Research Article

Transcriptional Profiling of ESTs from the Biocontrol Fungus Chaetomium cupreum

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1. Introduction

One of the reasons for environmental disorder is that modern agriculture is an ecologically unbalanced system which has been destroyed by chemical fungicides. Biocontrol is highly interesting alternative method of chemical plant disease control. C. cupreum Ames is an ascomycete fungus with considerable biocontrol potential to plant fungal pathogens, especially several notorious examples belonging to the genera of Pythium, Rhizoctonia, and Pyricularia [1]. In Thailand and China, its biological products have been applied in agricultural disease management [1, 2]; nevertheless, the genetic basis of the defense mechanisms of C. cupreum is not well understood thus inhibiting its application.

ESTs analysis has been proven to be an efficient and valuable tool in obtaining coding gene information, understanding the pathways involved in a given physiological or environmental stimulus [3, 4]. To date, several ESTs studies have been carried out on fungal biocontrol agents, especially to species of Trichoderma. For example, analysis of 8,710 ESTs of T. harzianum CECT 2413 from eight cDNA libraries including those simulating mycoparasitism [5] and of ESTs from four different Trichoderma strains grown under conditions related to biocontrol [6].

In the present study, we performed comparative analysis of two cDNA/ESTs libraries from C. cupreum. An obvious difference in gene expression and metabolic pathways were detected between libraries. This research contributes to elucidating further the mycoparasitic molecular mechanisms involved in C. cupreum and will help to develop novel strategies in fungal disease management.

2. Materials and Methods

2.1. Fungal Strains and Culture Conditions. The C. cupreum isolate was kindly provided by King Mongkut’s Institute of Technology of Ladkrabang, Thailand. Rhizoctonia solani was stored in our laboratory.

C1 was constructed from mycelia of C. cupreum grown on potato dextrose agar (PDA) culture. Library C2 was constructed with mycelia of Rhizoctonia solani cell wall (RsCW) as the carbon source, this was designed to resemble the phases of interaction with R. solani. For C1 library construction, mycelia of C. cupreum were cultured on potato dextrose agar, and then mycelium was harvested and ground into mycelial powder. The C2 library was constructed with mycelial powder of C. cupreum grown on agar as the carbon source. Mycelial powder was ground and used to inoculate rice agar plates. After the mycelium covered the entire surface of the agar plates, the plates were meticulously cut into small pieces and used to inoculate cDNA libraries. The cDNA libraries were constructed as described previously [3].
clones were sequenced using a T3 primer from the 5' end using the pBluescript II plasmid system. Fragments of cDNA construction followed the procedures of Zhang as described in the Oligotex mRNA Kit (Qiagen). The course of cDNA library construction was confirmed with Consed [8]. All unigenes were compared to public nonredundant (nr) protein databases using a BlastX search. According to KEGG (Kyoto Encyclopedia of Genes and Genomes) [9], unigenes were assigned to different metabolic pathways with the same criteria as described by Zhang [7]. All high-quality ESTs were submitted to the GenBank database under accession numbers DV544375-DV544865.

3. Results

3.1. ESTs Clustering and Function Assignment. A total of 5,538 cDNA clones with an insert size of more than 700 bp were selected for sequencing, resulting in 4285 ESTs (3066 from C1 and 1219 from C2) after removing sequences representing ribosomal, vector, and low-quality sequences. Minimum, average, and maximum lengths of ESTs were 102, 518, and 795 bp, respectively, with a large fraction falling between 500 and 700 bp (2110 from C1 and 795 from C2) after removing sequences representing ribosomal, vector, and low-quality sequences. Mini- 

2.2. Construction of the cDNA Libraries and DNA Sequencing. Total RNA was extracted using Trizol reagent from mycelia of C. cupreum. Polyadenylated RNA was purified using an Oligotex mRNA Kit (Qiagen). The course of cDNA library construction followed the procedures of Zhang as described before [7]. Unidirectional cDNA libraries were constructed using the pBluescript II plasmid system. Fragments of cDNA clones were sequenced using a T3 primer from the 5' end with MegaBase1000 DNA sequencer.

