DOI 10.1002/pmic.200800540

Proteomics 2009, *9*, 2892–2902

Dataset Brief

Proteomic analysis of the phytopathogenic fungus *Botrytis cinerea* during cellulose degradation

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The ascomycete *Botrytis cinerea* is a phytopathogenic fungus infecting and causing significant yield losses in a number of crops. Moreover, in the last few years, *B. cinerea* has been adopted as an important model system in molecular phytopathology. In spite of these contributions, the molecular basis of the infection cycle remains unclear. Proteomic approaches have revealed significant information about the infective cycle of several pathogens, including *B. cinerea*. The main aim of this study is to make available a proteomic database containing a significant number of identified proteins from *B. cinerea*. In brief, three independent *B. cinerea* cultures supplemented with carboxymethylcellulose were used, and the extracted proteins were independently separated by 2-D PAGE to obtain the proteome map from *B. cinerea*. Two hundred and sixty-seven spots were selected for MALDI TOF/TOF MS analysis, resulting in 303 positive identifications, mostly representing unannotated proteins. Identified proteins were then classified into categories using the PANTHER classification system (www.pantherdb.org), showing the relevance of protein metabolism and modification process and oxidoreductase activity. Since cellulose is one of the major components of the plant cell wall, many of the identified proteins may have a crucial role in the pathogenicity process. In brief, this proteomic map of *B. cinerea* will be a useful basis for exploring the proteins involved in the infection cycle, which will in turn provide new targets for crop diagnosis and focused fungicide design.

Received: July 4, 2008
Revised: January 12, 2009
Accepted: January 21, 2009

Keywords:
Botrytis cinerea / Fungal phytopathogens / Fungal proteomics

In the last few years, *Botrytis cinerea* has been adopted as an important model system in molecular phytopathology. Several approaches have been applied to this fungus to unravel its mechanism of infection [1]. These studies have revealed a wide variety and complexity of infection strategies, such as the use of active oxygen species (AOS), toxin production and the production of cell wall degrading enzymes, among others. In spite of these findings, neither the molecular basis of the synthesis and production of different virulence factors nor the mechanisms of phytopathogenicity used by this fungus are yet well understood [2, 3]. These "black boxes" are being studied by more than a dozen teams [4], improving transformation protocols, vectors, mutants, *etc.* but few articles have been published about the proteins produced by *B. cinerea* [3, 5, 6]. This study is intended to establish a proteomic map of *B. cinerea* during cellulose degradation. The ability of this fungus to secrete a large number of cell wall degrading enzymes has been well studied [7]. Though genes for some cell wall degrading enzymes involved in the degradation of plant cell wall have been characterized, their role in pathogenesis has not been

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Abbreviations: AOS, active oxygen species; **CMC,** carboxymethylcellulose; **TCA,** trichloroacetic acid deeply studied in *B. cinerea*. For this reason we have focused our study on these proteins.

Recently, a large amount of genomic information has become available, including the DNA sequences of *B. cinerea* (strains B05.10 and T4) and its neighbor species *Sclerotinia sclerotinium* [8]. These resources have stimulated the development of high-throughput functional analyses such as transcriptomics and proteomics. Studies in *Saccharomyces cerevisiae* have clearly demonstrated the complementary nature of these approaches by revealing significant differences between the amounts of transcripts and the levels of protein expression [9]. Although protein function cannot always be predicted from gene sequences, especially in organism-specific pathways, ongoing DNA sequencing projects provide a tremendous resource for proteomics, facilitating the generation of protein catalogs correlated to samples or phenotypes for future data mining [10]. The intensive description of the protein 2-DE map of *B. cinerea* may elucidate the "black boxes" of its biology, infection strategies and life cycle. Moreover, the identification of proteins expressed will provide a large number of target candidates to design new molecules for disease control. Despite the increasing number of publications providing molecular information about the pathogenicity mechanisms of *B. cinerea*, at present, there is no fungicide on the market that was developed on the basis of targeted molecular research [4]. The characterization of the *B. cinerea* proteome may result in new targets for antifungal drug screening. Moreover, the definition of a *B. cinerea* 2-DE map provides a basis for comparing proteome analyses between labs.

B. cinerea strain 2100 was obtained from the Spanish Type Culture Collection. Conidial stock suspensions were prepared and conserved as reported previously [11]. The whole fungal proteome of *B. cinerea* was prepared from fungal mycelia from 5-day-old liquid cultures. Three 250 mL flasks each containing 100 mL of minimal salts medium (50 mM NH_4Cl, 7.3 mM KH_2PO_4, 4.2 mM $MgSO_4$, 6.7 mM KCl and 0.07 mM $FeSO_4$) [12] supplemented with 1% carboxymethylcellulose (CMC) (Panreac, Barcelona, Spain) were inoculated with three 0.5 cm agar plugs taken from the outer edge of 3-day-old colonies of fungal mycelia on potato dextrose agar (Becton Dickinson, Sparks, MD, USA). To check the existence of a specific fungal response to cellulose, a control experiment using glucose as a sole carbon source was performed in parallel. The cultures were grown at 22°C with agitation (180 rpm) for 5 days; mycelia were harvested by filtration and washed with sterile water. A total protein fraction was isolated using a phenol-based procedure outlined previously [13] with some modifications. In short, three independent replicates of mycelia (2 g apiece) were ground to a fine powder under liquid nitrogen. The powder was transferred to 15 mL tubes and resuspended in 10 mL of cold acetone. After vortexing for 30 s, the suspension was centrifuged (10 000 × *g*, 5 min, 4°C), and the resulting pellet was washed once again with acetone and resuspended in 10 mL 20% w/v trichloroacetic acid (TCA) in acetone. After centrifugation, the pellets were sequentially washed twice with 20% w/v TCA in acetone, once with 20% w/v TCA and finally twice with 80% v/v acetone. This pellet was air-dried and the dry powder was resuspended in 5 mL "dense SDS buffer" (30% w/v sucrose, 2% w/v SDS, 0.1 M Tris-HCl, pH 8.0, 5% v/v 2-mercaptoethanol). Then, 5 mL Tris-

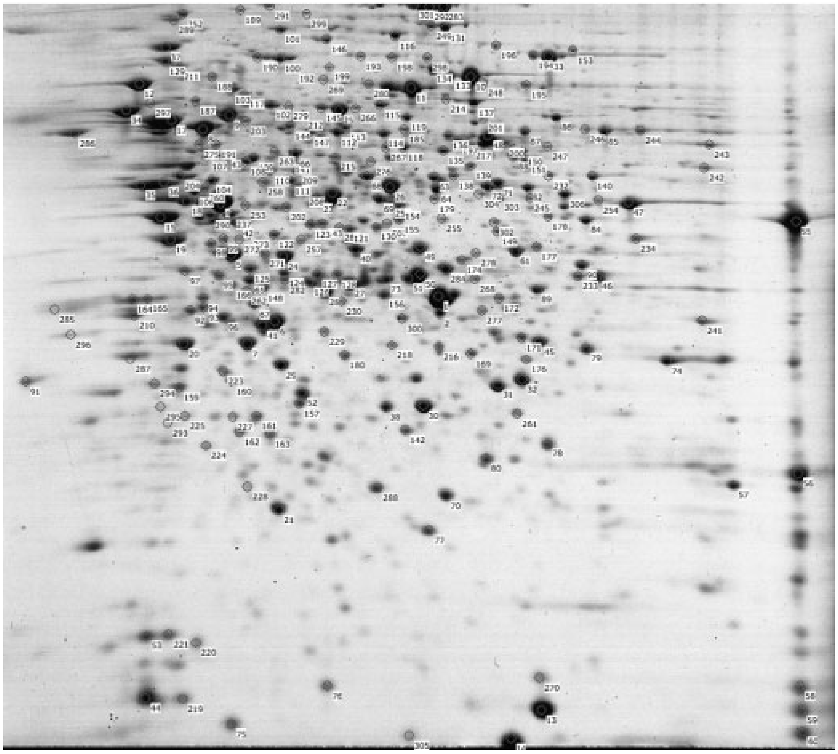

Figure 1. 2-DE CBB-stained gels from mycelial extracts of *B. cinerea* 2100 growing in CMC. Proteins were separated on 17 cm, pH 3–10 non-linear gradient IPG gels (IEF) and 14% polyacrylamide gels (SDS-PAGE).

