Genome sequence of the filamentous soil fungus Chaetomium cochliodes reveals abundance of genes for heme enzymes from all peroxidase and catalase superfamilies

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Abstract

Background: The ascomycetous family Chaetomiaceae (class Sordariomycetes) includes numerous soilborn, saprophytic, endophytic and pathogenic fungi which can adapt to various growth conditions and living niches by providing a broad armory of oxidative and antioxidant enzymes.

Results: We release the 34.7 Mbp draft genome of Chaetomium cochliodes CCM F-232 consisting of 6036 contigs with an average size of 5756 bp and reconstructed its phylogeny. We show that this filamentous fungus is closely related but not identical to Chaetomium globosum and Chaetomium elatum. We screened and critically analysed this genome for open reading frames coding for essential antioxidant enzymes. It is demonstrated that the genome of C. cochliodes contains genes encoding putative enzymes from all four known heme peroxidase superfamilies including bifunctional catalase-peroxidase (KatG), cytochrome c peroxidase (CcP), manganese peroxidase, two paralogs of hybrid B peroxidases (HyBpox), cyclooxygenase, linoleate diol synthase, dye-decolorizing peroxidase (DyP) of type B and three paralogs of heme thiolate peroxidases. Both KatG and DyP-type B are shown to be introduced into ascomycetes genomes by horizontal gene transfer from various bacteria. In addition, two putative large subunit secretory and two small-subunit typical catalases are found in C. cochliodes. We support our genomic findings with quantitative transcription analysis of nine peroxidase & catalase genes.

Conclusions: We delineate molecular phylogeny of five distinct gene superfamilies coding for essential heme oxidoreductases in Chaetomia and from the transcription analysis the role of this antioxidant enzymatic armory for the survival of a peculiar soil ascomycete in various harsh environments.

Keywords: Chaetomium cochliodes, Peroxidase-catalase superfamily, Peroxidase-cyclooxygenase superfamily, Peroxidase-chlorite dismutase superfamily, Peroxidase-peroxygenase superfamily, Heme-catalase super family

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Background
The ascomycetous family of Chaetomiaceae (class Sordariomycetes) includes numerous soil-sown, saprotrophic, endophytic and pathogenic fungi that apparently exhibit a large flexibility in their adaptation to various growth conditions and living niches. In Mycobank (www.mycobank.org) currently up to 451 members of this abundant fungal family are registered but only from two representatives (i.e. Chaetomium thermophilum and Chaetomium globosum) the completely sequenced genomes are available. Analysis of the genome of C. thermophilum [1] mainly focused on the presence of genes coding for nucleoporins of high thermal stability, whereas the draft genome of Chaetomium globosum [2] was mainly asked for diverse genes coding cellulyotic pathways.

The filamentous fungus Chaetomium cochliodes was long considered to be a variant of Chaetomium globosum (cf. the NCBI taxonomy database at www.ncbi.nlm.nih.gov/taxonomy) but already in very early studies e.g. [3] it was shown that C. cochliodes produces the antibiotic chaetomin which was shown to be highly active mainly against Gram-positive bacteria. Additionally, studies from our laboratory revealed differences between C. globosum and C. cochliodes in the primary sequence and expression profile of peroxisomal catalase-peroxidases [4]. These findings – together with the fact that peroxidases participate in diverse fungal secondary metabolism pathways [5–9] – prompted us to sequence the entire genome of Chaetomium cochliodes strain CCM-F232 for detailed comparative studies.

Here we release the draft genome of C. cochliodes, reconstruct its phylogeny and analyse the occurrence of abundant genes coding for heme containing peroxidases and catalases with respect to the recently described four distinct heme peroxidase superfamilies [10] and the heme catalase super family [11]. Interestingly, representatives from all five (super)families were found including putative bifunctional catalase-peroxidase, cytochrome c peroxidase, hybrid B peroxidases, cytochrome P450-like enzymes, dye-decolorizing peroxidases, heme thiolate peroxidases as well as large- and small-subunit mono-functional catalases. The occurrence of this large number and variability of genes encoding heme hydroperoxidases in C. cochliodes is discussed in comparison with related fungal genomes. We support our genomic findings with a first round of a quantitative expression analysis of selected genes from all mentioned superfamilies involved in the catabolism of H₂O₂.

Methods
Source and cultivation of Chaetomium cochliodes and isolation of genomic DNA
Chaetomium cochliodes CCM F-232 was obtained from Czech Collection of Microorganisms at the Masaryk University, Faculty of Natural Sciences in Brno, Czech Republic. The composition of the incubation medium and the growth conditions were the same as described previously [4].

Genomic DNA from 100 mg of frozen fungal mycelium was isolated with the method of Carlson [12] by using 2 % CTAB in a modification suitable for genome sequencing described in [13]. Finally, extracted DNA was completely dissolved in TE buffer (10 mM Tris–HCl 1 mM EDTA, pH 8.0) to a final volume of 100 μL. The concentration of obtained sample was measured in Nanodrop 2000 (Thermo Scientific, Waltham, MA, USA).

Library preparation for DNA sequencing
Approximately 1 μg of high quality genomic DNA was fragmented in 50 μl Low TE buffer (10 mM Tris pH 8.0, 0.1 mM EDTA) by BioRuptor UCD-200 sonication system (Life Technologies, Carlsbad, CA, USA) to obtain a population of ~190 bp long fragments. The length and the quantity of generated fragments were assessed by Bioanalyzer chip technology (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer’s instructions. The protocol of the Library Builder™ System (Life Technologies, Carlsbad, CA, USA) was used for adaptor ligation, nick repair and fragment purification. The selection of 270 bp long fragments was conducted by the Pippin Prep instrument (Sage Science, Beverly, MA, USA) according to the manufacturer’s instructions. Library quantification was carried out using the TaqMan qPCR protocol of Life Technologies.