2.3. Data Processing and Bioinformatics Analysis. Vector sequences, sequences shorter than 100 bp and containing more than 5% ambiguous bases, were discarded using the Cross-match program. High-quality sequences were assembled using Phrap (http://www.phrap.org/) and accuracy of contigs was confirmed with Consed [8]. All unigenes were compared against public nonredundant (nr) protein databases using a BlastX search. According to KEGG (Kyoto Encyclopedia of Genes and Genomes) [9], unigenes were assigned to different metabolic pathways with the same criteria as described by Zhang [7]. All high-quality ESTs were submitted to the GenBank database under accession numbers DV544375-DV544865.

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Using the Phrap and Consed programs, ESTs from both libraries were arranged into 585 contigs and 1577 singletons, giving a total of 2162 unigenes. Each unigene was subjected to analysis against homologous sequences in public protein databases using the BlastX algorithm. Approximately 1211 (56%) of the unigenes were assigned a function with an E-value of 10^{-5} or lower. The remaining 951 clones had no high homology to genes of known function. A total of 1138 (52.6%) and 691 (32%) unigenes were unique and only expressed in C1 or C2, respectively.

3.2. Exploration of Highly Expressed ESTs. Contigs containing 4 or more ESTs from each library are listed in Table 1. Of the 26 clusters, more than one third were (10/26) expressed only in the C1 library, 2 only in the C2 library, and half (14/29) in both but at a different level.

Glyceraldehyde-3-phosphate dehydrogenase was the most highly expressed transcript (109 ESTs) in the C1 library, occurring four times more than in the C2 library. The most highly represented transcripts in the C2 library coded for a putatively major facilitator superfamily (MFS) monosaccharide transporter; no such expression was observed in library C1. The expression of coproporphyrinogen oxidase, thiazole biosynthetic enzyme, glutamine synthetase, ATP citrate lyase, and aspartate aminotransferase were higher in the C1 library. It should be noted that many hits similar to predicted or unknown function proteins were detected in libraries. They are ideal candidates for future study.

3.3. Metabolic Pathways Analysis. Pathways analysis of KEGG was carried out using genes homologous to known functional sequences. A total of 65 and 61 different metabolic pathways were found in the C1 and C2 libraries, respectively. These results show evident difference in metabolic pathways between the libraries (Table 2).

Glycolysis/glucogenesis was the most represented pathway within each library. The second and third most enriched functional pathways in the C1 library were porphyrin and chlorophyll metabolism, which involved 180 ESTs (17.1%), and the citrate cycle, which involved 47 ESTs (4.5%). In the C2 library, the respective pathways were peptidoprotein biosynthesis and d-arginine and ornithine metabolism.

It should be noted that the types of genes involved in the same metabolic pathways were greatly different between libraries. For instance, in the glycolysis/glucogenesis pathway, the enzymes in the C1 library were enolase, glyceraldehyde 3-phosphate dehydrogenase, fructose 1,6-biphosphate aldolase, and pyruvate decarboxylase, which were assigned to glycolysis; however, in the C2 library, they were fructose-1,6-bisphosphatase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase, which were assigned to gluconogenesis. Because glucose is a very important source of nutrition, we speculate that the upregulated genes related to gluconogenesis observed in the C2 library may be necessary for mycoparasitism, that is, maintenance of fast cell growth rate in response to the competition with the plant fungal pathogen.

3.4. Genes Induced by the Mycoparasitic Process. Differences were observed in gene groups associated with degradation of the cell wall, proteolysis, and toxins production. Seven contigs were presented in both libraries, four were specific to library C1, and eight to library C2 (Table 3), the latter appearing to be induced by the mycoparasitic process directly. Two sequences similar to the 48-KDa endochitinase (GenBank accession nos. DV546055, DV544732, and DV544989) of Aspergillus nidulans and 46-KDa endochitinase (DV547883 and DV547485) of Hypocrea vires were identified in libraries C1 and C2, respectively. Four ESTs from library C1 (DV546459, DV546294, DV544423, and
### Table 1: The difference of high-redundancy genes expression between cDNA libraries from C. cupreum.