Table 1. List of identified proteins from *Botrytis cinerea* growing in CMC

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
1	BC1G_11968	5.9	36.7	75.8	280	241	2	*B. cinerea* glyceraldehyde 3-phosphate dehydrogenase (339 nt)
2	BC1G_11968	5.9	36.7	80.5	320	69	2	*B. cinerea* glyceraldehyde 3-phosphate dehydrogenase (339 nt)
4	BC1G_00350	5.1	47.3	66.1	177	318	2	*B. cinerea* enolase (439 nt)
5	BC1G_08198	5.4	41.7	65.2	246	166	2	*B. cinerea* actin (376 nt)
6	BC1G_04836	5.3	39.3	75.6	233	178	1	*B. cinerea* fructose-bisphosphate aldolase (361 nt)
7	BC1G_16357	5.1	23.8	89.4	177	301	2	*B. cinerea* short chain dehydrogenase (218 nt)
8	BC1G_10846	5.1	46	64	226	172	2	*B. cinerea* hypothetical protein similar to hsp70 (425 nt)
9	BC1G_11661	5.7	73.5	52.4	314	198	2	*B. cinerea* hypothetical protein similar to 70 kDa HSP (680 nt)
10	BC1G_12307	6	86.3	71.1	392	122	2	*B. cinerea* hypothetical protein similar to cobalamin-independent methionine synthase (768 nt)
11	BC1G_16294	4.9	34.6	54.9	130	123	2	*B. cinerea* hypothetical protein (319 nt)
12	BC1G_00350	5.1	47.3	37.8	60	206	2	*B. cinerea* enolase (439 nt)
12	BC1G_07315	4.7	79.6	58.3	289	137	2	*B. cinerea* hypothetical protein similar to hsp84 (702 nt)
13	BC1G_12729	6.6	18.2	94.6	215	181	2	*B. cinerea* woronin body major protein (167 nt)
14	BC1G_01740	6.4	19.7	72.5	108	160	2	*B. cinerea* peptidyl-prolyl cis-trans isomerase (182 nt)
15	BC1G_11962	4.6	21.1	79.8	71	18	1	*B. cinerea* ATP synthase beta chain (193 nt)
17	BC1G_04390	4.8	73.2	1.8	x	100	1	*B. cinerea* dnaK-type molecular chaperone BiP (671 nt)
17	BC1G_06164	4.8	59.9	68.8	254	125	2	*B. cinerea* heat shock 70 kDa protein 2 (551 nt)
18	BC1G_12086	5	54.4	36.6	62	x	x	*B. cinerea* hexokinase (492 nt)
19	BC1G_00466	5	45	57.9	180	216	3	*B. cinerea* eukaryotic initiation factor 4A (399 nt)
20	BC1G_00555	4.2	11.2	84.2	81	127	2	*B. cinerea* transaldolase (101 nt)
21	BC1G_08882	5.4	27.1	89.9	230	296	2	*B. cinerea* hypothetical protein (248 nt)
22	BC1G_06362	5.7	53.7	73	348	199	2	*B. cinerea* hypothetical protein (497 nt)
23	BC1G_05991	5.4	51.4	67	327	201	2	*B. cinerea* hypothetical protein similar to ATP-citrat-lyase (476 nt)
24	BC1G_03241	5.5	48.9	66	285	177	2	*B. cinerea*-conserved hypothetical protein (450 nt)
25	BC1G_08475	5.4	67	64.4	339	205	2	*B. cinerea*-conserved hypothetical protein (613 nt)
26	BC1G_02744	5.9	54.8	65.7	261	111	2	*B. cinerea* 6-phosphogluconate dehydrogenase (495 nt)
27	BC1G_05297	6.2	46.5	44.9	125	113	2	*B. cinerea* hypothetical protein (408 nt)
28	BC1G_04443	9.3	44.3	67.9	309	114	2	*B. cinerea* ketol-acid reductoisomerase. Mitochondrial precursor (399 nt)
29	BC1G_11823	6.7	26.6	64.4	140	79	2	*B. cinerea* inorganic pyrophosphatase (236 nt)
30	BC1G_07409	8.9	35.7	86.3	286	142	2	*B. cinerea*-conserved hypothetical protein (342 nt)
31	BC1G_10724	6.2	34.5	77.5	268	207	2	*B. cinerea*-conserved hypothetical protein (333 nt)
32	BC1G_10054	6.6	35	93.7	356	249	2	*B. cinerea* guanine nucleotide-binding protein beta subunit (317 nt)
33	BC1G_09731	5.9	85.9	60.1	316	174	2	*B. cinerea* hypothetical protein similar to elongation factor 2 (775 nt)
34	BC1G_04390	4.8	73.2	59.5	323	261	2	*B. cinerea* dnaK-type molecular chaperone BiP (671 nt)
35	BC1G_05299	4.8	54.2	48.1	214	173	2	*B. cinerea* hypothetical protein (489 nt)
36	BC1G_05299	4.8	54.2	47.4	166	111	2	*B. cinerea* hypothetical protein (489 nt)
36	BC1G_05600	4.8	50.1	54.8	165	82	1	*B. cinerea* tubulin alpha chain (451 nt)
37	BC1G_07510	4.9	90.1	49.5	260	194	2	*B. cinerea* hypothetical protein similar to valosin-containing protein (824 nt)
38	BC1G_07233	8.8	32.2	63.4	171	143	2	*B. cinerea* hypothetical protein (298 nt)
40	BC1G_12890	6.3	44.7	68.5	264	232	2	*B. cinerea* phosphoglycerate kinase (419 nt)
41	BC1G_15343	5.5	37.6	78.7	322	154	2	*B. cinerea* hypothetical protein (347 nt)
42	BC1G_01334	5.9	52.9	75.1	285	181	2	*B. cinerea* mitochondrial processing peptidase beta subunit. Mitochondrial precursor (481 nt)
43	BC1G_04759	5.6	55	58.5	252	239	2	*B. cinerea*-conserved hypothetical protein (511 nt)
44	BC1G_05503	4.9	14.6	95.2	185	99	1	*B. cinerea* hypothetical protein (126 nt)
45	BC1G_06394	6.8	37.5	70.5	183	108	1	*B. cinerea* hypothetical protein (352 nt)
46	BC1G_00206	9.4	41.4	64.9	234	52	2	*B. cinerea* isocitrate dehydrogenase subunit 1. Mitochondrial precursor (379 nt)
46	BC1G_15577	9.2	34.7	39.8	90	x	x	*B. cinerea* hypothetical protein (329 nt)
47	BC1G_07780	9.6	60.8	7.1	x	263	3	*B. cinerea* hypothetical protein similar to mitochondrial ATP synthase alpha subunit (562 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
48	BC1G_05305	6.9	56.7	54.1	181	168	2	*B. cinerea* hypothetical protein (518 nt)
49	BC1G_04602	5.9	43.2	74	300	153	2	*B. cinerea* S-adenosylmethionine synthetase (396 nt)
50	BC1G_12319	8.8	27	77.2	252	177	2	*B. cinerea* NAD-dependent formate dehydrogenase (246 nt)
51	BC1G_08723	6	41.8	68.2	192	102	2	*B. cinerea* hypothetical protein (377 nt)
51	BC1G_09640	5.9	38.9	66.3	175	135	2	*B. cinerea* hypothetical protein (365 nt)
51	BC1G_12319	8.8	27	67.1	71	123	1	*B. cinerea* NAD-dependent formate dehydrogenase (246 nt)
52	BC1G_06836	5.4	55.3	28.7	85	94	2	*B. cinerea* hypothetical protein (522 nt)
53	BC1G_09782	4.9	17.9	18.7	26	126	2	*B. cinerea* translation initiation factor eIF-5A (166 nt)
55	BC1G_09492	9.8	50.1	46.9	136	110	2	*B. cinerea* elongation factor 1-alpha (461 nt)
56	BC1G_02099	10	34	61.5	127	272	3	*B. cinerea* ADP/ATP carrier protein (314 nt)
56	BC1G_15613	11	29.4	77.2	200	56	1	*B. cinerea* 40S ribosomal protein S4 (263 nt)
57	BC1G_04187	9.4	30.1	89.8	291	111	2	*B. cinerea* outer mitochondrial membrane protein porin (284 nt)