Genomic DNA sequencing and ORF prediction
Whole genome sequencing was carried out using the Ion Proton Technology (including the Ion AmpliSeq library preparation kit, Template OT2 200 kit, Ion PI sequencing 200 kit, and the Ion PI chip kit version 2; Life Technologies, Carlsbad, CA), according to the instructions of the manufacturer. A total of 34.746 Mbp, with a median read length of 180 bp, were assembled into a draft genome containing 6036 contigs (N₅₀, 14,381). The genome assembly was performed with Newbler 2.9. Genome coverage of this sequencing was 316 x. The entire genome shotgun project was deposited at GenBank under accession LSBY00000000, BioProject PRJNA309375, BioSample SAMN0432217. For comparative genomic analyses of Chaetomium cochliodes genes Ensembl Fungi (http://fungiensembl.org/index.html) was used.

For gene prediction in sequenced C. cochliodes contigs, HMM-based methods FGENESH and FGENESH + located at www.softberry.com [14] trained for closely related C. globosum & C. thermophilum were used. For all peroxidase and catalase genes they were also curated manually.
Reconstruction of fungal phylogeny

Selected DNA sequence spanning the region from the 3′ end of the 18S rDNA, the complete ITS1, 5.8S rDNA, ITS2 and the 5′ end of the 28S rDNA from corresponding C. cochliodes contigs was aligned with 33 related sequences from Ascomycetes in exactly the same region obtained from GenBank (Table 1). This DNA alignment was performed with the Muscle program [15] implemented in MEGA 6 package with its default parameters and 100 iterations. For subsequent phylogeny reconstruction MEGA 6 program suite [16] was applied on this 2474 bp long DNA alignment containing the typical fungal barcode motif [17]. Maximum likelihood method with 1000 bootstrap replications and general time reversed substitution model were applied. Further, uniform rates of substitutions with invariant sites and involvement of all aligned sites with nearest-neighbour interchange and very strong branch swap filter were selected as optimised parameters. The resulting tree was rendered with Tree Explorer of the same MEGA

Table 1 List of all DNA sequences with their GenBank accession numbers used for phylogeny reconstruction in the region 18S, ITS1, 5.8S, ITS2, 28S-rDNA

| Abbrev. | Fungus | Taxonom. family | GB accession # | [bp] used for phyl. |
|---------|--------|----------------|----------------|---------------------|
| Ccoch   | Chaetomium cochliodes | Chaetomiaceae | KT895345 | 2217 |
| Celat   | Chaetomium elatum | Chaetomiaceae | M83257 | 2211 |
| Cg1     | Chaetomium globosum CBS148.15 | Chaetomiaceae | NT_166001 | 2245 |
| Cg2     | Chaetomium globosum (endophyt) | Chaetomiaceae | DQ234257 | 2219 |
| Cg3     | Chaetomium globosum isol. W7 | Chaetomiaceae | JQ686920 | 2219 |
| Cthe    | Chaetomium thermophilum | Chaetomiaceae | GCA_000221225 | 2237 |
| Coacu1  | Colletotrichum acutatum 1 | Glomerellaceae | AJ301905 | 2227 |
| Coacu2  | Colletotrichum acutatum 2 | Glomerellaceae | AJ301906 | 2225 |
| Cocir   | Colletotrichum circinans | Glomerellaceae | AJ301955 | 2216 |
| Ccocod | Colletotrichum coccosides | Glomerellaceae | AJ301957 | 2218 |
| Codem   | Colletotrichum dematiu | Glomerellaceae | AJ301954 | 2220 |
| Colup   | Colletotrichum lupini | Glomerellaceae | AJ301959 | 2200 |
| Cotri   | Colletotrichum trifolii | Glomerellaceae | AJ301942 | 2231 |
| Cotru   | Colletotrichum truncatum | Glomerellaceae | AJ301937 | 2213 |
| Fgram   | Fusarium graminearum | Nectriaceae | NC_026477 | 2188 |
| Gcin    | Glomerella cingulata | Glomerellaceae | AJ301952 | 2198 |
| Hgri    | Humicola grisea | Chaetomiaceae | AY706334 | 2202 |
| Lsak    | Lecanicillium sakensae | Cordycipitaceae | AB360363 | 2236 |
| Masp    | Madurella sp. TMMU3956 | Sordariaceae | EU815932 | 2271 |
| Mhni    | Myceliophthora hinnula | Chaetomiaceae | JQ067909 | 2099 |
| Mthe    | Myceliophthora thermophila | Chaetomiaceae | NC_016478 | 2217 |
| Mgram   | Mycosphaerella graminicola | Mycosphaerellaceae | NC_018212 | 2195 |
| Matr    | Myrothecium atroviride | Stachybotriaceae | AJ302002 | 2223 |
| Mcin1   | Myrothecium cinctum 1 | Stachybotriaceae | AJ301996 | 2204 |
| Mcin2   | Myrothecium cinctum 2 | Stachybotriaceae | AJ302004 | 2202 |
| Mver    | Myrothecium verrucaria | Stachybotriaceae | AJ301999 | 2222 |
| Ncr     | Neurospora crassa | Sordariaceae | FJ360521 | 2230 |
| Pan     | Podospora anserina | Lasiophaealiaceae | FO904938 | 2196 |
| Sfir    | Sordaria fimicola | Sordariaceae | X69851 | 2256 |
| Taus    | Thielavia australiensis | Chaetomiaceae | JQ067908 | 2160 |
| Tter    | Thielavia terrestris | Chaetomiaceae | NC_016459 | 2233 |
| Tasp    | Trichocladium asperum | Chaetomiaceae | AY706336 | 2202 |
| Tatr    | Trichoderma atroviride | Hypocreaceae | NW_014013638 | 2251 |
| Vcil    | Volutella ciliata | Nectriaceae | AJ301967 | 2214 |
package. For additional verification, the same 2474 bp long DNA alignment was subjected to phylogeny reconstruction using MrBayes 3.2 [18]. Majority consensus tree was obtained from all credible topologies sampled by MrBayes over 200,000 generations (with a standard deviation of split frequencies below 0.01) by using the same GTR substitution model with gamma distributed rate variation across sites and a proportion of invariable sites.

