P084160.1	BcPKA2	PKA catalytic subunit	211/212/213	E(t)(y)(t)LCGTPEYLAPEVIQSK
BC1G_10590.1	BcPKAR	PKA regulatory subunit	57, 60, 171/ 172/ 174	SV(p)SPG(p)TANRPR, (t)(s)V(s)AESLNPTASSNDNWSPPFHQK
BC1G_14873.1	BPK2	Sch9-like protein kinase	368, 369, 425, 427, 908, 913, 914	TD(p)S(p)SMGGTVVPPK.GPQSEEDDEDGN(p)SH(p)SPGGIPIGGIPIGGIPITR, GF(p)TFVDE(p)S(p)SIEENMKDR
BC1G_05432.1	BPK3	CLK1-like protein kinase	571	TGS(pS)PSTASAIK
BC1G_13633.1		RhoGAP protein	597, 599	(p)SK(p)SFGGPVDENPR
BC1G_09755.1		RhoGAP protein	545, 636	SISGPLLQ(pS)PIDDMVPPNR, NT(pS)SEASTSAR
BC1G_09755.1		RhoGAP protein	651, 680	SV(pS)ETTAR, DIS(pS)PISMSSPNPGDDPALLR
BC1G_06319.1		RhoGAP protein	128, 671, 685	LVEDIEEENKLAQDPET(pS)PK, AT(pS)PAPSPR, DR(pS)GPPAAETR
BC1G_15133.1		RhoGAP protein	392, 396/398	SF(pS)HGP(s)L(s)QHIVAPVVSPTNPSTSPDFNTWSPR
BC1G_01690.1		RhoGAP protein	145	SS(pS)NASAGADSVR
BC1G_09394.1	BcCDC24	RhoGEF protein	753, 852, 853	LGGDSYF(pS)PIGESPAPSAR, SY(p)S(p)TPDINAQQGVR
BC1G_07524.1		RhoGEF protein	112, 113, 268, 270	Y(p)Y(p)SGNAPALASDPYR, (p)SI(p)SSTAGGSSR
BofuT4_P012000.1	M3M1	Histidine kinase - group I	1136	AIE(pS)PTPNALVEK
BC1G_00374.1	BOS1	Histidine kinase - group III	1290/1293/1295	AYTTTGPINHG(s)AE(s)P(s)LVTADAEDPLAR
BC1G_07872.1	BRRG1	Response regulator	506/507/508	NSMGSLGALGGQGG(s)(s)(s)K
BC1G_03001.1	BcSAK1	MAPK	171, 173	IQDPQM(pT)G(pY)VSTR
BC1G_07144.1	BMP3	MAPK	186, 188	GFSVDPEENAGYM(pT)E(pY)VATR
BC1G_13966.1	BMP1	MAPK	183	SAASQEDNSGFM(p)TEYVATR
BC1G_07633.1	BOS5	MAPKK	399, 597, 601	ICDFGVSGNLVA(pS)IAK, APLDTV(pS)PLP(pS)PRE
BC1G_03809.1	BcSTE7	MAPKK	358, 365, 377, 381/383	(pS)HLAPQL(pS)PASR, GDD(p)SPNA(s)Q(t)PTSGDIPIK
BC1G_11713.1	BcMKK1	MAPKK	106, 167	SG(pS)FGPMDGK, DG(pS)MNGLEAFDK
BC1G_04606.1	BOS4	MAPKKK	1138, 1149	TLVTATTA(pS)IANK, SMTG(pT)PMYMSPEVIK
BC1G_06557.1	BcSTE11	MAPKKK	108	YGIM(pS)PAEQMK
BC1G_07478.1	BcPKC1	Protein kinase C	675, 686, 687, 688, 690, 691, 719	AESYGAPSSSEATQAAQAMYQQSTT(pS)PQQRPAQPD(p)T(p)S(p)TS(p)S(p)SATAAAAAATAAMGGR, A(p)STDYTNQVGAQR
BC1G_00093.1	BcCRZ1	CN-regulated TF	403	AV(pS)DPYNSPR
BC1G_07228.1	BcCMK1	Ca2+/CaM-dependent kinase	90, 116/117	L(pT)QDPHSDLVLADFGIAK, DEVL(t)(t)MAGSFGYAAPEVMLK