58	BC1G_03437	10	17	64.2	99	77	2	*B. cinerea* 40S ribosomal protein S17 (148 nt)
58	BC1G_12468	11	20.8	83.4	139	85	2	*B. cinerea* 60S ribosomal protein L18 (175 nt)
58	BC1G_15154	11	18.4	50.9	33	43	1	*B. cinerea* 60S ribosomal protein L21 (161 nt)
59	BC1G_00988	12	18.5	66.7	134	94	2	*B. cinerea* 40S ribosomal protein S11 (162 nt)
59	BC1G_08345	11	20.1	46	137	104	2	*B. cinerea* 60S ribosomal protein L11 (176 nt)
60	BC1G_03667	9.9	17.9	36.7	33	96	1	*B. cinerea* 60S ribosomal protein L12 (166 nt)
60	BC1G_15344	10	19	62.4	102	44	1	*B. cinerea* 40S ribosomal protein S10 (170 nt)
61	BC1G_02443	8.7	33.2	56	130	109	1	*B. cinerea* hypothetical protein (302 nt)
63	BC1G_08348	6.6	58.5	47	139	188	2	*B. cinerea* glucose-6-phosphate 1-dehydrogenase (508 nt)
63	BC1G_11051	6.1	41.7	53.9	101	124	2	*B. cinerea* hypothetical protein (401 nt)
64	BC1G_15108	5.6	38.2	53.1	151	129	2	*B. cinerea* hypothetical protein (343 nt)
65	BC1G_00185	5.1	39.7	41.6	135	181	2	*B. cinerea* predicted protein (368 nt)
66	BC1G_11347	5.6	62.8	67.5	294	289	2	*B. cinerea* hypothetical protein (573 nt)
67	BC1G_08819	5.4	37.4	85.2	195	23	1	*B. cinerea* fructose-1.6-bisphosphatase (344 nt)
67	BC1G_14880	5.4	39.4	69.6	247	76	2	*B. cinerea* hypothetical protein (369 nt)
68	BC1G_06102	5.5	30.7	46	74	104	2	*B. cinerea* hypothetical protein (272 nt)
68	BC1G_06103	5.6	53.6	43	127	x	x	*B. cinerea* hypothetical protein (472 nt)
68	BC1G_08348	6.6	58.5	51.8	165	176	2	*B. cinerea* glucose-6-phosphate 1-dehydrogenase (508 nt)
69	BC1G_11376	6.7	54.5	45	213	195	2	*B. cinerea* dihydrolipoyl dehydrogenase. Mitochondrial precursor (511 nt)
70	BC1G_00059	6.1	25.7	77.4	330	117	2	*B. cinerea*-conserved hypothetical protein (230 nt)
71	BC1G_01968	6.3	57.6	80	366	199	2	*B. cinerea* hypothetical protein (510 nt)
72	BC1G_10557	6.6	58.7	78.7	335	158	2	*B. cinerea* hypothetical protein similar to UDP-glucose pyrophosphorylase (526 nt)
73	BC1G_10112	5.7	41.5	67.5	148	40	2	*B. cinerea* cyanide hydratase (372 nt)
74	BC1G_03920	9.2	38.2	76.1	309	166	2	*B. cinerea* hypothetical protein (339 nt)
75	BC1G_05133	5.2	16.4	39.5	71	192	2	*B. cinerea* hypothetical protein (157 nt)
76	BC1G_14488	6.4	21.9	50	156	177	2	*B. cinerea* hypothetical protein (192 nt)
77	BC1G_00383	6.3	24.6	71.7	150	111	2	*B. cinerea* GTP-binding nuclear protein ran-1 (219 nt)
78	BC1G_04230	7.7	30.9	72.8	261	141	2	*B. cinerea* hypothetical protein (290 nt)
79	BC1G_14078	6.9	35.8	76.1	248	185	2	*B. cinerea* hypothetical protein (331 nt)
80	BC1G_04783	6.5	24.5	47.7	111	194	2	*B. cinerea* hypothetical protein (235 nt)
82	BC1G_10557	6.6	58.7	75.1	269	126	2	*B. cinerea* hypothetical protein similar to UDP-glucose pyrophosphorylase (526 nt)
82	BC1G_12462	6	28.8	68.1	94	277	3	*B. cinerea* hypothetical protein (254 nt)
84	BC1G_06851	7.8	52.8	69	299	205	2	*B. cinerea* hypothetical protein similar to serine hydroxymethyltransferase (478 nt)
85	BC1G_05989	9.4	57.7	63.1	253	114	2	*B. cinerea* ATP-citrate synthase subunit 1 (531 nt)
86	BC1G_02144	6.7	74.4	64.9	315	214	2	*B. cinerea* hypothetical protein (667 nt)
87	BC1G_16089	9.6	52.9	40.8	171	149	2	*B. cinerea* fumarate reductase flavoprotein subunit (485 nt)
88	BC1G_12242	6.5	64.1	58.9	275	143	2	*B. cinerea* hypothetical protein (574 nt)
88	BC1G_13616	6.4	61	45.4	139	61	1	*B. cinerea* isocitrate lyase (549 nt)
89	BC1G_13706	6.7	49	56.8	241	192	2	*B. cinerea* hypothetical protein (440 nt)
90	BC1G_02472	9.4	48.2	60.4	186	249	2	*B. cinerea* ubiquinol-cytochrome-c reductase complex core protein 2 (462 nt)
91	BC1G_03337	4.6	17.8	58.5	70	243	2	*B. cinerea* hypothetical protein (159 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
92	BC1G_06110	5.1	35.6	52.2	160	214	2	*B. cinerea* hypothetical protein (312 nt)
93	BC1G_00266	5.5	34.9	43.7	96	111	2	*B. cinerea* hypothetical protein (325 nt)
94	BC1G_00033	7.7	45.2	42	100	44	1	*B. cinerea* hypothetical protein (419 nt)
94	BC1G_01219	5.2	42.9	55.1	115	332	2	*B. cinerea* hypothetical protein (381 nt)
95	BC1G_03473	5.5	39.4	30.1	58	240	3	*B. cinerea* hypothetical protein similar to oxaloacetate acetylhydrolase (366 nt)
95	BC1G_13829	5.3	44.1	61.3	200	151	2	*B. cinerea* actin-related protein 2 (390 nt)
96	BC1G_06571	5.3	37.8	61.9	192	96	1	*B. cinerea* adenosine kinase (349 nt)
97	BC1G_15906	5.8	48	50.4	192	87	2	*B. cinerea* ATP-specific succinyl-CoA synthetase beta subunit (446 nt)
98	BC1G_01354	5.2	40.9	54.4	175	203	2	*B. cinerea* saccharopine dehydrogenase (373 nt)
99	BC1G_12601	5.4	42.2	50	125	199	2	*B. cinerea* hypothetical protein (382 nt)
100	BC1G_00769	5.4	86	37	160	163	2	*B. cinerea* hypothetical protein (791 nt)
100	BC1G_13753	4.9	46.6	37.1	65	x	x	*B. cinerea* hypothetical protein (428 nt)
101	BC1G_05626	5.6	38.6	35.4	70	187	3	*B. cinerea* hypothetical protein (339 nt)
101	BC1G_16318	8.9	23.3	70.4	94	116	2	*B. cinerea* hypothetical protein (206 nt)
102	BC1G_06515	5.4	74.7	50.8	177	230	2	*B. cinerea* hypothetical protein (666 nt)
103	BC1G_02363	5.2	82	24.7	103	55	1	*B. cinerea* hypothetical protein (728 nt)
103	BC1G_03729	5.3	78.8	41.7	212	74	1	*B. cinerea* hypothetical protein (714 nt)
104	BC1G_10065	5.2	51.8	44.4	136	155	2	*B. cinerea* hypothetical protein (471 nt)
104	BC1G_11238	5.2	58.6	35.8	99	x	x	*B. cinerea* hypothetical protein (542 nt)
104	BC1G_11314	5.4	40.7	4.9	x	178	1	*B. cinerea* hypothetical protein (371 nt)
106	BC1G_09341	5.5	61.2	49.1	187	234	2	*B. cinerea* hsp60 (583 nt)
106	BC1G_10581	5	53.2	60.5	170	85	2	*B. cinerea*-conserved hypothetical protein (479 nt)
107	BC1G_04759	5.6	55	21.1	35	91	2	*B. cinerea*-conserved hypothetical protein (511 nt)
