STUDY ON GENE TRANSFORMATION AND EXPRESSED SEQUENCE TAGS OF *Chaetomium globosum*

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ABSTRACT

In order to improve the bio-control fungus- *Chaetomium globosum,* and develop a new bio-fungicide, the resistance gene to benzimidazole fungicides was transformed into the fungus, and the expressed sequence tags (ESTs) of the fungus were sequenced. The results showed that the plasmid constructed in this study could improve the transformation rate significantly, (i.e. nearly 9 times higher than before). A cDNA library was constructed from the mycelium of the fungus and 3507 clones were randomly selected for sequencing. 1381 sequences were obtained, 868 of them are new genes. Analysis of the identified clones indicated sequence similarity to the genes encoding proteins such as enzymes, regulatory factors and the product related to plant disease bio-control.

Keywords: *Chaetomium globosum*, benzimidazole resistance, gene transformation, expressed sequence tags

1. INTRODUCTION

Chaetomium spp. have a world wide distribution. Their potential in plant disease biological control is well known. They protect plants from diseases by producing many kinds of antibiotics and ergosterols that can suppress plant pathogens, stimulate growth of plants and induce resistance of plants (Soytong and Soytong, 1997; Yang, *et al.*, 2000; Dipietro *et al.*, 1992). They can be used to control many soil born diseases, especially of vegetable and fruits. However, although they are very effective plant disease bio-control micro-organisms, they are very sensitive to benzimidazole fungicides, and can not be used in the field where chemical fungicides such as benzimidazole compounds are employed. Therefore, when the plant pathogenic micro-organisms develop resistance to these chemical fungicides, serious economic losses can occur.

One method to improve those plant disease bio-control agents is by producing organisms with the resistant to benzimidazole compounds. Various ways to achieve these resistant organisms were tried but none were successful. To overcome this difficulty, work on *C. globosum* with genetic engineering methods has been carried out since 1995. Isolates of *C. globosum* with high level and stable resistance to benzimidazoles were achieved in this study.

Since the genetic background of *C. globosum* was not clear, especially the genetic information about the bio-control related products from the fungus, the EST of the fungus were sequenced in this study. The information achieved will provide full support to develop new generation of bio-fungicides for plant disease control in future. This work will be able to promote plant disease bio-control in practice not only in China but also in the world.

2. MATERIALS AND METHODS

Materials

Isolates of *C. globosum* were collected and stored in the laboratory of Life Science and Engineering Department, Harbin Institute of Technology.

The plasmid pTA-*TUB2* containing the carbendazim-resistance gene was constructed by incorporating the resistance gene from pRB129 (Stanford University in USA) into plasmid pTA in order to improve the gene transformation rate.

PDA and PD media were used for growing the fungus in both gene transformation and genome study experiments.

Carbendazim (50% WP) was applied in the resistance test and transformant selection.

Test of the original resistance level of *Chaetomium globosum.*

Chaetomium globosum was cultured on PDA containing different concentrations of carbendazim (0, 0.05, 0.10, 0.25, 0.4, 0.6 μg/ml) at 28 °C, every concentration was repeated 3 times.

Bio-control regulated genes of *C. globosum*

Chitinase 3 gene (BP099205), cyclic peptide HC-toxin gene (BP099113) and fucose-specific lectin gene (BP099087) may all produce different antibiotics to suppress the plant pathogens. The aflatoxin biosynthesis ketoreductase (BP113123) and aflatoxin efflux pump genes (BP099694) are also possibly related to the production of the antibiotics.

Ergosterol biosynthesis gene (BP113299) (Soytong, 2000) produced the substances that can help to improve the humus layer soils which leads to higher soil fertility and induction of immunity of plants. Nodulin gene (BP113274) (Kouchi and Hata, 1993) were expressed long before the onset of nitrogen fixation. BP113299 and BP113274 may promote the growth of different plants.

Multidrug resistance protein (BP099630) and proliferating cell nuclear antigen (BP113075) probably produce induction of plant immunity for disease resistance.

Protoplast preparation.

