Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 331 (2005) 253–260
BBRC
www.elsevier.com/locate/ybbrc

# Discovery of a novel superfamily of type III polyketide synthases in *Aspergillus oryzae* ☆

Yasuyo Seshime [a], Praveen Rao Juvvadi [a], Isao Fujii [b], Katsuhiko Kitamoto [a,*]

[a] *Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*
[b] *Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

Received 23 March 2005
Available online 2 April 2005

**Abstract**

Identification of genes encoding type III polyketide synthase (PKS) superfamily members in the industrially useful filamentous fungus, *Aspergillus oryzae*, revealed that their distribution is not specific to plants or bacteria. Among other Aspergilli (*Aspergillus nidulans* and *Aspergillus fumigatus*), *A. oryzae* was unique in possessing four chalcone synthase (CHS)-like genes (*csyA*, *csyB*, *csyC*, and *csyD*). Expression of *csyA*, *csyB*, and *csyD* genes was confirmed by RT-PCR. Comparative genome analyses revealed single putative type III PKS in *Neurospora crassa* and *Fusarium graminearum*, two each in *Magnaporthe grisea* and *Podospora anserina*, and three in *Phenarocheate chrysosporium*, with a phylogenic distinction from bacteria and plants. Conservation of catalytic residues in the CHSs across species implicated enzymatically active nature of these newly discovered homologs.


*Keywords:* *Aspergillus oryzae*; Type III polyketide synthase; Chalcone synthase; Fungal secondary metabolism

Chalcone synthases (CHSs), the well-known representatives of type III polyketide synthase (PKS) superfamily, catalyze the condensation of 4-hydroxycinnamoyl-CoA and three malonyl-CoA molecules to form the chalcone derivative, naringenin chalcone, which is the first committed step in the phenylpropanoid pathway of plants leading to the biosynthesis of flavonoids, isoflavonoids, and anthocyanins [1]. Plant phenylpropanoids and flavonoids serve as antimicrobial and pigmentation agents, and as protectants from detrimental effects of UV light [2–4]. While the CHSs are functionally well characterized and traditionally supposed to be plant-specific until recently, the existence and functionality of bacterial type III PKSs have been reported [5–7]. Recently chalcone–flavanone isomerases, considered as downstream enzymes acting on the products of CHSs in plants, have been identified in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Neurospora crassa* [8]. Considering the absence of a CHS ortholog whenever a chalcone–flavanone isomerase is present in bacteria or ascomycetes, it was proposed that a flavonoid/isoflavonoid pathway akin to plants may not exist in these organisms and alternatively other unknown isoflavonoid-like compounds may be synthesized [8].

Among different microbes, filamentous fungi are used as sources of important metabolites that find wide usage in agriculture, industry, medicine, and nutrition [9,10]. While the primary metabolites are common to fungi, the secondary metabolites such as aflatoxins, antibiotics, pigments, etc., produced by the activity of specific PKSs represent infrequent chemical structures [9–12]. Little is known about their genetic architecture and physiological role despite the fact that some fungi have been exploited industrially for over several decades [13]. The well-characterized fungal secondary metabolites are

☆ *Abbreviations:* PKS, polyketide synthase; CHS, chalcone synthase; PFAM, protein families database of alignments.

* Corresponding author. Fax: +81 3 5841 8033.
*E-mail address:* akitamo@mail.ecc.u-tokyo.ac.jp (K. Kitamoto).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.160

aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and their biosynthetic pathway includes transcriptional activators, other genes, and genes encoding type I PKSs, that are arranged as a cluster in the genome [14]. While the *koji* mold, *Aspergillus oryzae*, is well recognized as a source of many useful enzymes and proteins in Japanese fermentation industry [13,15], recent completion of its genome sequencing revealed the presence of several genes encoding type I PKSs and cytochrome P450s pointing to its inherent secondary metabolic potential [16]. Although the existence of an aflatoxin biosynthesis gene cluster in *A. oryzae* and its non-functionality due to mutations in some of the key regulatory genes has been reported [17], the presence of putative type III PKSs in this fungus or any other fungi has not been examined. In accordance with these findings, we herein report for the first time on the occurrence of genes encoding type III PKSs in *A. oryzae* highlighting a further possibility of its metabolic diversity.