107	BC1G_14947	5.4	59.9	57.8	263	136	2	*B. cinerea* T-complex protein 1. Theta subunit (550 nt)
108	BC1G_10814	5.4	56.7	48.2	187	154	2	*B. cinerea* hypothetical protein (515 nt)
109	BC1G_04759	5.6	55	55.8	208	248	2	*B. cinerea*-conserved hypothetical protein (511 nt)
110	BC1G_04232	5.4	57.7	75.9	354	211	2	*B. cinerea* vacuolar ATP synthase subunit B (518 nt)
111	BC1G_09363	5.5	57.4	57.2	316	164	2	*B. cinerea* hypothetical protein (514 nt)
112	BC1G_07795	6.4	72.8	71.3	332	233	2	*B. cinerea*-conserved hypothetical protein (663 nt)
113	BC1G_10523	5.6	119	33.7	234	153	2	*B. cinerea* hypothetical protein (1048 nt)
114	BC1G_08339	5.9	65.1	75.1	389	237	2	*B. cinerea* hypothetical protein (595 nt)
115	gi|85083792	6	81.6	25.5	81	121	1	NADH-ubiquinone oxidoreductase 78 kDa subunit precursor (CI-78KD) [*Neurospora crassa* OR74A]
116	BC1G_12439	6.4	34.5	69.7	143	128	2	*B. cinerea* alpha-ketoglutarate dehydrogenase E1 component (300 nt)
117	BC1G_12797	5.3	66.6	40.3	170	124	2	*B. cinerea* translation initiation factor eIF3 (601 nt)
118	BC1G_06097	5.7	52	60.5	249	221	2	*B. cinerea* hypothetical protein (471 nt)
119	BC1G_06223	5.9	70	59.4	261	150	2	*B. cinerea* hypothetical protein (608 nt)
121	BC1G_11707	5.7	44	41.4	66	136	2	*B. cinerea* translation elongation factor EF-Tu (399 nt)
121	BC1G_12973	9.2	58.5	53.7	139	94	2	*B. cinerea* fumarate hydratase (540 nt)
122	BC1G_00939	5.3	47	56.3	240	67	1	*B. cinerea* hypothetical protein (416 nt)
123	BC1G_01188	5.8	38.3	56	120	151	2	*B. cinerea* hypothetical protein (359 nt)
124	BC1G_10921	9.6	57.4	34.1	96	191	2	*B. cinerea* hypothetical protein (507 nt)
125	BC1G_11550	5.9	11.6	97.2	144	219	2	*B. cinerea* hypothetical protein (108 nt)
125	gi|85792456	5.3	43	84.6	386	289	3	Mannitol-1-phosphate dehydrogenase [*Botryotinia fuckeliana*]
126	BC1G_05297	6.2	46.5	30.6	63	163	3	*B. cinerea* hypothetical protein (408 nt)
126	BC1G_16075	5.7	29.3	51.1	107	133	2	*B. cinerea* phosphatidylinositol/phosphatidylcholine transfer protein (264 nt)
127	BC1G_13607	5.8	40.3	44.6	92	90	1	*B. cinerea* peptidyl-prolyl *cis–trans* isomerase (372 nt)
127	BC1G_15180	5.6	40.2	29.3	49	42	1	*B. cinerea* L-arabinitol 4-dehydrogenase (375 nt)
128	BC1G_14194	5.7	41.1	57.7	189	271	2	*B. cinerea* hypothetical protein (383 nt)
129	BC1G_09769	4.9	79.3	39.2	144	140	2	*B. cinerea* hypothetical protein (712 nt)
130	BC1G_02852	8.8	45.6	66.1	237	231	2	*B. cinerea* hypothetical protein (410 nt)
131	BC1G_15638	6.2	106	35.9	201	101	2	*B. cinerea* translation elongation factor eEF-3 (947 nt)
133	BC1G_12307	6	86.3	44.5	163	122	2	*B. cinerea* hypothetical protein similar to cobalamin-independent methionine synthase (768 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
134	BC1G_01508	6	92.9	49.4	330	193	2	*B. cinerea* phosphoketolase (822 nt)
135	BC1G_06374	6.5	53.9	66	318	187	2	*B. cinerea* hypothetical protein (477 nt)
136	BC1G_05305	6.9	56.7	50.4	167	79	2	*B. cinerea* hypothetical protein (518 nt)
137	BC1G_07090	5.9	72.2	39.9	202	214	2	*B. cinerea* hypothetical protein (652 nt)
138	BC1G_04076	6.2	53.8	53.5	248	113	2	*B. cinerea* hypothetical protein (499 nt)
139	BC1G_00483	6.4	55.7	46.8	207	68	2	*B. cinerea* hypothetical protein (494 nt)
140	BC1G_09443	7.8	60.9	64.8	273	116	2	*B. cinerea* malate synthase (543 nt)
141	BC1G_12883	5.7	61.9	47.8	223	205	2	*B. cinerea* hypothetical protein (540 nt)
142	BC1G_02778	6.1	31.7	57.5	182	209	2	*B. cinerea* hypothetical protein (285 nt)
143	BC1G_02852	8.8	45.6	63.4	235	226	2	*B. cinerea* hypothetical protein (410 nt)
144	BC1G_05616	5.5	73.3	50.1	240	236	2	*B. cinerea* predicted protein (681 nt)
145	BC1G_08475	5.4	67	44.5	194	207	2	*B. cinerea*-conserved hypothetical protein (613 nt)
146	BC1G_01538	5.5	106	56	374	164	2	*B. cinerea* hypothetical protein (953 nt)
147	BC1G_14651	5.5	62.1	38	192	232	2	*B. cinerea* phosphoenolpyruvate carboxykinase (561 nt)
148	BC1G_12011	5.5	40.7	41.2	115	267	2	*B. cinerea* glutamine synthetase (369 nt)
149	BC1G_02594	6.9	45.5	5.4	x	130	2	*B. cinerea* hypothetical protein (429 nt)
150	BC1G_12988	6.9	112	37.4	271	123	1	*B. cinerea* hypothetical protein (1045 nt)
151	BC1G_07479	9.1	65.7	48.4	275	188	2	*B. cinerea*-conserved hypothetical protein (589 nt)
153	BC1G_00557	9	105	42.4	189	68	2	*B. cinerea* hypothetical protein (940 nt)
153	BC1G_06628	6.5	67.2	35	102	121	2	*B. cinerea* hypothetical protein similar to septin 2 (588 nt)
154	BC1G_13490	5.9	49.1	61.4	336	144	2	*B. cinerea* NADP-specific glutamate dehydrogenase (451 nt)
155	BC1G_15049	5.9	49.4	31.8	153	138	2	*B. cinerea* saccharopine reductase (449 nt)
156	BC1G_11968	5.9	36.7	71.7	203	150	2	*B. cinerea* glyceraldehyde 3-phosphate dehydrogenase (339 nt)
157	BC1G_08301	9.2	40.8	14.5	x	415	3	*B. cinerea* ascorbate peroxidase (373 nt)
159	BC1G_04780	4.9	34.1	63.2	223	115	2	*B. cinerea* hypothetical protein (299 nt)
159	BC1G_13374	5	32.5	32.2	71	60	1	*B. cinerea* hypothetical protein (304 nt)
160	BC1G_08794	7.7	40.8	40.5	138	133	2	*B. cinerea* pyruvate dehydrogenase E1 component beta subunit (373 nt)
161	BC1G_08983	5.3	35.4	29.9	67	246	2	*B. cinerea* hypothetical protein similar to spermidine synthase (311 nt)
162	BC1G_05298	5.4	29.5	66.7	225	70	2	*B. cinerea* hypothetical protein (264 nt)
163	BC1G_05298	5.4	29.5	66.7	206	81	2	*B. cinerea* hypothetical protein (264 nt)
164	BC1G_01804	4.6	31.8	63.6	189	169	3	*B. cinerea* 40S ribosomal protein S0 (297 nt)
165	BC1G_01804	4.6	31.8	62.3	188	181	3	*B. cinerea* 40S ribosomal protein S0 (297 nt)
166	BC1G_11454	5.2	36.3	69.4	293	162	2	*B. cinerea* hypothetical protein (324 nt)
169	BC1G_11083	6.3	36.1	60.4	161	130	2	*B. cinerea* alcohol dehydrogenase (341 nt)