Chaetomium globosum was grown in shake cultures of PD for 24h (170 rev/min, 28°C). Mycelium was collected by centrifugation (3000 rev/min, 5 min) and washed in DM (1.2M sorbitol, 10mM tris, pH 7.0), re-suspended in 0.8% Novozym 234 in DM and digested for 1 h at 30 °C; then the protoplasts were separated from cellular debris by filtration through lens paper and centrifuged (3000 rev/min, 5min), washed in the DM and TM (1.0M sorbitol, 10mM Tris, pH 7.5, 20 mM $CaCl_2$), and then re-suspended in TM.

The resisstance gene transformation

Transformation protocols were a modification of the method of Mark (Yang, 1995; Mark, *et al.*, 1990). Protoplasts (1×10^6) in 10 μl TM were mixed with circular pRB129 (1.5 μg in 10 μl) TE and incubated on ice for 30 min. Twenty five μl of 60% PEG in TM were added and the protoplasts were incubated on ice for an additional 10 min. After the addition of 0.5 ml PEG and 10 min incubation at room temperature, the protoplasts were diluted in 2 ml of RM. Aliquots were mixed in 10 ml of molten (46 °C) RA, put in plates and incubated for 24 h at 25 °C. The plates were then overlaid with 10 ml of RA containing 10 μg/ml carbendazim. The plates were incubated at 25 °C to select the carbendazim-resistant transformants.

Bioassay of the transformants.

The transformants were tested by culturing on PDA containing 0, 50, 100, 200, 300, 500, 1000 μg/ml carbendazim to test the transformants resistance level. Then the transformants were subcultured in unselective medium for 10 times before testing their resistance level to different concentrations of carbendazim to examine for resistance stability.

RNA Isolation and DNA library construction

Chaetomium globosum was grown in PD medium for 2 days, then the mycelia were collected and ground under liquid nitrogen using a mortar and pestle. Total RNA was extracted by the guanidium-isothiocyanate method, polyATtract® mRNA isolation System (Promega). First-strand cDNA was synthesized using an oligo-dT linker-primer with a XhoI cloning site. After the other end of each cDNA was ligated to an adaptor with an EcoRI-compatible overhang, cDNA was ligated directionally into the EcoRI and XhoI sites of the pBluescript II sk(+) vector (Stratagene).

EST Sequencing

The obtained plasmid library was used to transform *Escherichia coli* strain $DH5_{\alpha}$ by electroporation. The bacteria were grown for 60 min at 37 °C and then plated at low density on Luria-Bertani medium containing, X-Gal, IPTG, and Ap (60 mg/ml). After cultivation at 37 °C overnight, individual colonies were selected randomly for plasmid DNA purification and sequencing.

All sequencing reactions contained the standard T3 sequencing primer, which read into the presumed 5'end of each cDNA. Reactions were run and analyzed on capillary automated sequencing machines (MegaBACE 1000, Amersham Pharmacia).

EST Sequence analysis

The original sequence data analysis was performed on a Dawning 3000 super computer. The peak value files obtained from sequence measurement were analyzed with the Phred programs, and the sequences and the quality files were then carried out. The cross match file was used attempt alignments between the insert sequences and the sequence of the vector, and then the sequence of the expressed gene tags of the mycelium were identified by removing the sequence of the vector. The obtained ESTs were processed by Phrap software, and the contigs and singlets were obtained.

Homology study

Each EST was translated in all six reading frames and compared with the non-redundant database at the NCBI by using the BLASTX program, the sequences were then translated into the protein sequences. Default BLAST parameter values were used except the following settings: E-value = 1e-5 and Alignments =25 %.

Sequences without significant similarity were compared again with BLASTN, with E-value = 1e-5 and Alignments =30 %. Homologies to negative reading frames were disregarded, except the reverse insert clones. Putative identifications for the ESTs were assigned by the results of the BLAST searches.

3. RESULTS

The original sensitivity level of *C. globosum* to carbendazim

The results (**Table 1**) showed that carbendazim inhibited the growth of *C. globosum* effectively. The EC_{50} of the chemicals is 0.206~0.208 μg/ml. The fungi can not survive when the concentration was 0.6 μg/ml.