| Contig no. | Annotation                                      | ESTs | %   | ESTs | %   |
|------------|------------------------------------------------|------|------|------|------|
| Contig525  | Glyceraldehyde-3-phosphate dehydrogenase        | 109  | 7.41 | 14   | 1.69 |
| Contig531  | Coproporphyrinogen oxidase                      | 72   | 4.89 | 5    | 0.6  |
| Contig30   | Predicted protein                               | 56   | 3.81 | 0    | 0    |
| Contig433  | C-4 sterol methyl oxidase                       | 54   | 3.67 | 0    | 0    |
| Contig313  | Predicted protein                               | 36   | 2.45 | 0    | 0    |
| Contig523  | Predicted protein                               | 27   | 1.84 | 0    | 0    |
| Contig39   | Predicted protein                               | 23   | 1.56 | 0    | 0    |
| Contig507  | Xylulose-5-phosphate phosphoketolase            | 21   | 1.43 | 3    | 0.36 |
| Contig152  | Predicted protein                               | 20   | 1.36 | 16   | 1.93 |
| Contig523  | EF1-alpha translation elongation factor         | 19   | 1.29 | 12   | 1.45 |
| Contig42   | Thiazole biosynthetic enzyme                     | 19   | 1.29 | 2    | 0.24 |
| Contig520  | Actin                                          | 17   | 1.16 | 4    | 0.48 |
| Contig495  | Glutamine synthetase                           | 17   | 1.16 | 1    | 0.12 |
| Contig517  | Aspartic protease                               | 16   | 1.09 | 3    | 0.36 |
| Contig317  | Ammonium transporter                           | 16   | 1.09 | 0    | 0    |
| Contig419  | ATP synthase protein                           | 16   | 1.09 | 0    | 0    |
| Contig4    | Alcohol dehydrogenase                          | 15   | 1.02 | 4    | 0.48 |
| Contig474  | β-1,3-exoglucanase                             | 14   | 0.95 | 2    | 0.24 |
| Contig494  | ATP citrate lyase                               | 14   | 0.95 | 1    | 0.12 |
| Contig412  | Histone H2B                                    | 13   | 0.88 | 0    | 0    |
| Contig298  | Pyruvate kinase                                | 13   | 0.88 | 0    | 0    |
| Contig310  | Aspartate aminotransferase                     | 11   | 0.75 | 1    | 0.12 |
| Contig408  | Heat shock protein 30                          | 11   | 0.75 | 0    | 0    |
| Contig489  | ADP-ATP translocase                            | 11   | 0.75 | 8    | 0.97 |
| Contig165  | MFS monosaccharide transporter                 | 0    | 0    | 20   | 2.42 |
| Contig162  | Phosphoenolpyruvate carboxykinase              | 0    | 0    | 12   | 1.45 |

Relative values (%): ESTs numbers/all ESTs numbers in C1 or C2 library.

*DV546484* and 1 (*DV548260*) from library C2 shared similarity with serine proteases (Figure 1). One-gene homologue of MAP kinase A (*DV548513*) was identified in library C2 only (Figure 2).

## 4. Discussion

It has been demonstrated that the cell wall of the fungal pathogen can simulate some aspects of the mycoparasitic interactions between biocontrol fungi and its targets [10].

Only a limited amount of overlap (333 unigenes) was observed in both libraries. A total of 1138 and 691 unigenes were unique and only expressed in C1 or C2, respectively. The lack of significant overlap between the individual libraries also suggests a high level of flexibility at the level of gene expression under the examined conditions, some of which may reflect particular requirements for phases of mycoparasitism.

The analysis of the frequency of specific ESTs that form individual contigs can give information about the expression levels of particular genes under different experimental conditions [11]. The most abundant transcripts in library C2 but no expression in C1 were MFS monosaccharide transporters. MFS transporters transport uni-, sym-, and antiporters of sugars, peptides, drugs, and organic and inorganic ions with 12 or 14 transmembrane spanners [12]. In the present study, the high proportion of ESTs expressing a homology to MFS monosaccharide transporters implies that they may be responsible for transport of monosaccharides derived from the degradation of RsCW. This was not consistent with the results of a study of *T. harzianum* CECT 2413 [13], in which abundant expression of peptide transporter 2 (PTR2) was found in a cDNA library of *T. harzianum* CECT 2413 when interacted directly with *Botrytis cinerea*. However, only one EST similar to PTR2 (*DV547977*) was detected in library C2. We speculate that this may have been caused by the different cultivation times of the two fungi.