171	BC1G_04987	6.7	38.3	91.4	344	184	2	*B. cinerea* hypothetical protein (338 nt)
172	BC1G_06124	6.1	38.9	48.6	97	218	2	*B. cinerea* hypothetical protein similar to mannose-1-phosphate guanylyltransferase (354 nt)
172	gi|111065380	10	50.2	6.3	x	153	2	Hypothetical protein SNOG_06669 [*Phaeosphaeria nodorum* SN15]
174	BC1G_12319	8.8	27	53.7	84	128	1	*B. cinerea* NAD-dependent formate dehydrogenase (246 nt)
174	BC1G_16156	10	28.6	68.3	171	267	2	*B. cinerea* NADH-ubiquinone oxidoreductase 40 kDa subunit (252 nt)
176	BC1G_07285	9.3	41.7	11.7	33	241	3	*B. cinerea* hypothetical protein (385 nt)
177	BC1G_02443	8.7	33.2	8.9	22	63	1	*B. cinerea* hypothetical protein (302 nt)
178	BC1G_07492	6.8	44.8	65.7	251	195	2	*B. cinerea* hypothetical protein (417 nt)
179	BC1G_04985	6.1	54.4	43.1	133	90	1	*B. cinerea* hypothetical protein (485 nt)
179	BC1G_07094	6.6	56.4	31.8	60	x	x	*B. cinerea* hypothetical protein (493 nt)
180	BC1G_02348	6.1	30.9	72.6	247	161	2	*B. cinerea* hypothetical protein (285 nt)
185	BC1G_01327	5.3	50.8	44.2	176	244	2	*B. cinerea* hypothetical protein (455 nt)
187	BC1G_07142	5.1	68.4	64.2	292	183	2	*B. cinerea* hypothetical protein (617 nt)
188	BC1G_03226	5.1	77.1	47.8	261	170	2	*B. cinerea* hypothetical protein similar to NADPH cytochrome P450 oxidoreductase (693 nt)
189	BC1G_11655	5.8	115	23.8	111	113	2	*B. cinerea* hypothetical protein (1050 nt)
190	BC1G_02682	5.6	95	50.6	288	112	2	*B. cinerea* hypothetical protein (842 nt)
191	BC1G_06804	5.3	61.8	52.8	242	76	2	*B. cinerea* hypothetical protein (591 nt)
192	BC1G_04169	5.5	81.5	41.7	174	261	2	*B. cinerea* hypothetical protein (762 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
193	BC1G_03007	6.2	40.3	65.4	182	176	2	*B. cinerea* hypothetical protein (358 nt)
194	BC1G_09731	5.9	85.9	49.2	216	205	2	*B. cinerea* hypothetical protein similar to elongation factor 2 (775 nt)
195	BC1G_14312	5.4	58.3	69.4	228	212	2	*B. cinerea* hypothetical protein (510 nt)
196	BC1G_04451	6.3	101	42.8	307	146	2	*B. cinerea* hypothetical protein (940 nt)
197	BC1G_09030	6.3	58.2	54.7	248	172	2	*B. cinerea* hypothetical protein (548 nt)
198	BC1G_03007	6.2	40.3	80.2	267	161	2	*B. cinerea* hypothetical protein (358 nt)
199	BC1G_05496	5.7	89.4	39.9	253	91	2	*B. cinerea* hypothetical protein (797 nt)
200	BC1G_14217	6.2	58.9	62.1	281	222	2	*B. cinerea* acetyl-CoA hydrolase (531 nt)
201	BC1G_04159	6.3	72.4	48.1	321	118	2	*B. cinerea* hypothetical protein (643 nt)
202	BC1G_04300	5.6	53.6	46	120	210	2	*B. cinerea* hypothetical protein (487 nt)
202	BC1G_09350	5.5	51.5	41.6	111	181	3	*B. cinerea*-conserved hypothetical protein (471 nt)
203	BC1G_09514	6	75.4	39.6	258	131	2	*B. cinerea* hypothetical protein (670 nt)
204	BC1G_01381	5	51.4	40.8	92	90	2	*B. cinerea* hypothetical protein (468 nt)
204	BC1G_13687	5.4	48.4	53.4	139	45	1	*B. cinerea* dihydrolipoamide acetyltransferase E2 of pyruvate dehydrogenase complex (464 nt)
207	BC1G_01554	5.7	47.5	34	80	x	x	*B. cinerea* hypothetical protein (424 nt)
207	BC1G_03240	5.9	41.3	44.3	102	193	2	*B. cinerea* hypothetical protein (368 nt)
207	BC1G_12069	6	46.2	40.4	64	98	1	*B. cinerea* hypothetical protein (416 nt)
208	BC1G_06362	5.7	53.7	69.2	286	203	2	*B. cinerea* hypothetical protein (497 nt)
209	BC1G_04232	5.4	57.7	68.3	361	152	2	*B. cinerea* vacuolar ATP synthase subunit B (518 nt)
210	BC1G_08895	4.8	28.8	50.4	89	205	2	*B. cinerea* hypothetical protein (262 nt)
211	BC1G_11866	4.9	85.3	39.5	145	93	2	*B. cinerea* hypothetical protein (745 nt)
212	BC1G_06967	5.5	72.7	67	403	192	2	*B. cinerea* hypothetical protein similar to fimbrin (651 nt)
214	BC1G_00347	6.3	77.7	48.4	207	93	2	*B. cinerea* hypothetical prot similar to glucosamine-fructose-6-phosphate aminotransferase (702 nt)
214	BC1G_13832	6.4	46.3	58.5	163	204	3	*B. cinerea* hypothetical protein (422 nt)
215	BC1G_09186	6.6	65.2	49.8	265	198	2	*B. cinerea* hypothetical protein (610 nt)
216	BC1G_04788	6.1	35.6	85.8	261	271	2	*B. cinerea* alcohol dehydrogenase (324 nt)
217	BC1G_07745	6.3	59.2	38.6	234	112	2	*B. cinerea*-conserved hypothetical protein (541 nt)
218	BC1G_06844	8.8	38	49.7	143	205	2	*B. cinerea* hypothetical protein (354 nt)
219	BC1G_07699	4.9	17.7	43.8	58	182	3	*B. cinerea*-conserved hypothetical protein (160 nt)
220	BC1G_05706	5.3	19.1	86.9	161	16	1	*B. cinerea* hypothetical protein (198 nt)
221	BC1G_09782	4.9	17.9	25.9	57	138	2	*B. cinerea* translation initiation factor eIF-5A (166 nt)
223	BC1G_00152	6.4	25.7	68.8	125	170	2	*B. cinerea* hypothetical protein (234 nt)
223	BC1G_07903	4.9	18.3	67.3	119	257	2	*B. cinerea* hypothetical protein (165 nt)
223	BC1G_14077	5.1	34.3	35.3	63	x	x	*B. cinerea* hypothetical protein (312 nt)
224	BC1G_15795	5.1	30.2	28.9	91	92	2	*B. cinerea* 20S proteasome subunit Y7 (280 nt)
225	BC1G_02778	6.1	31.7	25.6	74	128	2	*B. cinerea* hypothetical protein (285 nt)
225	BC1G_12159	5.1	32.7	45.4	100	80	1	*B. cinerea* hypothetical protein (293 nt)
227	BC1G_02778	6.1	31.7	54.4	112	180	3	*B. cinerea* hypothetical protein (285 nt)
227	BC1G_09132	6.4	29.8	66.2	162	96	2	*B. cinerea* hypothetical protein (272 nt)
228	BC1G_14431	5.5	27.6	43.5	155	165	2	*B. cinerea* glucose 1-dehydrogenase (260 nt)
229	BC1G_04452	5.7	37.5	79.5	315	139	2	*B. cinerea* eukaryotic translation initiation factor 3 (336 nt)
230	BC1G_10614	5.8	39.4	72.9	338	143	2	*B. cinerea* hypothetical protein (351 nt)
232	BC1G_01297	6.6	54.5	24.4	64	173	3	*B. cinerea* hypothetical protein (475 nt)
233	BC1G_00206	9.4	41.4	32.7	70	x	x	*B. cinerea* isocitrate dehydrogenase subunit 1. Mitochondrial precursor (379 nt)