Table 1. Growth inhibition (%) of *C. globosum* by carbendazim

Treatment (μg/ml)	0	0.05	0.10	0.25	0.40	0.60
1	0	21.0	39.3	56.5	90.7	100
2	0	22.1	43.3	53.3	90.9	100
3	0	20.0	41.1	59.1	91.5	100
Mean	0	21.0	41.2	56.3	91.0	100
S_n-1	0	1.1	2.0	2.9	0.4	0

The resistance level of the transformants

The transformants were obtained from those selected with carbendazim after the resistance gene was transformed for 24 h. The transformants can survive on PDA containing 10 μg/ml carbendazim. The results of repeated experiments in this study showed that the transformation rate of fungus was 231 transformant/μg palsmid pTA-*TUB2*. This transformation rate was 9 times higher than that with plasmid pRB129 (27 transformant /μg palsmid pRB129).

The plating of transformants on PDA containing 0, 50, 100, 200, 500, 1000 μg/ml carbendazim indicated that the transformants of *C. globosum* could grow well on concentrations of carbendazim.

The resistance of transformants of the bio-control fungus were very stable after subculturing on nonselective medium for 10 times followed by culturing on PDA containing different concentrations of carbendazim .

RNA isolation and cDNA library construction

Before the mycelia were squeezed to complete dryness, they were used for mRNA isolation, since the excessive high density of the mycelia made it difficult to remove the polysaccharide from the cell walls, which could affect purification of mRNA. The titer of the constructed cDNA library was 2.25×10^4 clones/ml (un-amplified), the colony Blue/white ratio of the library was 17/450 (recombinant colonies were 96.4%). A library containing more than 112,500 recombinants was constructed. Seven thousand clones were selected for isolation sequencing of ESTs. The rest of the cDNA library was preserved under -80 °C in a freezer after amplification. The quality of the library was assessed by examining the insert sizes of 384 randomly-selected recombinant clones by specific PCR amplification with the T_7 and T_3 primers. Insert sizes ranged between 400 and 3000 bp with an average insert size of 1000 bp. Sequence analysis of 3507 randomly-selected clones from the library indicated that the library had no contaminating rRNA sequences. In addition, both full-length and near full-length sequences detected, indicate that the cDNA library was suitable for the collection of expressed sequence tags in this study.

EST sequencing

Of 4368 sequencing reactions conducted, 3507 readable sequences were achieved with single pass sequencing. The leading vector, trailing and poor-quality sequences were edited out. 3'vector and linker sequences were removed if poly(A+) were included in the sequencing results. The following three classes of anomalous sequences were also excluded: (1) sequences without inserts at all; (2) sequences with reverse inserts; (3) sequences with incorrect adapter.

Analysis of EST sequences

For achieving high quality of the ESTs, only the clones with an insert gene longer than 400 bp were selected for sequencing. The BLAST result of each comparison was screened manually. Sequences shorter than 100 bp were excluded. Finally, 3141 EST fragments were selected after screening. Putative identification of the EST fragments was attributed to each EST fragment. Three thousand one hundred and forty one EST fragments were then pieced together and 1381 sequences were created, in which 387 were contigs and 994 were singlets. Five hundred and thirteen sequences were annotated, indicating that they were all known genes; The 868 un-annotated sequences were new genes. After removing the repeated sequences in the annotated genes, there were 466 genes left. The homology between a *C. globosum* EST and the known sequence, deduced amino acid sequence and nucleotide sequence was deemed significant if the similarity score was greater than 80 (Altschul *et al.*, 1990). All *C. globosum* ESTs have been submitted to the International DNA Database (DDBJ) in Japan.