Comparison analysis illustrated variations in the proportions of different pathways. Metabolic pathways of ubiquinone biosynthesis; electron transport and oxidative phosphorylation; purine metabolism; pyrimidine metabolism; alanine and aspartate metabolism; valine, leucine, and...
Table 2: The metabolic pathways difference between cDNA libraries from *C. cupreum*.

| Map no. | Pathways                                                                 | ESTs in C1 | ESTs in C2 |
|--------|---------------------------------------------------------------------------|------------|------------|
| 00010  | Glycolysis/gluconeogenesis                                                 | 188        | 31         |
| 00020  | Citrate cycle                                                             | 47         | 9          |
| 00030  | Pentose phosphate cycle                                                    | 35         | 6          |
| 00040  | Pentose and glucuronate interconversions                                   | 6          | 4          |
| 00051  | Fructose and mannose metabolism                                            | 4          | 10         |
| 00052  | Galactose metabolism                                                       | 7          | 7          |
| 00053  | Ascorbate and aldarate metabolism                                          | 5          | 5          |
| 00061  | Fatty acid biosynthesis (path 1)                                            | 3          | 1          |
| 00062  | Fatty acid biosynthesis (path 2)                                            | 0          | 1          |
| 00071  | Fatty acid metabolism                                                      | 24         | 12         |
| 00100  | Sterol, vitamin K, vitamin E, carotenoids biosynthesis                     | 1          | 0          |
| 00120  | Bile acid biosynthesis                                                     | 19         | 12         |
| 00130  | Ubiquinone biosynthesis                                                    | 14         | 3          |
| 00150  | Androgen and estrogen metabolism                                           | 4          | 12         |
| 00190  | Electron transport and oxidative phosphorylation                           | 34         | 6          |
| 00220  | Urea cycle and metabolism of amino groups                                  | 10         | 0          |
| 00230  | Purine metabolism                                                          | 41         | 1          |
| 00240  | Pyrimidine metabolism                                                      | 6          | 1          |
| 00251  | Glutamate metabolism                                                       | 24         | 4          |
| 00252  | Alanine and aspartate metabolism                                           | 14         | 1          |
| 00260  | Glycine, serine, and threonine metabolism                                  | 2          | 8          |
| 00271  | Methionine metabolism                                                      | 12         | 5          |
| 00272  | Cysteine metabolism                                                        | 6          | 1          |
| 00280  | Valine, leucine, and isoleucine degradation                                 | 6          | 6          |
| 00290  | Valine, leucine, and isoleucine biosynthesis                               | 12         | 1          |
| 00300  | Lysine biosynthesis                                                        | 4          | 1          |
| 00310  | Lysine degradation                                                         | 6          | 5          |
| 00330  | Arginine and proline metabolism                                            | 15         | 6          |
| 00340  | Lysine degradation                                                         | 4          | 4          |
| 00350  | Tyrosine metabolism                                                        | 25         | 9          |
| 00360  | Phenylalanine metabolism                                                   | 9          | 6          |
| 00361  | Gamma-hexachlorocyclohexane degradation                                    | 5          | 6          |
| 00380  | Tryptophan metabolism                                                       | 22         | 14         |
| 00400  | Phenylalanine, tyrosine, and tryptophan biosynthesis                       | 13         | 13         |
| 00410  | B-alanine metabolism                                                       | 5          | 5          |
| 00440  | Aminophosphonate metabolism                                                | 4          | 1          |
| 00450  | Selenoamino acid metabolism                                                | 14         | 5          |
| 00472  | D-arginine and ornithine metabolism                                        | 2          | 15         |
| 00480  | Glutathione metabolism                                                     | 8          | 2          |
| 00500  | Starch and sucrose metabolism                                              | 16         | 11         |
| 00520  | Nucleotide sugars metabolism                                               | 7          | 4          |
| 00530  | Aminosugars metabolism                                                     | 7          | 5          |
| 00550  | Peptide protein biosynthesis                                               | 17         | 0          |
| 00561  | Glycerolipid metabolism                                                   | 5          | 9          |
Table 2: Continued.