## Materials and methods

*Strains, media, and culture conditions*. The genomic DNA and cDNAs were derived from *A. oryzae* (RIB40). DPY medium was used for culturing *A. oryzae* [13]. *Escherichia coli* DH5α and pT7 Blue vector were utilized for subcloning experiments.

*Isolation of RNA and RT-PCR analysis*. Conidia of *A. oryzae* RIB40 strain were cultivated as submerged cultures in DPY medium at 30 °C with shaking at 120 rpm for 1–7 days. Total RNA was extracted from freeze-dried mycelia using ISOGEN (Nippon Gene) according to the manufacturer's protocol. First strand cDNA synthesis reaction contained 1 μg of total RNA, 4 μl of 5× ReverTraAce buffer, 8 μl dNTPs (2.5 mM), 1 μl oligo(dT)$_{12-18}$ Primer (Invitrogen), and 3.5 μl DEPC treated $H_2O$. Reaction was heated to 70 °C for 10 min followed by cooling to room temperature, addition of RNase inhibitor and 1 μl of ReverTraAce reverse transcriptase (Toyobo). First strand synthesis proceeded at 42 °C for 1 h followed by heat inactivation for 5 min at 99 °C. PCRs contained 1 μl of the first strand reaction mixture, 1× *Ex Taq* buffer (TaKaRa), 8 μl dNTPs, 0.5 μM each of primers designed specifically for CSYA (5′-ATGGCGCCCTTAATTCATGGTAAC-3′ and 5′-TCAGCGATATCTGGTAGCTTGG-3′), CSYB (5′-TGCCCT TGTTCTCAGCAATGG-3′ and 5′-ACGCATCGTAGCTCGCTCT C-3′), CSYC (5′-CCCTGGATTCGACTGCACACTTTCT- 3′ and 5′-GATCATCCTCGGAACGTCC T-3′), and CSYD (5′-CTATCTCG CCATTGTCGTCATTCTCC-3′ and 5′-AGGTCCAATGCTAAGCT CGC-3′), and 2.5 U *Ex Taq* DNA polymerase. The reaction mixtures were heated to 94 °C for 30 s followed by 35 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min. γ-Actin expression was taken as a control using the primers 5′-GTTGCTGCTCTCGTCATTGAC-3′ and 5′-GTAATCGGTCAAATCACGGCC-3′.

*Cloning and sequencing of csyA and csyB cDNAs*. The 5′ and 3′ ends of csyA and csyB cDNAs were obtained by RACE-PCR using the respective gene-specific primers: 5′ CSYA (5′-CTCTGAACGAGC TCCTCTCTGGAA-3′) and 3′ CSYA (5′-CGGATGTTGAACTGG ATGCTGGAAG-3′) for csyA, 5′ CSYB (5′-TGAACACTGGCACC GACCGACTA-3′) and 3′ CSYB (5′-GCCGCTGTTCCGATGCCT TATGG-3′) for csyB, and reactions were performed using GeneRacerTM RACE kit (Invitrogen). PCR-amplified cDNA fragments were cloned into the plasmid, pCR 4Blunt-TOPO (Invitrogen), and sequenced on an ABI PRISMTM 310NT Genetic Analyzer.

*Sequence data analyses*. CHS-like protein sequences were obtained from fungal and yeast genome databases; (1) http://www.tigr.org/tdb/e2k1/afu1/, (2) http://www.broad.mit.edu/annotation/fungi/aspergillus/, (3) http://www.broad.mit.edu/annotation/fungi/fusarium/, (4) http://www.broad.mit.edu/annotation/fungi/magnaporthe/, (5) http://www.broad.mit.edu/annotation/fungi/neurospora_crassa_7/index. html, (6) http://podospora.igmors.u-psud.fr/index.html, (7) http://www.broad.mit.edu/annotation/fungi/rhizopus_oryzae/, (8) http://www.broad.mit.edu/annotation/fungi/ustilago_maydis/, (9) http://www.broad.mit.edu/annotation/fungi/coprinus_cinereus/, (10) http://genome.jgi-psf.org/whiterot1/whiterot1.home.html, (11) http://www.candidagenome.org/, (12) http://www.broad.mit.edu/annotation/fungi/cryptococcus_neoformans/index.html, (13) http://www.yeastgenome.org/, and (14) http://www.genedb.org/genedb/pombe/index.jsp. Pairwise alignments were performed using BLAST and ClustalW program for generating multiple alignments and dendrogram. The genome sequence data of *A. oryzae* will be available publicly after publication [16].