Identification of EST genes

Analysis of 1334 sequence revealed that 27.2 % of them were homologous to peptide sequences and nucleotide sequences present in the NCBI protein and nucleotide databases (**Table 2**). Of the remaining 72.8 %, 65.1 % of them did not show similarity to any sequence on the databases according to the search criteria used, and thus were interpreted as possibly representing new genes not only in *C. globosum* but also in all the organisms. The remaining 7.7 % although showing homology to previously-identified genes in the databases, were meaningless (i.e., similarity scores below 80) and thus they were putatively identified on the basis of sequence similarity only. Of the 1334 sequences analyzed, 26.3 % showed significant deduced amino acid sequence and nucleotide sequence homology to previously identified fungal genes. Twenty six sequences, although similar to fungal genes, did not have scores above 80 and thus could not be considered as homologous. As only 9 previously identified *Chaetomium* genes were registered with GenBank at the time of the database searches, all putative sequence identities to fungi genes came from fungus other than *Chaetomium*. Of the 325 identified homologous sequences, 69.2 % showed homology to model fungi species such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Neurospora crassa*. As expected, these proteins gave high similarity scores. Forty four ESTs (3.3 %) showed sequence similarity to previously identified genes from species other than fungus, and 2.85 % of these were considered homologous (**Table 2**). The targeted species were widely distributed from bacteria to human.

Apart from providing an efficient method for gene discovery, EST data can also provide information on gene expression (Ewing, *et al.*, 1999; Mekhedov, *et al.*, 2000; Ohlrogge and Benning, 2000). In the fungus *Rhizopus nigricans* the heat shock protein 70 (BP099021, Hsp70) transcripts were inducible with deoxycorticosterone and testosterone as well as with heat stress, ethanol, $CuSO_4$, and H_2O_2 (Cernila, *et al.*, 1999). An endogenous circadian biological clock controls the temporal aspects of life in most organisms, including rhythmic control of genes involved in clock output pathways. In the fungus *Neurospora crassa*, one pathway known to be controlled by the clock is asexual spore (conidia) development (Deborah Bell-Pedersen, *et al.*, 1996).

Table 2. *C. globosum* ESTs with sequence homology or similarity to known Non-fungus genes.

EST NO. Putative Identification and database Accession	Alignment	Score	E-value	Identity	Organism
BP099039 ribosomal protein S3 (AY098949)	66.05	211	3.00E-54	82.95	AT
BP113064 ribosomal protein L23a (BC026656)	54.19	170	1.00E-41	66.67	MM
BP099096 ribosomal protein S5 (AF429979)	58.56	214	5.00E-55	65.03	SF
BP099043 ribosomal protein L33-A (L37A)	63.89	150	2.00E-36	63.3	
BP113137 ribosomal protein L39 (X95458)	64.71	75.1	3.00E-13	66	ZM
BP099637 ribosomal protein L37A(AF052737)	62.64	127	9.00E-29	62.64	OO
BP113228 heat-shock protein 80 (AF221856)	35.4	78.6	7.00E-30	73.08	EE
BP098964 short-chain oxidoreductase (AL590464)	42.67	112	2.00E-24	44	SC
BP099030 caleosin (AF109921)	34.57	124	8.00E-28	51.67	SI
BP113074 dUTP pyrophosphatase-like protein (AL096859)	54.82	187	9.00E-47	66.91	AT
BP098973 oxidoreductase(AE001711)	40.12	152	3.00E-36	41.26	TM
BP099135 oxidoreductase (AL591791)	34.52	113	1.00E-24	35.15	SM
BP113179 oxidoreductase (AL590464)	25.74	114	2.00E-24	29.51	SC
BP098943 epoxide hydrolase (AL603642)	36.27	97.8	2.00E-20	36.92	SM
BP113110 ubiquitin (X61053)	61.94	99	1.00E-20	100	TP
BP099095 Cyanate hydratase (AP003585)	47.95	125	5.00E-28	47.3	Nsp.
BP113122 5'-nucleotidase (NC_001264)	37.01	137	7.00E-32	37.25	DR
BP113141 glutathione-S-transferase12(AE007268)	26.86	69.3	2.00E-11	34.31	SM
BP113157 major seed albumin (M17147)	26.84	99.4	4.00E-20	29.25	PS
BP113209 tripeptidyl-peptidase I precursor(AF111172)	31.26	190	2.00E-47	40.14	MM
BP113248 NAD-specific glutamate dehydrogenase	37.5	79.7	8.00E-15	92.68	
BP113274 nodulin (AF065435)	37.33	96.3	6.00E-20	39.71	GM
BP099264 S-adenosylhomocysteine hydrolase (M61831)	92.59	107	4.00E-23	92.59	HS
BP099288 ubiquitin-conjugating enzyme E2 (AF091621)	30.07	102	2.00E-29	71.88	CR
BP099357 quinone oxidoreductase (AE007020)	28.55	96.7	1.00E-19	47.32	MT

Table 2. *C. globosum* ESTs with sequence homology or similarity to known Non-fungus Genes (cont.).