| Map no. | Pathways                                      | ESTs in C1 | ESTs in C2 |
|---------|-----------------------------------------------|------------|------------|
| 00562   | Inositol phosphate metabolism                  | 4          | 2          |
| 00600   | Sphingoglycolipid metabolism                   | 4          | 3          |
| 00620   | Pyruvate metabolism                            | 35         | 10         |
| 00626   | Nitrobenzene degradation                       | 1          | 1          |
| 00630   | Glyoxylate and dicarboxylate metabolism        | 9          | 4          |
| 00640   | Propanoate metabolism                          | 9          | 6          |
| 00643   | Styrene degradation                            | 0          | 1          |
| 00650   | Butanoate metabolism                           | 13         | 6          |
| 00670   | One-carbon pool by folate                      | 6          | 3          |
| 00680   | Methane metabolism                             | 35         | 3          |
| 00740   | Riboflavin metabolism                          | 5          | 5          |
| 00750   | Vitamine B6 metabolism                         | 0          | 1          |
| 00760   | Nicotinate and nicotinamide metabolism         | 4          | 3          |
| 00770   | Pantothenate and CoA biosynthesis              | 9          | 1          |
| 00790   | Folate biosynthesis                            | 4          | 2          |
| 00860   | Porphyrin and chlorophyll metabolism           | 180        | 6          |
| 00920   | Sulfur metabolism: reduction and fixation      | 2          | 0          |
| 00950   | Alkaloid biosynthesis I                        | 7          | 0          |
| 00970   | Aminoacyl-tRNA biosynthesis                    | 11         | 2          |
| 03020   | RNA polymerase                                 | 3          | 1          |
| 03030   | DNA polymerase                                 | 4          | 0          |
| 03130   | Cytochrome C oxidase                           | 1          | 0          |
| 03140   | Cytochrome C reductase                         | 10         | 4          |
| 03150   | Succinate dehydrogenase                        | 3          | 1          |

Table 3: The different expression of genes related to biocontrol between cDNA libraries from *C. cupreum*.

| Annotation                          | C1 ESTs | C2 ESTs |
|-------------------------------------|---------|---------|
| 48 KDa endochitinase                | 3       | 0       |
| 46 KDa endochitinase                | 0       | 2       |
| β-N-acetylg glucosaminidase         | 0       | 1       |
| β-1,3-exoglucanase                  | 22      | 2       |
| β-1,3-endoglucanase                 | 1       | 0       |
| β-glucosidase D                     | 1       | 1       |
| β-glucosidase 5                     | 6       | 0       |
| β-glucosidase 6                     | 1       | 0       |
| Serine protease                     | 4       | 1       |
| Aspartic proteinase                 | 20      | 5       |
| Immune antigen 1                    | 0       | 1       |
| C-8 sterol isomerase                | 2       | 2       |
| Sterol C-22 desaturase              | 1       | 1       |
| Sterol-C-5-desaturase               | 2       | 2       |
| β-endo-1,4-xylanase                 | 0       | 3       |
| Peptide transporter 2               | 0       | 1       |
| MAP kinase A                        | 0       | 1       |
| ABC transporters                    | 0       | 1       |

isoleucine biosynthesis; porphyrin and chlorophyll metabolism were proportionately more represented in library C1. In contrast, pentose and glucuronate interconversions; fructose and mannose metabolism; galactose metabolism; androgen and estrogen metabolism; glycine, serine, and threonine metabolism; valine, leucine, and isoleucine degradation; arginine and proline metabolism; histidine metabolism; tryptophan metabolism; d-arginine and ornithine metabolism; glycerolipid metabolism were overrepresented in library C2. Metabolic pathways of sterol, vitamin K, vitamin E, carotenoids biosynthesis; sulfur metabolism: reduction and fixation; DNA polymerase; cytochrome C oxidase were only observed in library C1, while those of fatty acid biosynthesis (path 2), styrene degradation, and Vitamine B6 metabolism were only observed in library C2.