*Accession numbers*. GenBank Accession Nos. for selected plant sequences were: STS (*Arachis hypogaea*, Stilbene synthase, AB027606); CTAS (*Hydrangea macrophyll a*, *p*-coumaryl triacetic acid synthase, AB011468); CHS2 (*Medicago sativa*, CHS, ALFCHS2A); BAS (*Rheum palmatum*, benzalacetone synthase, AF326911); ACS2 (*Ruta graveolens*, acridone synthase, RGR297788); At-15217605 (*Arabidopsis thaliana*, CHS and STS family protein, NP_171707); At-15236691 (*Arabidopsis thaliana*, CHS and STS family protein, NP_191915); Os-15341587 (*Oryza sativa*, Putative CHS, AAK95679); and Os-7433785 (*Oryza sativa*, CHS homolog, T03612). Bacterial sequences: DhpA (*Amycolatopsis orientalis*, dihydroxyphenylacetate synthase, T17474); PhlD (*Pseudomonas fluorescens*, PhlD protein, AAB48106); RppA (*Streptomyces griseus*, 1,3,6,8-tetrahydroxynaphthalene synthase, BAA33495); PKS11 (*Mycobacterium tuberculosis*, possible CHS, NP_216181); and PKS18 (*Mycobacterium tuberculosis*, hypothetical protein, NP_215888). The genomic DNA sequences pertaining to the *csyA*, *csyB*, *csyC*, and *csyD* have been deposited in DDBJ and their accession numbers are listed in Table 1.

Table 1
List of putative type III polyketide synthases in fungi

| Fungi | Number | Gene | Database* |
|---|---|---|---|
| *Aspergillus oryzae* | 4 | *csyA* (AB206758)<br>*csyB* (AB206759)<br>*csyC* (AB206760)<br>*csyD* (AB206761) | This study |
| *Aspergillus fumigatus* | 0 | | (1) |
| *Aspergillus nidulans* | 0 | | (2) |
| *Fusarium graminearum* | 1 | FG08378.1 | (3) |
| *Magnaporthe grisea* | 2 | MG04643.4<br>MG06254.4 | (4) |
| *Neurospora crassa* | 1 | NCU04801.1 | (5) |
| *Podospora anserina* | 2 | Contig1186<br>Contig1894 | (6) |
| *Rhizopus oryzae* | 0 | | (7) |
| *Ustilago maydis* | 0 | | (8) |
| *Coprinus cinereus* | 0 | | (9) |
| *Phanerochaete chrysosporium* | 3 | PC132.3.1<br>PC132.5.1<br>PC16.58.1 | (10) |
| *Candida albicans* | 0 | | (11) |
| *Cryptococcus neoformans* | 0 | | (12) |
| *Saccharomyces cerevisiae* | 0 | | (13) |
| *Schizosaccharomyces pombe* | 0 | | (14) |

* Refer to Materials and methods for database addresses.

## Results and discussion

### *Identification and cloning of putative chalcone synthase homologs in A. oryzae*

CHSs being the prominent members of type III PKS superfamily have received significant attention in plants and bacteria by virtue of their products with agricultural and pharmaceutical values [18,19]. While type I PKSs in filamentous fungi have been well documented with their relevance for spore pigmentation and secondary metabolite production [13,20,21], the type III PKSs have always been considered restricted to either plants or bacteria [3]. Since filamentous fungi are remarkable producers of natural products [9–12], and in contrast to bacteria exhibit morphological differentiation it was of interest to examine the existence of type III PKSs in fungi.