EST NO. Putative Identification and database Accession	Alignment	Score	E-value	Identity	Organism
BP099358 lysyl-tRNA synthetase (AF285758)	53.44	101	2.00E-21	73.33	HS
BP099981 alpha-galactosidase (AL110470)	47.08	100	6.00E-21	53.09	SC
BP100051 aspartate aminotransferase (U39645)	25	70.5	3.00E-26	58.49	CE
BP099592 actin-related protein 13E (L25314)	35.79	92.4	1.00E-18	40.17	DM
BP099566 aspartate aminotransferase 1 (AF470319)	25.44	65.9	1.00E-10	63.04	MA
BP099573 ascorbate peroxidase (AB077953)	43.15	169	2.00E-41	48.57	EG
BP099484 flavoprotein C26F1.14C (Q10499)	37.86	100	5.00E-21	52	
BP099851 casein kinase I (AP003273)	65.08	101	3.00E-21	73.21	OS
BP099862 DNA repair protein RAD5 protein (AB006699)	41.38	106	1.00E-22	45.39	AT
BP099867 ADP-ribosylation factor 3 (BC028402)	50.51	140	2.00E-33	82.5	HS
BP099872 transitional endoplasmic reticulum ATPase2 (Z48045)	74.86	268	2.00E-71	75.82	CE
BP099731 phosphoinositide 3-kinase (U23480)	49.8	180	5.00E-47	58.22	DD
BP099776 deoxycytidylate deaminase (AE009697)	46.7	148	1.00E-35	52.38	BM
BP100102 minor allergen ALT A 7 (X78225)	47.66	146	4.00E-35	63.55	AA
BP100084 cyclododecanone monooxygenase (AY052630)	52.94	91.7	2.00E-18	57.53	RR
BP113161 RIKEN cDNA 1300013B24 (XM_135568)	49.56	107	3.00E-22	45.19	MM
BP099105 putative protein Y55F3AM.4 (AC024826)	25.18	92.4	3.00E-18	43.12	CE
BP113113 putative protein (D64005)	34.78	95.1	3.00E-19	41.18	Ssp
BP099909 putative protein SC1E6.19c (AL033505)	26.38	67.4	7.00E-11	36.13	SC
BP099456 putative protein (AP004594)	33.5	84	3.00E-16	34.07	OI
BP099401 putative protein ZK1055.6a (AF068721)	26.09	67.4	7.00E-11	33.87	CE
BP099545 putative protein (AP003597)	29.46	75.9	2.00E-13	34.27	Nsp.
BP100094 putative protein (AP003591)	33.24	104	3.00E-22	35.4	Nsp.
BP100040 putative protein Y71H2AM.11(AC024859)	30.08	72.4	1.00E-12	48.65	CE
BP099003 putative protein (NZ_AAAP01003506)	37.97	130	9.00E-30	43.01	MaM

Table 2. *C. globosum* ESTs with sequence homology or similarity to known Non-fungus Genes (cont.).