233	BC1G_02655	9.4	48	30.4	54	x	x	*B. cinerea* acyl-CoA dehydrogenase family protein (438 nt)
233	BC1G_04778	9.2	41.1	36.5	87	109	2	*B. cinerea* GTP-binding protein (370 nt)
234	BC1G_15535	8.6	43.9	28.4	61	137	3	*B. cinerea* hypothetical protein (402 nt)
237	BC1G_13777	5.3	52	15.3	19	30	1	*B. cinerea* secretory pathway Rab GDP dissociation inhibitor (472 nt)
241	BC1G_15301	9.8	46.1	67.6	262	145	3	*B. cinerea* glutamate oxaloacetate transaminase 2 (426 nt)
242	BC1G_07909	9.5	50.6	38.7	179	47	1	*B. cinerea* hypothetical protein (447 nt)
243	BC1G_14663	10	34.3	30.5	63	62	2	*B. cinerea* hypothetical protein (302 nt)
244	BC1G_05989	9.4	57.7	50.3	175	146	2	*B. cinerea* ATP-citrate synthase subunit 1 (531 nt)
245	BC1G_15101	6.4	51.2	51.1	155	299	2	*B. cinerea* hypothetical protein (470 nt)
246	BC1G_05989	9.4	57.7	50.3	180	94	1	*B. cinerea* ATP-citrate synthase subunit 1 (531 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
247	BC1G_14516	7.8	71.9	34.8	68	348	5	*B. cinerea* hypothetical protein (633 nt)
248	BC1G_11336	6.4	89.3	52.2	304	125	2	*B. cinerea* hypothetical protein (788 nt)
249	BC1G_15638	6.2	106	31.3	153	21	1	*B. cinerea* translation elongation factor eEF-3 (947 nt)
252	BC1G_00471	4.8	55.4	50.8	108	73	2	*B. cinerea*-conserved hypothetical protein (504 nt)
252	BC1G_00472	9.3	50.4	49.7	93	x	x	*B. cinerea*-conserved hypothetical protein (465 nt)
252	BC1G_03668	6	68.7	39.6	88	131	2	*B. cinerea* protein transport protein sec23s (621 nt)
253	BC1G_07152	5.4	48.6	55	194	152	3	*B. cinerea* hypothetical protein (447 nt)
254	BC1G_07780	9.6	60.8	59.8	269	203	2	*B. cinerea* hypothetical protein similar to mitochondrial ATP synthase alpha subunit (562 nt)
255	BC1G_15049	5.9	49.4	59.7	226	146	2	*B. cinerea* saccharopine reductase (449 nt)
257	BC1G_05226	5.9	33	62.3	133	94	2	*B. cinerea* hypothetical protein (300 nt)
257	BC1G_05617	5.7	50.4	58.5	172	169	2	*B. cinerea* hypothetical protein (458 nt)
258	BC1G_09363	5.5	57.4	25.5	65	30	1	*B. cinerea* hypothetical protein (514 nt)
259	BC1G_07012	5.9	49	65.3	116	36	1	*B. cinerea* hypothetical protein (458 nt)
259	BC1G_07967	6.6	92.7	21.6	43	57	1	*B. cinerea* hypothetical protein (850 nt)
259	BC1G_11228	8.9	61.2	71.2	282	181	2	*B. cinerea* hypothetical protein (553 nt)
260	BC1G_02079	5.2	54.3	77.7	229	65	1	*B. cinerea* hypothetical protein (503 nt)
261	BC1G_01271	6.4	33.1	66.3	181	142	2	*B. cinerea*-conserved hypothetical protein (294 nt)
262	BC1G_01260	5.4	38.3	67.1	223	164	2	*B. cinerea*-conserved hypothetical protein (359 nt)
263	BC1G_11347	5.6	62.8	56.7	202	113	1	*B. cinerea* hypothetical protein (573 nt)
266	BC1G_07147	6.7	52.8	56.2	196	161	2	*B. cinerea* hypothetical protein (475 nt)
266	BC1G_08475	5.4	67	41.6	112	178	2	*B. cinerea*-conserved hypothetical protein (613 nt)
267	BC1G_14004	5.4	59.2	73.8	326	186	2	*B. cinerea* hypothetical protein (545 nt)
268	BC1G_01594	6	39.3	31	63	114	2	*B. cinerea* hypothetical protein similar to endochitinase (364 nt)
269	BC1G_01292	6.3	88.7	28.4	164	58	1	*B. cinerea* hypothetical protein (805 nt)
270	BC1G_05448	5.7	31.8	45.7	181	130	2	*B. cinerea* hypothetical protein (293 nt)
271	BC1G_03241	5.5	48.9	64.2	225	146	2	*B. cinerea*-conserved hypothetical protein (450 nt)
272	BC1G_08581	5.3	43.3	56.7	161	151	2	*B. cinerea* hypothetical protein (386 nt)
272	BC1G_13183	5.4	48	39.8	83	41	1	*B. cinerea* hypothetical protein (450 nt)
273	BC1G_00939	5.3	47	59.4	232	215	2	*B. cinerea* hypothetical protein (416 nt)
275	BC1G_10846	5.1	46	32.9	45	104	2	*B. cinerea* hypothetical protein similar to hsp70 (425 nt)
275	BC1G_11821	9	61.6	50.5	168	266	2	*B. cinerea* hypothetical protein (572 nt)
275	BC1G_14762	4.7	39.6	39.5	76	26	1	*B. cinerea* D-ribulokinase (362 nt)
276	BC1G_02094	5.7	54.9	32.1	110	159	1	*B. cinerea* hypothetical protein (489 nt)
277	BC1G_00027	6.2	29.8	56.7	141	248	2	*B. cinerea* hypothetical protein (275 nt)
278	BC1G_00284	6.7	50	64	228	117	2	*B. cinerea* hypothetical protein (456 nt)
279	BC1G_11963	5	24.8	56.6	141	136	2	*B. cinerea* glycogen-branching enzyme (212 nt)
280	BC1G_12426	5.7	86.5	22.7	68	126	2	*B. cinerea* predicted protein (774 nt)
281	BC1G_06095	5.8	51.1	57.6	222	143	2	*B. cinerea* hypothetical protein (472 nt)
282	BC1G_05297	6.2	46.5	27.9	27	236	4	*B. cinerea* hypothetical protein (408 nt)
283	BC1G_05327	6.1	133	47.6	383	x	x	*B. cinerea* pyruvate carboxylase (1210 nt)
284	BC1G_11051	6.1	41.7	26.7	82	x	X	*B. cinerea* hypothetical protein (401 nt)
285	BC1G_06849	4.8	43	19.8	104	169	1	*B. cinerea* hypothetical protein similar to aspartic proteinase precursor (399 nt)
286	BC1G_02223	4.5	57.6	26.9	182	35	1	*B. cinerea* hypothetical protein (532 nt)
287	BC1G_08294	4.7	30.9	35.1	96.5	x	x	*B. cinerea* 14-3-3 protein homolog (276 nt)
288	BC1G_00383	6.3	24.6	41.6	68	104	1	*B. cinerea* GTP-binding nuclear protein ran-1 (219 nt)
289	BC1G_04759	5.6	55	38.4	115	89	1	*B. cinerea*-conserved hypothetical protein (511 nt)
290	BC1G_00121	5	45.1	31.3	104	x	x	*B. cinerea* hypothetical protein (406 nt)
291	BC1G_13197	5.7	125	30	255	x	x	*B. cinerea* L-aminoadipate-semialdehyde dehydrogenase large subunit (1131 nt)
292	BC1G_05327	6.1	133	43.3	432	x	x	*B. cinerea* pyruvate carboxylase (1210 nt)
293	BC1G_08174	9.6	26	33.2	99	x	x	*B. cinerea* hypothetical protein (235 nt)
294	BC1G_10819	5	30.4	26.3	90	x	x	*B. cinerea* hypothetical protein (304 nt)
295	BC1G_02778	6.1	31.7	27.7	73	x	x	*B. cinerea* hypothetical protein (285 nt)
296	BC1G_09983	4.6	23.2	19.7	44	x	X	*B. cinerea* nascent polypeptide-associated complex alpha polypeptide (213 nt)
297	BC1G_11887	4.7	67.4	14.7	76	x	x	*B. cinerea* hypothetical protein (605 nt)