Reconstruction of molecular phylogeny of protein superfamilies
Selected protein sequences translated from C. cochliodes contigs (Table 2B) and similar protein sequences coding for various peroxidases and catalases (i.e. hydroperoxidases deposited at PeroxiBase http://peroxibase.toulouse.inra.fr with direct links to GenBank & UniProt) were aligned with the Muscle program [15] using default parameters and 100 iterations. Obtained alignments were inspected and ambiguously aligned regions were excluded from further analysis. Resulting alignments were subjected to protein phylogeny reconstruction using MEGA 6 [16] with optimized parameters according to lowest Bayesian information criterion scores (Additional file 1: Table S1). Maximum likelihood method with 100 bootstraps was chosen using the best substitution model for each alignment (WAG in three cases and LG in two cases cf. Additional file 1: Table S1 for details), gamma distribution of rates (four categories) and the presence of invariant sites. Nearest-neighbour interchange was used as heuristic method and very strong branch swap filter was applied. The same protein alignments were subjected to phylogeny reconstruction using MrBayes 3.2 [18]. Majority consensus tree was obtained from all credible topologies sampled by MrBayes over 500,000 generations (with a standard deviation of split frequencies below 0.01) by using the same substitution model as in MEGA. Resulting trees were rendered with FigTree graphic suite available at http://tree.bio.ed.ac.uk/software/figtree as cladograms with transformed branches.

Transcriptional analysis of genes involved in peroxide catabolism with RT-qPCR
To study the level of expression of genes involved in peroxide catabolism either non-induced C. cochliodes samples or samples induced in the early exponential phase of growth with 5 mM H$_2$O$_2$ or 5 mM PAA (final concentration, added only for last 30 min.) were used for total RNA isolation with RNeasy Plus Mini kit (Qiagen, Netherlands). Obtained RNA samples were directly subjected to RT-qPCR assays in AriaMx6 device (Agilent Technologies, Sancta Clara CA, USA) using the Brilliant III Ultra Fast SYBR Green Master Mix (also from Agilent Technologies) with specific primers for selected genes listed in Table 3.

Results and discussion
Overview of the sequenced genome of Chaetomium cochliodes CCM F-232
In total 6036 contigs were obtained from the genomic DNA of C. cochliodes strain CCM F-232 deposited at GenBank under accession L5BY00000000, BioProject PRJNA309375, BioSample SAMN04432217. 4141 of these contigs were larger than 500 bp. The genome size of the complete assembly was determined to be 34,745,808 bp. This value is very near to previously determined size of closely related C. globosum (updated to 34.9 Mb) [2]. The GC content of the entire genome of C. cochliodes was estimated to 55.95 % which is a slight difference to the corresponding value for C. globosum (55.6 %). The average size of C. cochliodes large genomic contigs (>500 bp) in this experiment was determined as 8256 bp, the N$_{50}$ contig size was 14,381 bp and the largest assembled contig comprised 109,425 bp. As a quality control Phred quality scores were determined according to Illumina device: the portion of Q40+ bases was 34,112,976 (99.83 % of the whole genome sequence draft) whereas Q39+ bases portion was only 59,430 (0.17 %). Prediction of all possible ORFs of C. cochliodes with Chaetomia-optimised FGENESH suite [14] led in both DNA strands to a total value of 10,103. This count is lower than the estimation for mesophilic C. globosum [2] but much higher than the estimation for C. thermophilum [1] or related thermophilic fungi. A brief comparison of three related fungal genomes is presented in Table 4. The average count of exons per predicted C. cochliodes gene was calculated as 3 with FGENESH.

Phylogeny reconstruction in the 18S rDNA – ITS1 – 5.8S rDNA – ITS2 – 28S rDNA region
First, we were interested in the exact phylogenetic position of Chaetomium cochliodes. For this purpose we have reconstructed the DNA phylogeny of its 2217 bp region spanning the region from the 3’ end of the 18S rDNA, the complete ITS1, 5.8S rDNA, ITS2 and the 5’ end of the 28S rDNA containing the highly conserved locus described as universal fungal barcode [17]. Besides all corresponding DNA sequences for species of the Chaetomiaeae family currently available in GenBank, also sequences from related ascomycetous families were included in this reconstruction (Table 1). The DNA alignment used for the phylogeny reconstruction (Additional file 2: Figure S1) reveals clear differences (i.e. substitutions, insertions and deletions) in the sequence of C. cochliodes if compared with corresponding sequences of C. globosum in the entire region. The phylogenetic output presented in Fig. 1 (obtained by two independent methods) clearly
Table 2 List of potentially all genes coding for enzymes involved in H2O2 metabolism in contigs of C. cochliodes genome