BC1G_07228.1	BcCMK1	Ca2+/CaM-dependent kinase	288, 290, 294, 298, 282	N(p)TG(p)SSSA(p)SGST(p)SPGK, MQEDDGEESDVPGNAAAAASDAIPG(pS)PERK
BC1G_06577.1	BcCMK3	Ca2+/CaM-dependent kinase	144	SA(pS)DIHINEQR
BC1G_13713.1		Ca2+/H+ antiporter	37, 38, 39	(p)S(p)S(p)TFPAQVPDEEALR
BC1G_01673.1		Ca2+/H+ antiporter	566	TG(pS)FTNYVMTK
BofuT4_P084940.1		Protein phosphatase 2C	335, 337, 339	GPGVHHNFDD(pS)D(p)SG(pY)DVDMDQK
BC1G_01244.1		Protein phosphatase PP-Z	35, 84	GSDNLSSYP(pS)FSK, NSIAGIGSLVDSNDKSDVA(pS)LK
BC1G_14507.1		AC-associated protein	222	SD(pS)QSSINSNR
BC1G_00082.1		Calreticulin/ calnexin	570	ASEAVA(pS)GTDAVK
BofuT4_P154620.1		cNMP-binding protein	270/271	EATIHA(s)(s)VR
BC1G_10054.1	BcGBL1	G β-like protein	288, 292, 297	EPECV(pS)LAW(pS)ADGQ(pT)LFAGYTDNIIR
BC1G_06083.1		Myosin	1571	MEYTQDDIAEQ(pS)DNEEK

BC1G_10821.1		Myosin	1145/1146	(s)(t)PTPSLAGGLAEALR
BC1G_05708.1		Penta-EF-hand protein	71, 91	RL(pS)PQMHPGNYGASPPGR, GYA(pS)PPPGQFQAGR
BC1G_13037.1		Protein kinase	44, 313, 430	(pS)DPNVMAHQAHQVPGGDAYSVANEDNK,IVWDTQTM(pT)PCGTVGYTapeIVKDER, GSEPTYAALDAPPLA(pT)PAVER
BC1G_13037.1		Protein kinase	453, 517, 537	AFNPDLLE(pS)PGAGR, GTAALNPLNPIDDEFDE(pS)DEENYDENGVPsk, SGQ(pS)DVAQVGDALR
BC1G_13227.1		Protein kinase	64, 175, 255	RP(pS)GNGASTNTPPQPQYTSPLVTTTTTAPVNSR, RAQ(pS)QENQYHER, SHPAGTTPAGLSHEA(pS)EVLNR
BC1G_13227.1		Protein kinase	175	RAQ(pS)QENQYHER
BC1G_13227.1		Protein kinase	255	SHPAGTTPAGLSHEA(pS)EVLNR
BC1G_13227.1		Protein kinase	510/ 511, 733	RGDLMQ(t)(s)CGSPCYAAPELVVSDSLYTGR, AG(pS)QGPVEISGAPQGSR
BC1G_00346.1		Protein kinase	97	DSQALS(pS)PVR
BC1G_15362.1		RasGEF protein	701/702	(t)(s)QPDFSAPAFLAVEDYEK
BC1G_04274.1		Rho-like GTPase	97, 101, 102/104	SNQNTSSS(pT)PPG(pT)(s)G(s)QGVVIPK
BC1G_08670.1		RhoGDI protein	37	MDAEDE(pS)LQR
BC1G_07505.1	BcSTE5	Scaffold protein	12, 341	AFESGTAYPE(pS)DADDEYER, SDQQP(pT)PGR
BC1G_12009.1	BcSNF1	Snf1-like protein kinase	209, 216/217	IADFGLSNIM(pT)DGNFLK(t)(s)CGSPNYAAPEVINGK

(pS): Phosphorylated site, (p)S: Peptide found monophosphorylated at different sites, (s),(y), or (t) undistinguishable site