EST NO. Putative Identification and database Accession	Alignment	Score	E-value	Identity	Organism
BP099233 putative protein (NZ_AAAC01000304)	54.64	205	2.00E-52	55.14	BF
BP099295 putative protein (NZ_AAAC01000308)	34.91	107	4.00E-23	37.5	BF
BP099950 putative protein (NZ_AAAC01000306)	27	74.7	2.00E-13	47.37	BF
BP099574 putative protein (NZ_AABC01000199)	26.06	66.2	7.00E-11	32.74	NP
BP113343 putative protein (NZ_AABE01000028)	61.82	72.4	1.00E-12	64.15	CH
BP099646 putative protein (NZ_AAAP01003823)	30.89	75.1	4.00E-13	49.35	MaM
BP099693 putative protein (NZ_AAAT01000048)	64.59	288	4.00E-77	65.53	MD
BP099711 putative protein (NZ_AABC01000188)	46.06	163	1.00E-39	51.66	NP
BP113324 putative protein (NZ_AABE01000063)	40.91	112	4.00E-34	62.79	CH
BP099780 putative protein (NZ_AAAD01000065)	38.25	67	5.00E-11	52.46	AV
BP100082 agCP1709 (AAAB01008859)	63.01	178	1.00E-44	65.19	AG
BP099595 agCP3603 (AAAB01008807)	38.78	109	3.00E-30	54.29	AG
BP099980 agCP11613 (AAAB01008964)	45.56	118	2.00E-26	69.74	AG
BP099102 agCP13791 (AAAB01008844)	29.14	100	1.00E-20	30.34	AG
BP099053 agCP6162 (AAAB01008960)	39.66	107	7.00E-23	42.2	AG
BP113154 AT5g19580/T20D1_100 (AY078955)	32.67	150	1.00E-35	35.8	AT
BP099091 AT3g14410/MLN21_19 (AF410286)	32.54	196	4.00E-49	37.63	AT
BP099698 CG5366-PA (AE003628)	44.96	115	1.00E-25	47.83	DM
BP099986 BcDNA.GH07774 (AF145645)	25.7	79.3	2.00E-14	27.56	DM
BP099402 T25N20.16 (AC005106)	41.7	133	8.00E-31	47.33	AT
BP099335 Y52B11C.1 (AL023846)	34.32	90.5	3.00E-18	44.25	CE
BP099266 Crm1-K1(AB027498)	43.9	128	5.00E-31	54.55	SyC
BP099620 RPN-11 protein (AF077534)	29.33	67.8	3.00E-11	58.33	CE
BP099303 GCIP-interacting protein p29 (AY033432)	26.45	74.3	3.00E-13	44.19	MM
BP099460 TAG factor protein (AF538688)	62.5	143	4.00E-34	65.42	LA

Note: AT, *Arabidopsis thaliana*; MM, *Mus musculus*; SF, *Spodoptera frugiperda*; ZM, *Zea mays*; OO, *Ostertagia ostertagi*; EE, *Euphorbia esula*; SC, *Streptomyces coelicolor*; SI, *Sesamum indicum*; TM, *Thermotoga maritime*; SM, *Sinorhizobium meliloti*; TP, *Tetrahymena pyriformis*; Nsp., *Nostoc* sp.; DR, *Deinococcus radiodurans*; PS, *Pisum sativum*; GM, *Glycine max*; HS, *Homo sapiens*; CR, *Catharanthus roseus*; MT, *Mycobacterium tuberculosis*; CE, *Caenorhabditis elegans*; DM, *Drosophila melanogaster*; MA, *Musa acuminata*; EG, *Euglena gracilis*; OS, *Oryza sativa*; DD, *Dictyostelium discoideum*; BM, *Brucella melitensis*; AA, *Alternaria alternata*; RR, *Rhodococcus rubber*; Ssp., *Synechocystis* sp.; OI, *Oceanobacillus iheyensis*; MaM, *Magnetospirillum magnetotacticum*; BF, *Burkholderia fungorum*; NP, *Nostoc punctiforme*; CH, *Cytophaga hutchinsonii*; MD, *Microbulbifer degradans*; AV, *Azotobacter vinelandii*; AG, *Anopheles gambiae*; SyC, Synthetic construct; LA, *Lupinus angustifolius*

Abundantly expressed genes

Redundancy of the cDNA from *C. globosum* shown in **Table 3,** was highest for glucose-repressible protein (BP113143) which reached 97 clones. The glucose-repressible mRNA message levels were found to increase within minutes following the onset of glucose deprivation and rise 50% during the first 90 min of the non-depression period (Mcnally and Free, 1988). The second highest redundancy was the clock controlled gene-6 (BP113131), which reached 84 clones. The gene redundancy of pyruvate decarboxylase (BP113191 and BP11319) was 45 clones. The gene redundancy of glyceraldehydes phosphate dehydrogenase (BP113197) was 30 clones, indicating that metabolism was very active during mycelium growth stage. The gene redundancy of elongation factor 1-α was also high, with 47 clones, indicating an active protein bio-synthesis.