Table 1. Continued

Spot no.	Acc. number	p*I*	MW	Cov (%)	Mascot MS	Mascot MS/MS	MS/MS peptides	Protein
298	BC1G_03007	6.2	40.3	36.6	100	x	x	*B. cinerea* hypothetical protein (358 nt)
299	BC1G_15149	5.3	109	16.1	112	x	x	*B. cinerea* cytoskeleton assembly control protein (962 nt)
300	BC1G_05074	7.8	34.5	29.7	124	x	x	*B. cinerea* hypothetical protein similar to succinyl-CoA synthetase (330 nt)
301	BC1G_05327	6.1	133	43.4	295	x	x	*B. cinerea* pyruvate carboxylase (1210 nt)
302	BC1G_06400	6.4	47.7	57.7	173	x	x	*B. cinerea*-conserved hypothetical protein (440 nt)
303	BC1G_07147	6.7	52.8	16.2	64.6	x	x	*B. cinerea* hypothetical protein (475 nt)
304	BC1G_01161	6.2	53	18.4	70	x	x	*B. cinerea* hypothetical protein (499 nt)
305	BC1G_00558	5.9	16	34.8	85	x	x	*B. cinerea* superoxide dismutase Cu-Zn (155 nt)
306	BC1G_06628	6.5	67.2	12.9	54	x	x	*B. cinerea* hypothetical protein similar to septin 2 (588 nt)

Acc Number, accesion number of the identified protein in the *B. cinerea* database from the Broad Institute (www.broad.mit.edu); p*I*, isoelectric point (theoretical); MW, molecular weight (theoretical); Cov, percent of the residues in each protein sequence that has been identified (from PMF when a PMF identification was successful); Mascot MS, Mascot score of PMF identification; Mascot MS/MS, Mascot score of the peptide fragmentation fingerprint identification; MS/MS peptides, number of peptides from MS/MS search.

buffered phenol, pH 8.0 (Biomol, Hamburg, Germany) was added, and the resulting mixture was vortexed for 30 s. The phenol phase was separated by centrifugation and transferred to a fresh tube. After addition of five volumes of cold 0.1 M ammonium acetate in methanol, the proteins were precipitated from the phenol phase overnight at −20°C. The precipitated proteins were recovered by centrifugation, washed twice with cold 0.1 M ammonium acetate in methanol and twice with 80% v/v acetone. The final pellet was air-dried and stored at −80°C. A separately precipitated aliquot was dissolved in a small volume of 8 M urea for protein quantification using the Bradford assay (Bio-Rad, München, Germany) with BSA as standard. The few 2-DE proteomic approaches carried on *B. cinerea* mycelia proteins were made on the basis of an extraction protocol based on phosphate buffer solubilization and TCA/acetone precipitation [3, 5]. However, the combined use of TCA precipitation and phenol extraction improved better spot definition, reduced streaking and led to a higher number of detected spots (data not shown). 2-DE was performed as follows: the first dimension was run using 17 cm IPG strips (3–10 non-linear pH gradient, Amersham) on a Bio-rad Protean IEF cell according to the manufacturer's instructions. The second dimension was run on a Protean II xi Cell (Bio-rad) using self-cast 14% acrylamide SDS-PAGE gels [14]. The gels were stained with Coomassie PAGE Blue (Fermentas, St. Leon-Rot, Germany) or Sypro Ruby (Invitrogen, Paisley, UK) stains according to the manufacturer's instructions (Fig. 1). Only those spots that were present in all the replicates were selected for identification process.

2-DE gels of mycelia cultured with glucose displayed a characteristic profile with 262 ± 18 detected spots, whereas gels of mycelia cultured with cellulose showed 318 ± 5 (data not shown), indicating that the use of a particular carbon source, such as glucose or cellulose, induces a characteristic fungal response. These differences will be analyzed in a further study (manuscript in preparation).

Protein spots were selected visually and robotically excised from Coomassie-stained gels and digested using the Proteineer spII and dp systems (Bruker Daltonic, Bremen, Germany). Following robotic target preparation, PMFdata were collected on an UltraflexIII MALDI ToF/ToF spectrometer (Bruker). LIFT MS/MS spectra [15] were then collected on selected precursors for verification of PMF identifications and further elucidation. Both MS and MS/MS data were used to search against the *B. cinerea* protein database (http://www.broad.mit.edu/ August 2007 release) or the NCBI non-redundant database (http://www.ncbi.nlm.nih.gov May 2007 release) using MASCOT (http://www.matrixscience.com). MS acquisition and database search parameters are available in Supporting Information. Further experimental details are available from the authors upon request.