Preliminary effort to identify the type III PKSs in the *A. oryzae* genome revealed 4 CHS-like type III PKS sequences using the *Medicago sativa* CHS2 amino acid sequence, that were designated as *csyA*, *csyB*, *csyC*, and *csyD* (Fig. 1). The respective cDNA sequences for two putative type III PKSs (*csyA* and *csyB*) were determined by sequencing the 5′ and 3′ end partial cDNA fragments obtained by RACE-PCR together with the internal fragments. CsyA and CsyB showed a 45% amino acid identity between each other. While the *csyA* ORF spanning 1209 bp encoded a polypeptide of 402 amino acids with a calculated molecular mass of 43.5 kDa, the *csyB* ORF extended to 1194 bp with 397 amino acids and a calculated molecular mass of 43 kDa.

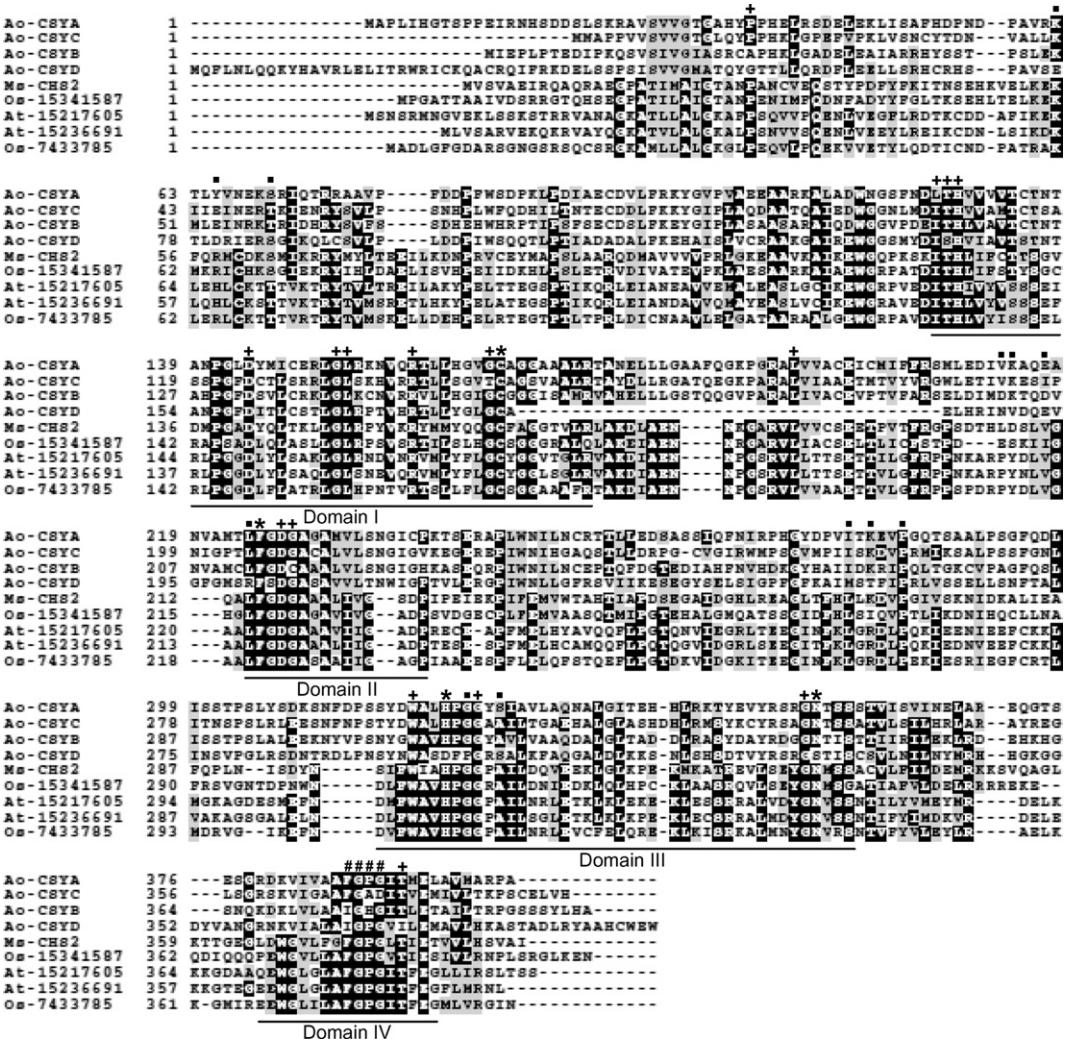


Fig. 1. Complete amino acid sequences of CHS-like proteins from *A. oryzae* (refer to Table 1 for GenBank accession numbers), aligned with representative CHSs from *M. sativa* (CHS2 GenBank Accession No. ALFCHS2A), *O. sativa* (CHS GenBank Accession No. AAK95679; CHS GenBank Accession No. T03612), and *A. thaliana* (CHS GenBank Accession No. NP_171707; CHS GenBank Accession No. NP_191915). Highly conserved residues in all the sequences are shaded in black and only in the fungal CHS sequences are highlighted in grey. Domains showing conservation are designated as I, II, III, and IV. Catalytic residues, other highly conserved residues, CoA-binding sites, and CHS-signature sequence residues are indicated as *, +, ., and #, respectively.

A comparative analysis of *A. oryzae* putative type III PKSs with plant CHSs showed conservation of the catalytic center residues Cys, Phe, His, and Asn at positions 164, 215, 303, and 336, respectively (Fig. 1; amino acid positions mentioned are with respect to *M. sativa* CHS2 sequence and are indicated by asterisks). Apart from this basic criterion required to classify a protein as a