The results showed that genes related to mycoparasitism were differentially expressed. Two different sequences similar to the 48-KDa endochitinase and 46-KDa endochitinase were identified in libraries C1 and C2, respectively. Since library C1 was obtained from cultivation on PDA medium, the 48 KDa endochitinase homolog might play a role in the dissolution and formation of the cell wall of *C. cupreum*. Similarly, because library C2 was constructed under conditions associated with mycoparasitism, the 46 KDa endochitinase homolog is expected to be involved in cell wall degradation of the fungal pathogen during the mycoparasitic process.
subtilisin-like serine protease [Acremonium sp. F11177]
Score = 345 bits (885), expect = 1e–115
Identities = 161/179 (90%), positives = 170/179 (95%)

Query: ASEHR5VQ4HTSRESERADLKKRQ1PLVDVFGLHHTYK1GQFGLYSQFDEDV1 213
Sbjct: ASAMHR5QV4HTSRESERADLKKRQ1PLVDVFGLHHTYK1GQFGLYSQFDEDV1 116

MAP kinase Mps1 [Magnaporthe grisea]
Length = 415 Score = 394 bits (1013), expect = 2e–136
Identities = 191/193 (99%), positives = 193/193 (100%), gaps = 0/193 (0%)

Query: MAUQ5K1PKVQNDVFDREYTVTGLQGAY1GVCAYYNow5TS5Q1A1KGY5NFSK 189
Sbjct: MSULQ5K1PKVQNDVFDREYTVTGLQGAY1GVCAYYNow5TS5Q1A1KGY5NFSK 60

ABC transporter [Myceliophthora thermophila ATCC 42464]
Length = 1482 Score = 140 bits (352), expect = 2e–37
Identities = 67/92 (73%), positives = 78/92 (85%), gaps = 0/92 (0%)

Query: FQAAWTS1LKPS3MSLMAYGVLKVPLLL1PFPRLC1AYNFCQPL1NNA1LSQ5EV1 206
Sbjct: FSQ51WNS1TEKPS3MSLMAYGVLKVPLLL1PFPRLC1AYNFCQPL1NNA1LSQ5EV1 307

Figure 1: DV546294 shared 90% identity with serine proteases of Acremonium sp.

Figure 2: DV548513 shared 98% identity with MAP kinase of Gibberella intermedia.

Figure 3: DV548480 shared 73% identity with ABC transporter of Myceliophthora thermophila ATCC 42464.
The conditions used for construction of library C2 were aimed at \textit{in vitro} simulation of the mycoparasitic process, which is triggered by the recognition of the structural character of the pathogenic fungal cell wall. As a result, the genes involved in signal transduction pathways of mycoparasitism were acquired. Examples include homologue of gene encoding an ABC transporter (ATP-binding cassette transporter, \textit{DV548480}, Figure 3) and MAP-kinase A (Tmk1 of \textit{T. atroviride}). Four ESTs have sequence homology to an ABC transporter, it was also observed previously in other fungal pathogens (\textit{Gibberella pulicaris} and \textit{Sclerotinia sclerotiorum}). Mehrabi et al. [14] as potential pathogenicity factors responsible for tolerance to phytoalexins or a pathogenicity factor for the host Fleissner et al. [15] and Li et al. [16]. Studies on signal transduction pathways from \textit{Trichoderma} strains revealed the involvement of MAP-kinases in the mycoparasitic interaction, including production of hydrolytic enzymes such as chitinases and secretion of antibiotic substances [17].

In this study, we sequenced and analyzed two independent cDNA libraries, providing the first comparative analysis of the transcriptome of \textit{C. cupreum} under different conditions. The findings provide an entry point for understanding further the molecular mechanisms of this fungus and will also help to advance our efforts in developing novel strategies for biocontrol of fungal diseases.

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