| Gene name                | In contig # | Seq. identity* | Closest neighbour** | # Introns | Gene-superfamily relations                                                                 |
|--------------------------|-------------|----------------|---------------------|-----------|------------------------------------------------------------------------------------------|
| A) genes coding for enzymes producing H2O2 |
| CcochCuZnSOD             | 0613        | 98 %           | CgCuZnSOD           | 4         | Copper/zinc superoxide dismutase superfamily (SODC)                                      |
| CcochDAAD                | 0702        | 85 %           | Mth_GZQLH3          | 3         | Flavin D-amino acid oxidase (peroxisomal)                                                 |
| CcochFeMnSOD1            | 0353        | 91 %           | ThFeMnSOD           | 2         | Iron/manganese superoxide dismutase superfamily                                          |
| CcochFeMnSOD2            | 1984 & 0805 | 93 %           | CgFeMnSOD1          | 3         | Iron/manganese superoxide dismutase superfamily                                          |
| CcochFeMnSOD3            | 0879        | 94 %           | CgFeMnSOD2          | 1         | Iron/manganese superoxide dismutase superfamily                                          |
| CcochFIOx1               | 0556        | 55 %           | Colgra_E3QSF0       | 1         | GMC superfamily (flavin oxidases)                                                          |
| CcochGLOx1               | 0600        | 53 %           | Scap_A0A084G823     | 7         | GMC superfamily (flavin oxidases): glucose oxidase                                         |
| CcochNOx1                | 0029        | 93 %           | CgNox2              | 2         | NADPH oxidase                                                                            |
| B) genes coding for enzymes degrading H2O2 |
| CcochkatG1               | 0012        | 93 %           | CgkatG1             | none      | peroxidase-catalase superfamily: bifunctional catalase-peroxidase                        |
| Ccochccp                 | 0676        | 95 %           | Cgccp               | 2         | peroxidase-catalase superfamily: cytochrome c peroxidase                                |
| Ccochpox2a               | 3115 & 3438 | 68 %           | Cthepox2a           | 1         | peroxidase-catalase superfamily: Family II prob. manganese-dependent                      |
| CcochhyBpox1             | 3712 & 3350 | 100 %          | CgyBpox1            | none      | peroxidase-catalase superfamily: hybrid B peroxidase                                      |
| CcochhyBpox2             | 0794        | 93 %           | CgyBpox2            | 1         | peroxidase-catalase superfamily: hybrid B peroxidase                                      |
| CcochcycOx1              | 2133 & 0418 | 83 %           | CgCycOx1            | 3         | peroxidase-cyclooxygenase superfamily: cyclooxygenase                                     |
| Ccochlds                 | 1074 & 4463 | 91 %           | Cglds1              | 5         | peroxidase-cyclooxygenase superfamily: linoleate diol synthase                           |
| Ccochdypx                | 0391        | 89 %           | Cgdypx              | none      | peroxidase-dismutase superfamily: Dyp_B peroxidase (fusion w. PFL)                      |
| Ccochhpt1                | 1650        | 92 %           | Cghpt1              | 3         | peroxidase-peroxygenase superfamily: heme-thiolate peroxidase                             |
| Ccochhpt2                | 2302        | 95 %           | Cghpt3              | 3         | peroxidase-peroxygenase superfamily: heme-thiolate peroxidase                             |
| Ccochhpt3                | 1018        | 85 %           | Cghpt4              | 2         | peroxidase-peroxygenase superfamily: heme-thiolate peroxidase                             |
| Ccochvcpa                | 0469 & 1446 | 93 %           | Cgvcpa              | 3         | non heme peroxidases: vanadium haloperoxidase                                            |
| Ccochgpox                | 0466        | 84 %           | Mthgpx              | 1         | non-metal peroxidases: glutathione peroxidase                                            |
| Ccoch1cysprx             | 1586        | 96 %           | Cg1cysprx           | 1         | non metal peroxidases: 1-cysteine peroxiredoxin                                          |
| Ccoch2cysprx             | 1595        | 99 %           | Cg2cysprx           | 2         | non-metal peroxidases: typical 2-cysteine peroxiredoxin                                  |
| Ccochprxl                | 0388 & 0197 | 95 %           | Cgprxl              | 1         | non-metal peroxidases: atypical 2-cysteine peroxiredoxin                                  |
| CcochkatA1               | 0438 & 2821 | 94 %           | Cgkat1              | 2         | heme catalase superfamily: large subunit heme catalase                                   |
| CcochkatA2               | 1883 & 2899 | 87 %           | Cgkat2              | 3         | heme catalase superfamily: large subunit heme catalase                                   |
| CcochKatB1               | 0511        | 86 %           | Cgkat3              | 2         | heme catalase superfamily: small subunit heme catalase                                   |
| CcochKatB2               | 0351        | 67 %           | SschkatE            | 2         | heme catalase superfamily: small subunit heme catalase                                   |

* - With closest known phylogenetic neighbour
** - Abbreviations of peroxidase & catalase gene names are explained in Additional file 3: Table S2, Additional file 5: Table S4, Additional file 6: Table S3, Additional file 7: Table S5 and Additional file 8: Table S6

segregates Chaetomium cochliodes from closely related C. elatum which is a root-colonizing fungus whose genome is not yet sequenced [19]. Both these fungi are separated from a sister clade represented by three different DNA sequences within this region coding for various C. globosum strains with a high statistical support. This figure clearly demonstrates that the thermophilic representatives (mainly C. thermophilum but also e.g. T. terestris and M. thermophila) of the Chaetoniaceae family can be considered as basal lineages of the Chaetomia clade thus suggesting that mesophily has evolved only secondarily in this lineage. Our results correlate with the previous work on thermophilic
fungi [20] and particularly on the thermostability of Chaeto-
momiaceae [21] where C. cochliodes was not included at that
time.

Putative heme peroxidases & catalases in Chaetomium
cochliodes
Intracellular hydrogen peroxide is a by-product of various physiological pathways but, unique among all reactive oxygen species, it serves also as an important signalling molecule in apoptosis and ageing [22]. In filamentous fungi hydrogen peroxide was shown to be implicated in essential proliferation and differentiation processes [23]. Thus we have performed this genomic screening for all possible ORFs coding for a) enzymes supposed to release H$_2$O$_2$ during their reaction and b) two main types of enzymes involved in the catabolism of hydrogen peroxide in a novel genome of a soil Ascomycete. With TBLASTX method we could identify 8 genes for various oxidoreductases producing H$_2$O$_2$ (Table 2A) and up to 20 distinct genes belonging to various heme and non-heme peroxidase superfamilies as well as to the heme catalase superfamily. Overview on all these genes together with their introns composition is presented in Table 2B. All presented sequences are from contigs of the genome project deposited at GenBank under accession LSBY00000000, BioProject PRJNA309375, BioSample SAMN04432217. From Table 2 it is obvious that genes coding H$_2$O$_2$ degradation exhibit a higher diversity than genes coding H$_2$O$_2$-releasing enzymes. Detected genes for non-heme peroxidases include vanadium-containing haloperoxidase, glutathione peroxidase as well as 1-cysteine and 2-cystein peroxiredoxins. This work focuses further on genes coding for heme peroxidases.