member of the CHS family, further evidence to support the nature of these CHS-like type III PKS sequences was obtained based on their conservation with other plant CHSs (Fig. 1). *A. oryzae* putative type III PKSs could be divided into four conserved domains that constituted first, a broad stretch of residues in the N-terminal half which included the active site with the highly conserved Cys residue apart from at least eight other residues (indicated by +) known to be highly conserved among the plant CHSs [22]. Second, a narrow stretch of residues which included the Phe active residue in addition to two other conserved residues, Asp and Gly. While the third conserved domain included the His and Asn active residues, and three other conserved residues (Trp and two Gly residues), the fourth domain included the CHS-family signature sequence G(F/L)GPG [22] at position 372–376 on the C-terminus with the four conserved residues (Phe, Pro, and two Gly residues; indicated by #), which indicated that these amino acids involved in substrate specific recognition were also conserved in the fungal CHS-like type III PKS sequences (Fig. 1). Moreover, in addition to the residues for substrate specificity and catalytic activity, the conservation in 16 (Fig. 1; indicated by +) of the 18 residues that are highly conserved in plant CHSs [23] confirmed that these sequences encoded functional chalcone synthases.

### *Phylogenetic analysis of fungal chalcone synthase-like genes*

Since *A. oryzae* contained putative homologs of plant CHSs, the occurrence of CHS-like type III PKS genes in other fungi was verified by scanning the available fungal genome databases using the *A. oryzae* putative type III PKS sequences as mentioned under Materials and methods. As shown in Table 1 and Fig. 2, putative type III PKSs were identifiable in *Neurospora crassa*, *Magnaporthe grisea*, *Phanerochaete chrysosporium*, *Fusarium*