4. DISCUSSION

This is the first report of the carbendazim resistance gene being transferred to *C. globosum* with plasmid pTA-*TUB2*. The resistance level of the transformants is as high 1000 μg/ml, which is much higher than that of original resistance level of the fungus. This resistance level is also much higher than the chemical dose used in plant disease control in the field. Therefore, it would be safe to use those transformants in an integrated plant disease management programme with chemicals such as benzimidazole fungicides. The resistance of transformants are very stable even after subculturing on unselective medium for 10 times, this feature is very useful for applying them in practice continuously.

The EST study on the fungus provided us with more than 1000 genes for further study. Of those genes, some of them have already been identified as plant disease bio-control related genes. After further study on them, they will be used as target gene in host micro-organisms to produce new generation of bio-fungicides. Then plant disease bio-control will be significantly promoted in practice.

Table 3. Identified *C. globosum* cDNA clones showing redundancy (EST no. and total cDNA clones)

BP098894	2	BP098992	2	BP099080	3	BP099164	6	BP113094	2	BP113158	19
BP098895	5	BP098997	2	BP099081	3	BP099166	6	BP113095	2	BP113159	7
BP098898	2	BP098999	2	BP099084	3	BP099168	6	BP113097	2	BP113161	8
BP098924	2	BP099003	2	BP099086	3	BP099169	6	BP113101	3	BP113162	9
BP098925	3	BP099005	2	BP099087	3	BP099176	8	BP113102	3	BP113163	10
BP098929	2	BP099007	2	BP099091	3	BP099192	11	BP113110	3	BP113167	11
BP098933	2	BP099009	2	BP099093	3	BP099202	13	BP113111	3	BP113168	11
BP098935	2	BP099011	2	BP099095	3	BP099205	15	BP113112	3	BP113169	13
BP098940	2	BP099013	2	BP099096	5	BP099218	21	BP113113	9	BP113174	13
BP098943	2	BP099014	2	BP099098	3	BP099307	3	BP113115	3	BP113175	13
BP098948	2	BP099021	5	BP099101	3	BP099331	2	BP113118	4	BP113179	14
BP098951	2	BP099028	2	BP099102	3	BP099393	2	BP113121	4	BP113181	14
BP098957	2	BP099030	2	BP099103	3	BP099499	2	BP113122	4	BP113184	15
BP098958	2	BP099032	3	BP099105	3	BP099583	3	BP113123	4	BP113186	17
BP098960	2	BP099034	2	BP099113	3	BP099608	2	BP113126	4	BP113190	19
BP098961	2	BP099036	2	BP099115	4	BP099630	2	BP113131	84	BP113191	21
BP098963	2	BP099039	2	BP099116	4	BP099648	4	BP113134	4	BP113195	25
BP098964	2	BP099042	2	BP099117	4	BP099798	2	BP113136	5	BP113196	28
BP098967	2	BP099043	2	BP099121	4	BP099984	2	BP113137	5	BP113197	30
BP098971	3	BP099044	2	BP099122	4	BP100043	2	BP113138	5	BP099146	5
BP098973	2	BP099045	2	BP099126	4	BP113064	2	BP113139	5	BP113203	47
BP098976	2	BP099050	2	BP099127	4	BP113069	2	BP113141	5	BP113207	78
BP098977	2	BP099051	2	BP099128	4	BP113071	3	BP113142	5	BP113209	8
BP098978	2	BP099052	2	BP099130	6	BP113072	2	BP113143	97	BP113277	2
BP098982	2	BP099053	2	BP099135	4	BP113074	2	BP113146	6	BP113317	2
BP098983	2	BP099059	3	BP099137	4	BP113075	2	BP113147	6		
BP098985	2	BP099066	3	BP099151	5	BP113077	2	BP113151	6		
BP098986	2	BP099075	3	BP099156	5	BP113080	2	BP113154	6		
BP098987	2	BP099076	3	BP099158	6	BP113083	2	BP113156	9		
BP098990	2	BP099079	3	BP099160	6	BP113091	2	BP113157	7		

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