As was presented recently, there are at least four heme peroxidase superfamilies and one heme catalase superfamily that arose independently during a convergent evolution. They differ in overall fold, active site architecture and enzymatic activities [10]. The following sections aim to discover all genes for representatives of all five superfamilies within the genome of C. cochliodes and to determine their exact phylogenetic positions. Heme peroxidases are found in all kingdoms of life and typically catalyse the one- and two-electron oxidation of a myriad of organic and inorganic substrates. In addition to the basal peroxidatic activity distinct families show pronounced catalase, cyclooxygenase, chlorite dismutase or peroxygenase activities.

**Table 3** List of primers for C.cochliodes peroxidase & catalase genes

| B | Primer description | Sequence in 5′→ 3′ direction | Tm [°C] | PCR product size [bp] |
|---|-------------------|-------------------------------|--------|----------------------|
| hyBpox1 | CcochhyBpox1Fwd | CGAGAAACAGATATTCAGGACAC | 60.1 | 116 |
| CcochhyBpox1Rev | TCTACCCGCATTTAAATGTTG | 56.5 | |
| hyBpox2 | CcochhyBpox2FWD | GTCTTTAGCAGGAGGTCAA | 60.3 | 119 |
| CcochhyBpox2aREV | TGTCCTGCTGAGTTAGTCAT | 58.4 | |
| cyox1 | CcochCyox1bFWD | GCCCTCAAAACTTCCAACAAAGA | 58.4 | 117 |
| CcochCyox1bREV | GTACCCGCATGAGGTTGATAT | 60.3 | |
| lds | CcochLD3FWD | AACTTACACCATTCTCCCGTGTC | 58.4 | 127 |
| CcochLD3REV | GTCGTAATGGGCGTCGTCATG | 60.3 | |
| dyPrx | CcochDyprxBfwd2 | AAAGGAATGTGAGCGAACAAGAA | 54.7 | 135 |
| CcochDyprxBrev1 | GCCGAGATCAAGCTCGGAATG | 58.4 | |
| htp1 | CcochHtp1Fwd2 | ATCTCAACGACATCTCTGG | 58.4 | 114 |
| CcochHtp1rev2 | GAAGCCATGTGACTCTATG | 59.8 | |
| katA2 | CcochkatA2_FWD | GAATCAACAGCAGGTGGT | 63.5 | 202 |
| CcochkatA2_1REV | TAGGTTGGTAGGCAAATGAGA | 63.3 | |
| katB1 | CcochkatB2_2REV | TAAACCAAGCTGCTTCC | 58.4 | 207 |
| CcochkatB2_1REV | TGAGAAGCCGCGTGATG | 61.4 | |
| katB2 | CcochkatB2_FWD | GGGGCGATGTTTGAGGACC | 63.5 | 198 |
| CcochkatB2_2REV | TAAACCAAGCTGCTTCC | 58.4 | |

**Table 4** comparison of three related Chaetomia genomes

| Organism | Reference Genome size [bp] | Comparison with C.coch. Predicted ORFs |
|----------|-----------------|-------------------|
| C. cochliodes | this work 34,745,808 | 10.103 |
| C. globosum | [2] 34,886,900 | 100.41 % 11.048 |
| C. thermophilum | 28,322,800 | 81.51 % 7.165 |
**Peroxidase-catalase superfamily**

The peroxidase-catalase superfamily is currently the most abundant peroxidase superfamily in various gene and protein databases. It is comprised of three distinct families (Families I, II and III formerly known as classes) and hybrid peroxidases that represent transition forms (clades) between these families. Here we present an updated reconstruction of the phylogeny of this largest known heme peroxidase superfamily analyzed previously [24, 25]. Our updated input included already 632 complete sequences and is presented in Fig. 2. We focus here on the phylogenetic positions of all representatives (ORFs) found in Chaetomium.

Family I of the peroxidase-catalase superfamily typically contains catalase-peroxidases (KatG), ascorbate peroxidases and cytochrome c peroxidases (CcP) [24]. A HGT-event from Bacteroidetes to filamentous Ascomycetes was previously reported as a peculiarity of katG gene family evolution [26]. Circular tree of the whole superfamily clearly demonstrates that all katG genes from the Chaetomiaceae family (cf. Additional file 3: Table S2 for abbreviations) apparently are late descendants of this HGT event (Fig. 2 left upper part). Within the upper clades we observe a basal position of the thermophilic variants from which their mesophilic counterparts descended. However, a question remains whether only the coding region of katGs was transferred from bacteria to fungi or whether some neighbouring regions were also included in such a transfer? We demonstrate for the gene encoding KatG1 in *C. cochliodes* (i.e. *CcochkatG1*) that the regulatory elements located on 5’ and 3’ regions embedding the ORF are clearly of

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**Fig. 1** Phylogenetic relationship among 34 Ascomycetes reconstructed from the conserved region spanning 18S-ITS1-5.8S-ITS2-28S rDNA genes. Maximum likelihood method from MEGA6 with 1000 bootstraps and MrBayes method over 200,000 generations were applied on the same DNA sequence alignment 2,474 bp long (Additional file 2: Figure S1). Bootstrap values above 50 & posterior probabilities are shown, respectively. Scale bar represents the frequency of ML substitutions.
The promoter region there is (besides the GC box) a typical regulatory sequence – the “CCAT” box involved in eukaryotic oxidative stress response [27]. In the 3’ untranslated region the poly-A site for corresponding mRNA formation can be predicted with a high probability. Thus, we can conclude that a prokaryotic katG was inserted in the fungal genome but received a typical eukaryotic transcription regulation during later evolution. The main physiological role of KatG in C. cochlodes is most propable dismutation of metabolically-generated hydrogen peroxide to molecular oxygen and water, similar to typical (monofunctional) catalases (see below) [24, 26]. In addition to KatG Chaetomia contain genes (ccp) encoding cytochrome c peroxidases (CcP, Fig. 2 – middle of the left part). The relationships among the fungi presented in the CcP phylogenetic analysis suggest that this protein has evolved vertically throughout Ascomycetes. For ccp genes from both C. globosum and C. cochlodes a basal lineage represented by C. thermophilum and M. thermophila is apparent in the reconstructed tree. The physiological role of CcP is still under discussion.