In constructing the 2-DE map of *B. cinerea*, 267 spots excised from the gel yielded protein identifications by MALDI-TOF/TOF MS/MS analysis. The identifications represent 303 positive hits to proteins and their respective isoforms (Table 1), representing approximately 2% of the predicted proteins in the annotated genome. This information is available in the public database World-2DPAGE (www.expasy.org/world-2dpage/) for the *B. cinerea* research community (Accession number: 0005-"*B. cinerea* mycelium cultured in carboxymethyl cellulose"). Proteins with an important role during plant infection have been identified (Table 1) (*i.e.* spot 14 – *B. cinerea* peptidyl-prolyl *cis–trans* isomerase) [16]. Moreover, other proteins with a suggested role in the infection process are listed too (*i.e.* spot 156 – glyceraldehyde 3-phosphate dehydrogenase) [3, 17]. However, a large number of the identified proteins were annotated as "*B. cinerea* hypothetical proteins." These proteins were annotated by BLAST searches, and the results are listed in Supporting Information Table 2.

Identified proteins from *B. cinerea* mycelia were distributed into categories according to their molecular functions and involvement in biological processes based on PANTHER classification system (www.pantherdb.org/). Classification by molecular function showed that more than 40% of the proteins fell into three categories: (i) oxidoreductase 20%, (ii) nucleic acid binding 12% and (iii) synthase and synthetase 10% (Fig. 2A). Oxidoreductase activity (catalysis of redox reactions) has been well documented in *B. cinerea*. During infection process, AOS seem to play an important role. Plants produce a lot of AOS as a defense mechanism, but the fungus seems to produce these radicals as well. However, the fungus also produces free radical scavenger proteins and metabolites that protect it from AOS attack, such as oxalic acid, laccase, catalase and several peroxidases [18]. Moreover, some of these proteins have been directly related to virulence and toxin production [3, 19]. Nucleic acid-binding activity implies nucleotide-binding enzymes or DNA-binding proteins. Among other functions, the relevance of such proteins in the cascade of signals has been deeply studied in *B. cinerea* [20]. Ten percent of identified proteins (synthase and synthetase) have a role in the synthesis of several molecules, especially nucleoside triphosphates. Other significantly represented categories include transferases (8%), hydrolases, lyases and ligases (6%). Most of the proteins (45%) categorized by biological process (Fig. 2B) were grouped into three categories: (i) protein metabolism and modification (21%), (ii) carbohydrate metabolism (15%) and (iii) amino acid metabolism (9%). One of the goals of proteomic approaches is to study the processes related with protein modifications. Metabolic processes that involve protein synthesis, modification or degradation may

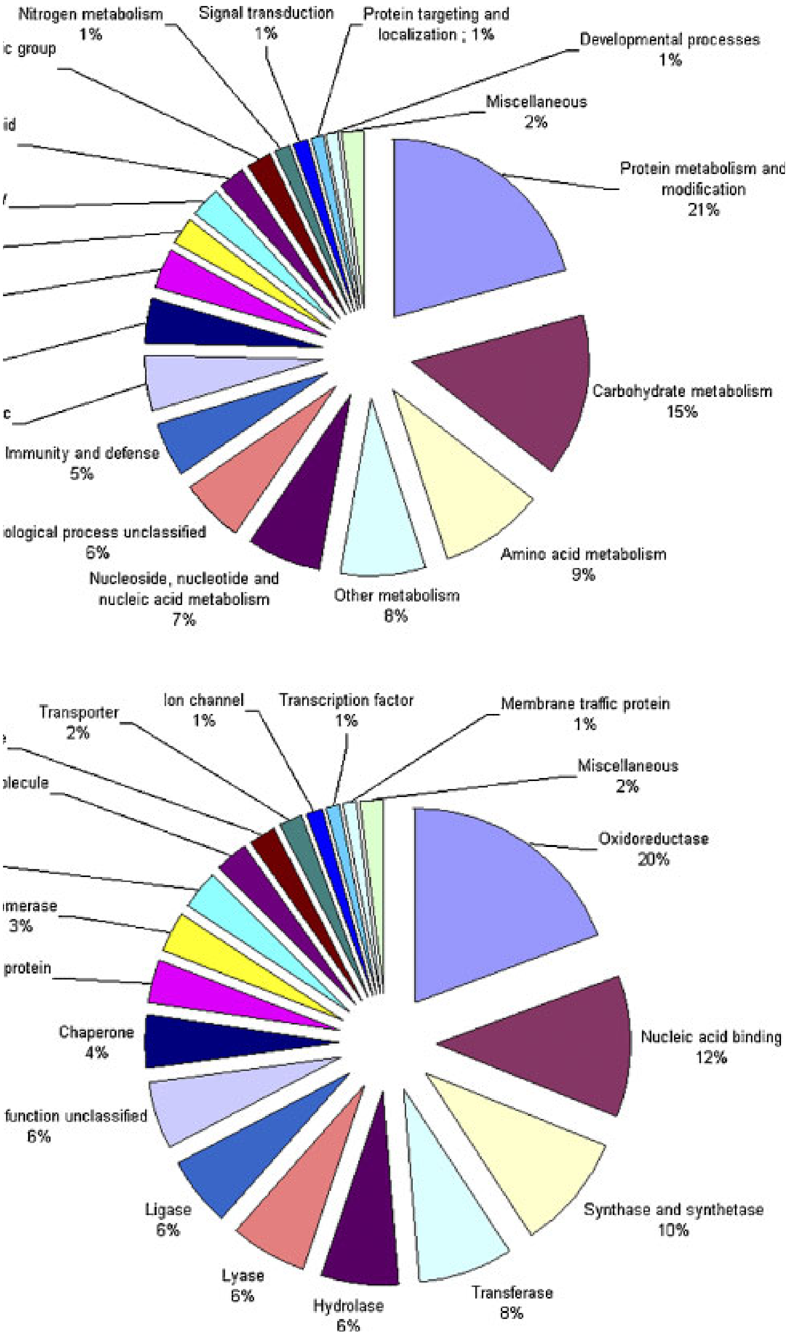

Figure 2. Representations of the distribution of identified *B. cinerea* proteins according to their (A) molecular function and (B) biological process. Categorizations were based on information provided by the online resource PANTHER classification system.

confer multiple biological functions to individual genes [21]. Thirty percent of the identified proteins are related with the biosynthesis, assembly, folding, translational regulation of proteins or amino acid biosynthesis transport, *etc*. The role of these modifications and their impact on virulence, intracellular signal cascades and other biological processes is an area for further investigation. Proteins related to carbohydrate metabolism (15%) are involved in the use of CMC as a sole carbon source. Since CMC is one of the major components of the plant cell wall, many of them may have a crucial role in the pathogenicity process.

In summary, this study describes the first proteomic analysis of the phytopathogenic fungus *B. cinerea*, containing an important number of identified proteins. Moreover, the use of CMC as a sole carbon source may reveal proteins involved in the infection cycle such as pathogenicity or virulence factors. The identification of these proteins, their PTMs, isoforms and subunits will provide a new source of knowledge to unravel the molecular basis of pathogenesis. Moreover, this *B. cinerea* 2-DE proteomic map can be a hypothesis driver for fungicide design based on new therapeutical targets. At present, there is no fungicide on the market that was developed on the basis of targeted molecular research [4]. Some of the identified proteins may also be used to develop a procedure for field diagnosis of plant infections [22].

This research has been financed by the Spanish DGICYT (MEC) (AGL2006-13401-C02-02/AGR) INTERNACIONALIZACION CICE - 2008 and by the Junta de Andalucia (P07-FQM-02689) Projects. The authors thank Ursula Wieneke for the excellent technical assistance.

The authors have declared no conflict of interest.

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