Further phylogenetic reconstruction of the peroxidase-catalase superfamily reveals that in C. cochlodes but not in C. globosum a Family II representative is present (Fig. 2 – lower part). This is very surprising for such closely related fungal species. However, the Family II representative from C. cochlodes has its closest neighbour in C. thermophilum. Family II ascomycetous genes code for hypotethical heme peroxidases with yet unknown reaction specificty but are closely related with well investigated basidiomycetous manganese and lignin peroxidases (Fig. 2, labelled violet). The latter are involved in oxidative degradation of lignin-containing soil.
debris and typically use Mn$^{2+}$ or small organic molecules as electron donors.

Additional representatives from the peroxidase-catalase superfamily in *C. cochliodes* include two paralogs of hybrid B heme peroxidases discovered as a new gene family only recently [25]. Hybrid-type B peroxidases are present solely in fungi but are related to Family III (comprised of numerous plant secretory peroxidases, labelled green in Fig. 2) and also to Family II (fungal secretory peroxidases mentioned above). The basal lineage for the first paralogs (Cc0chHyBpox1) together with its closely related *C. globosum* counterpart appears among mesophilic Sordariomycetes (Fig. 2 upper part). The second variant (Cc0chHyBpox2) containing besides the peroxidase domain also an additional C-terminal WSC (sugar binding) domain is not closely related with *C. globosum* ortholog (Fig. 2 right). Thus, both these HyBpox paralogs are not the result of a recent gene duplication but segregated rather early in the evolution of fungal genomes. Transcription analysis (Table 5 & Additional file 4: Figure S2) reveals a slight induction of both *hyBpox* genes selectively with peroxyacetic acid in the cultivation medium. In contrast, previous results [4] reveal a constitutive mode of expression for distantly related *katG1* gene with hydrogen peroxide and peroxyacetic acid.

**Peroxidase-cyclooxygenase superfamily**

Members of the peroxidase-cyclooxygenase superfamily (comprised of Families I - VII) are widely distributed among all domains of life. In many cases they are multidomain proteins with one heme peroxidase domain [10, 28]. Family IV is comprised of bifunctional cyclooxygenases possessing both peroxidase and cyclooxygenase activities. They are involved in various physiological and pathophysiological processes [29]. In mammals they are located in the luminal membrane of the endoplasmic reticulum and mediate the conversion of free essential fatty acids to prostanoids by a two-step process [30]. The structure and function of the two distinct human paralogs (constitutive COX-1 and inducible COX-2) were intensively investigated but a comprehensive analysis of their diverse paralogs among eukaryotic microbes or even among prokaryots was only recently reported [31]. Evolutionary relationships among fungal cyclooxygenase genes were not analysed in sufficient detail yet.

Our current reconstruction based on the phylogeny of selected members from the whole superfamily (comprising 204 unique genes) is presented in Fig. 4. Genome analysis suggests the occurrence of two representatives of this superfamily in Chaetomia, a cyclooxygenase-like enzyme and a linoleate diol synthase. Cyclooxygenase genes from *C. cochliodes* and *C. globosum* share their closest phylogenetic neighbour (Fig. 4 upper part left) in the genome of *M. mycetomi*, a human pathogenic fungus that grows optimally at room temperature [32]. No cyclooxygenase genes were found in thermophilic fungi so far. In contrast, the evolutionary reconstruction of another important subfamily of Family IV, linoleate diol synthases, reveals a very similar pattern for Chaetomiaceae as already described for the previous superfamily. Corresponding part of the tree (Fig. 4 – upper part right) demonstrates that genes encoding linoleate diol synthases (*lds*) from thermophilic fungi (*M. thermophila* and *C. thermophilum*) represent basal lineages for

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**Table 5** Transcription analysis of 9 selected genes for peroxide catabolism in *C. cochliodes* recorded with RT-qPCR. Quantitative values representing relative changes of the transcription level were obtained by comparison of the expression of a particular gene in 30 min induced vs. non-induced samples. The constitutively expressed ITS1 region was used as internal standard for normalization.

| Analyzed gene | Sample with 5 mM H$_2$O$_2$ | Sample with 5 mM PAA |
|---------------|-----------------------------|---------------------|
| *CcochHyBpox1* | 1.5 x                       | 3.0 x               |
| *CcochHyBpox2* | 0.3 x                       | 1.7 x               |
| *Ccochcyox1*  | 0.3 x                       | 2.3 x               |
| *Ccochlrls*   | 0.4 x                       | 1.8 x               |
| *Ccochdypx*   | 3.3 x                       | 18.5 x              |
| *Ccochhtp1*   | 2.7 x                       | 2.9 x               |
| *CcochkatA2*  | 1.1 x                       | 0.5 x               |
| *CcochkatB1*  | 0.4 x                       | 1.1 x               |
| *CcochkatB2*  | 0.6 x                       | 1.9 x               |

*Changes in the expression levels compared to the control sample (with the reference value of 1.0) were calculated as relative quantities due to the formula $RQ = 2^{-\Delta\Delta Cq}$, where $Cq$ is the quantification cycle of each RT-qPCR reaction. Presented are average values of triplicates for each listed gene and each inducer. Typical amplification plots and melting curves are presented in Additional file 4: Figure S2.*
corresponding genes in mesophilic Chaetomia. Only recently it was shown that fatty acid diol synthases are unique fusion proteins containing a N-terminal heme peroxidase domain joined with a C-terminal P450-heme thiolate domain for conversion of unsaturated fatty acids to dihydroxy-fatty acids [33]. These enzymes are an essential part of the psi factor sexual inducer cascade in various fungi [34]. Their exact physiological role within the life cycle of Chaetomiaceae needs to be elucidated in the future. Our first round of transcription analysis revealed around 2-fold induction of expression of both cyox1 and lds genes in a medium with peroxyacetic acid (Table 5 and Additional file 4: Figure S2).

Peroxidase-chlorite dismutase superfamily

Our next screening within the C. cochliodes genome focused on the presence of genes encoding dye-decolorizing peroxidases (DyPs). These heme enzymes were first isolated from soil basidiomycetes but were further shown to be present in a wide variety of fungi and bacteria [35]. DyPs catalyse the H$_2$O$_2$-mediated oxidation of a very broad substrate range. Originally, fungal representatives were found to degrade bulky dyes. A detailed structure- and sequence-based comparison demonstrated that DyPs together with chlorite dismutases and chlorite-dismutase like proteins (EfeB, HemQ) constitute the CDE superfamily [36], also designated as peroxidase-chlorite dismutase superfamily [10]. The reconstructed evolution of DyPs within this superfamily is shown in Fig. 5. In fungal genomes mainly representatives of the subfamilies DyP-type D and DyP-type B can be found as paralogs. Interestingly, in the genome of C. cochliodes only a fused version of DyP-PFL is present, i.e. an N-terminal DyP peroxidase domain connected
with a C-terminal pyruvate formate-lyase (PFL) domain known as a glycol radical containing region [37]. This unique gene fusion was detected also in other distantly related prokaryotic & eukaryotic genomes [38]. The PFL domain can be activated by PFL activase, a radical SAM superfamily member [39], but the significance of a PFL fusion with a peroxidase domain remains elusive. We could detect a putative PFL activase in *C. cochliodes* contig 00230 revealing 81 % identity with CHGG_03160 from *C. globosum* and other putative PFL activases from filamentous fungi. Thus, *C. cochliodes* possesses both components necessary for the glycol radical formation with yet unknown physiological function. A HGT event with a high bootstrap support in the clade of fused DyPs B can be observed between proteobacteria and ascomycetous fungi (Fig. 5 and Additional file 6: Table S4 for abbreviations). As the fused DyP B-PFL proteins are yet hypothetical, their physiological relevance has to be determined among Chaetomiaceae. Our first round of transcription analysis of *dyprx* gene exhibited the highest induction observed among all 5 superfamilies followed in this study with hydrogen peroxide (3-fold) and mainly
with peroxycetic acid (18.5-fold) in the cultivation medium (Table 5).

Peroxidase-peroxygenase superfamily
Heme-thiolate peroxidases from Fungi and Stramenopiles constitute the peroxidase-peroxygenase superfamily [10]. Enzymes encoded by htp genes represent probably the most versatile catalysts among peroxidase superfamilies thus catalysing on one side classical heme peroxidase reactions and on the other side monooxygenase (monohydroxylation) reactions like cytochrome P450s [40]. The reconstructed phylogenetic tree for the peroxidase-peroxygenase superfamily (Fig. 6) reveals the distribution of three gene paralogs of this superfamily within the Chaetomiaceae genome. The presence of multiple gene paralogs in genomes of ascomycetous fungi is frequent and occurred by repeated gene duplications of this rather short gene but the phylogenetic distribution of C. cochliodes paralogs is variable (Fig. 6). Whereas there is a thermophilic basal lineage for CcochHTP2 and CcochHTP3 and their corresponding counterparts in C. globosum, the situation for paralog CcochHTP1 is different. Corresponding genes from pathogenic fungi represent a basal lineage for closely related CcochHTP1 and CgHTP1. It is unknown so far whether these three putative heme-thiolate peroxidases exhibit different enzymatic properties but they were segregated early during the evolution of fungal genomes and thus they all may be interesting for biotechnological applications. We have also performed transcription analysis of htp1 gene paralog resulting in almost 3-fold induction both with hydrogen peroxide and peroxycetic acid present in the cultivation medium (Table 5).

Putative heme catalases in Chaetomi
Typical (monofunctional) heme catalases are enzymes that very efficiently dismutase hydrogen peroxide to
oxygen and water. In contrast with heme peroxidases they can both reduce and oxidize hydrogen peroxide and have negligible peroxidatic activity [41]. Heme catalases represent a monophyletic group that evolved as a distinct gene family from prokaryotes to almost all lineages of eukaryotes [11]. In fungi only representatives of Clade 2 (large subunit, secretory catalases) and Clade 3 (small subunit, mostly peroxisomal catalases) can be found. There are up to four gene paralogs of a catalase gene within C. cochliodes genome that underlines the importance of four gene paralogs of a catalase gene within C. cochliodes (Fig. 7 – on the right). In particular, CcK KatB1 and CcK KatB2 have a basal lineage among catalases from various soil and phytopathogenic fungi. Surprisingly, CcK KatB2 has no counterpart in the closely related genome of C. globosum. Putative catalase from a widely distributed soil fungus S. schenckii shares a common ancestor with this unique small subunit peroxisomal catalase of C. cochliodes (Fig. 7). Possible involvement of C. cochliodes four catalase isoforms in the defence against oxidative stress was analysed by RT-PCR. Obtained results in the early exponential phase of fungal growth show only a slight

Fig. 7 Reconstructed phylogeny of the heme catalase superfamily with focus on Clade 2 and 3 representing the distribution of Ascomycetous large subunit as well as small subunit catalases (labelled in different colors). The complete tree from 222 full length sequences is presented with 546 sites aligned. C. cochliodes paralogs are labelled red. Distinct clades are labelled in different colours. Values in nodes represent bootstrap values above 50 (from maximum likelihood analysis) and posterior probabilities (from Mr. Bayes), respectively. Abbreviations of peroxidase names are listed in Additional file 8: Table S6. Abbreviations of taxa: Ar, Archaea; Ac, Actinobacteria; AcI, Acidobacteria; Bi, Bacteroidetes; Chl, Chloroflexi (bacteria); Cy, Cyanobacteria; Dei, Deinococci; Fi, Firmicutes; Pb, Proteobacteria; Pmc, Planctomycetes; As, Ascomycota; Ba, Basidiomycota; Chy, Chytridiomycota; Zy, Zygomycota; Cn, Cnidaria; Ichh, Ichthyosporea; Chlph, Chlorophyta; BlMgno, basal Magnoliophyta; My, Mycetozoa; Cryp, Cryptogams, Eudi, Eudicotyledons, Mctd, Monocotyledons; De, Deuterostomia; Ec, Ecdysozoa

![Diagram of catalases](image-url)
induction of the paralog katB2 in the medium containing peroxyacetic acid (Table 5).

**Conclusions**

In conclusion, genomic sequence analysis revealed that *Chaetomium cochlioides* is closely related to *C. globosum* & *C. elatum*. These three filamentous fungi are mesophilic but probably have thermophilic ancestors as revealed from their basal lineage. *C. cochlioides* contains heme peroxidases and catalases from all so far described superfamilies. Ascomycetous genes encoding catalase-peroxidase and dye decolorizing peroxidase were obtained during the evolution by horizontal gene transfer from various bacteria. Several heme peroxidases of *Chaetomia* like hybrid heme B peroxidase, lipoate diol synthase and DyP-type B form fusions with additional functional domains that might enable a broader catalytic variability. Furthermore cytochrome c peroxidase, manganese and three paralogs of heme-thiolate peroxidases are found in addition to typical (monofunctional) catalases of large and small subunit architecture. Our transcription analysis reveals the highest induction of a fused *dyprx* gene with hydrogen peroxide and mainly with peroxyacetic acid in the cultivation medium followed by moderate inductions of *htp1* and *hyBpox1* genes.

**Additional files**

**Additional file 1: Table S1.** Substitution models with the lowest Bayesian information criterion scores for all 5 superfamilies analysed in this contribution. (XLXS 54 kb)

**Additional file 2: Figure S1.** DNA sequence alignment of genomic DNA from 94 ascomycetous fungi in the region covering 185 rDNA – ITS1 – 5.8S rDNA – ITS2 – 28S rDNA (fasta format). (FAS 85 kb)

**Additional file 3: Table S2.** Abbreviations of peroxidase gene names used for the peroxidase-catalase-superfamily. (XLXS 54 kb)

**Additional file 4: Figure S2.** A typical profile of real-time quantitative PCR analysis of transcripts from peroxidase genes obtained from *C. cochlioides* under oxidative stress. Upper panel: amplification plots for *hyBpox1*, *cyox1* and *lds* genes detected with SYBR Green Master Mix (Agilent Technologies). (PDF 450 kb)

**Additional file 5: Table S3.** Abbreviations of peroxidase gene names used for the peroxidase-cyclooxygenase superfamily. (XLXS 27 kb)

**Additional file 6: Table S4.** Abbreviations of peroxidase gene names used for the peroxidase-dismutase superfamily. (XLXS 23 kb)

**Additional file 7: Table S5.** Abbreviations of peroxidase gene names used for the peroxidase-peroxygenase superfamily. (XLXS 18 kb)

**Additional file 8: Table S6.** Abbreviations of catalase gene names used for the catalase superfamily. (XLXS 22 kb)

**Abbreviations**

CcP: Cytochrome c peroxidase; CldL: Chlorite dismutase-like protein; CTAB: Hexadecyltrimethylammonium bromide; HGT: Horizontal gene transfer; HimM: Hidden Markov model; KatG: Bifunctional catalase-peroxidase; LDS: Lipoate diol synthase; LIPOX: Lignin peroxidase; ML: Maximum likelihood phylogeny; MnPOX: Manganese peroxidase; ORF: Open reading frame; PAA: Peroxyacetic acid; PEG: Polyethylene glycol; PFL: Pyruvate formate-lyase; RT-qPCR: Quantitative real-time PCR; SOD: Superoxide dismutase; WSC: Cell-wall integrity & stress response component

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**Availability of data and materials**

All used DNA sequences are deposited in GenBank (Table 1). All protein sequences that were used for reconstruction of phylogenies are listed in Additional file 3: Table S2, Additional file 6: Table S4, Additional file 5: Table S5, Additional file 7: Table S5 and Additional file 8: Table S6. If possible their PeroxiBase accession number is given to find them at (http://peroxibase.toulouse.inra.fr) if no PeroxiBase accession numbers exist yet their Uniprot (http://www.uniprot.org) accession numbers are given.

**Authors’ contributions**

MZ selected the fungus, designed all experiments, performed all molecular phylogeny analyses and wrote the manuscript; AK cultivated the fungus and performed genomic & transcription analyses; KC optimised the isolation of fungal DNA and performed genomic & transcription analysis; KL prepared the genomic DNA for sequencing and contributed to the discussion; HT performed the sequencing and assembled the contigs; CO evaluated the classification and phylogeny of peroxidases & catalases and finalized the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Consent to publish**

Not applicable (this manuscript does not contain any individual persons data).

**Ethics approval and consent to participate**

Not applicable for this fungal genomic study. None of here analysed genes of *Chaetomia* was used in experimental cloning research (yet).

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