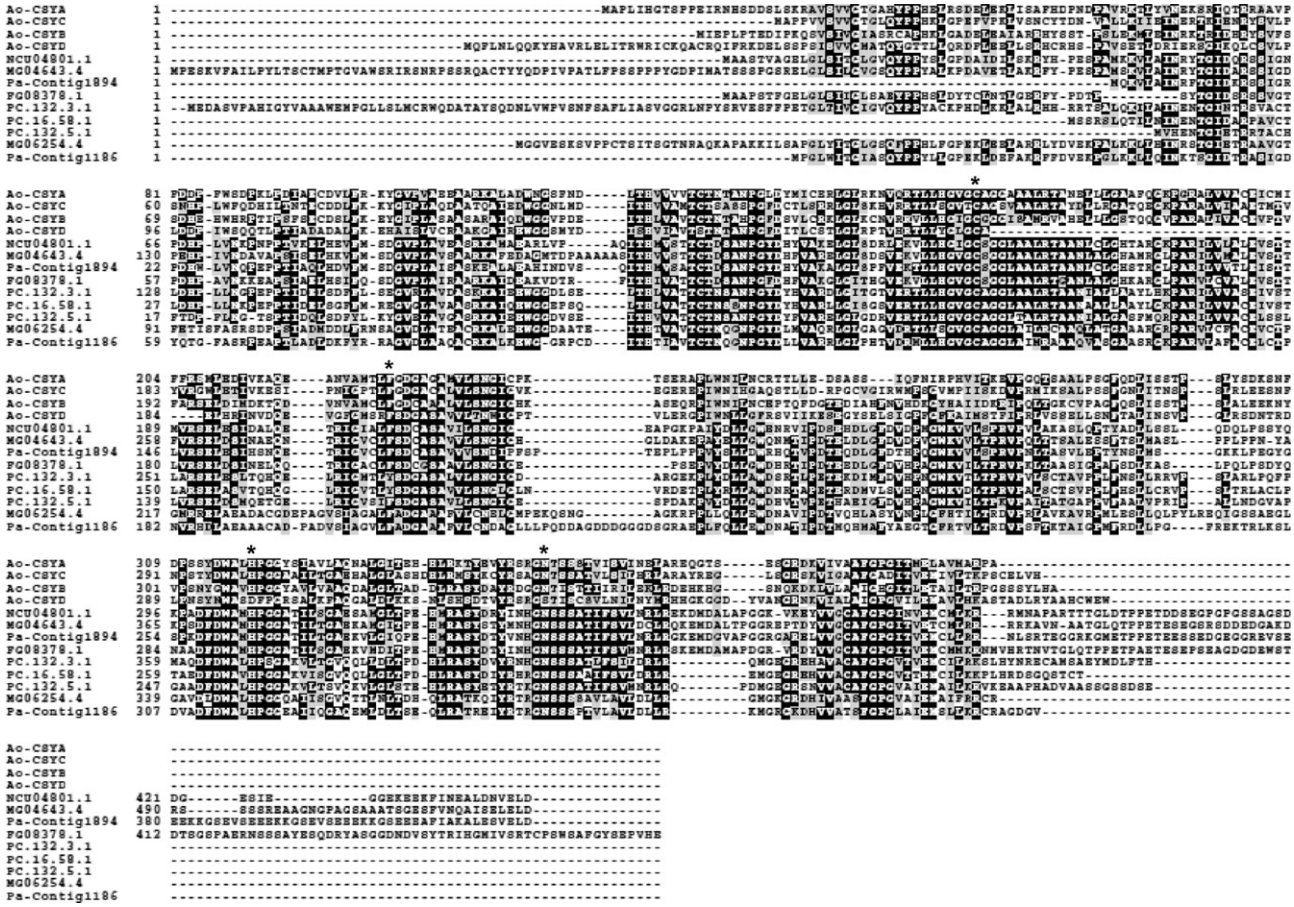

Fig. 2. Comparison of amino acid sequences of CHS-like type III PKSs from *A. oryzae* (refer to Table 1 for respective GenBank accession numbers) with other putative type III PKSs from *N. crassa*, *M. grisea*, *F. graminearum*, *P. chrysosporium*, and *P. anserina*. Catalytic residues are indicated as ∗.

*graminearum*, and *Podospora anserina*. The number of putative type III PKSs varied among these fungi with *N. crassa* and *F. graminearum* consisting of a single homolog each, *M. grisea* and *P. anserina* each with two homologues, and *P. chrysosporium* with three homologues, respectively. Comparison of *A. oryzae* putative type III PKSs with other fungal type III PKS sequences showed a predominant conservation in the catalytic residues and the CHS-family signature sequence at the C-termini (Fig. 2).

As listed in Table 1, it may be noted that a majority of these fungi are classified as plant pathogens pointing to their evolutionary significance. It is possible that these fungal CHS-like type III PKS genes have been acquired by lateral gene transfer from plants. Although *A. oryzae* is not a plant pathogen its close relatives, *A. flavus* and *A. parasiticus*, are opportunistic plant pathogens, [24]. This aspect is significant considering that recent completion of the *A. oryzae* genome sequencing revealed the presence of several other genes (encoding pisatin

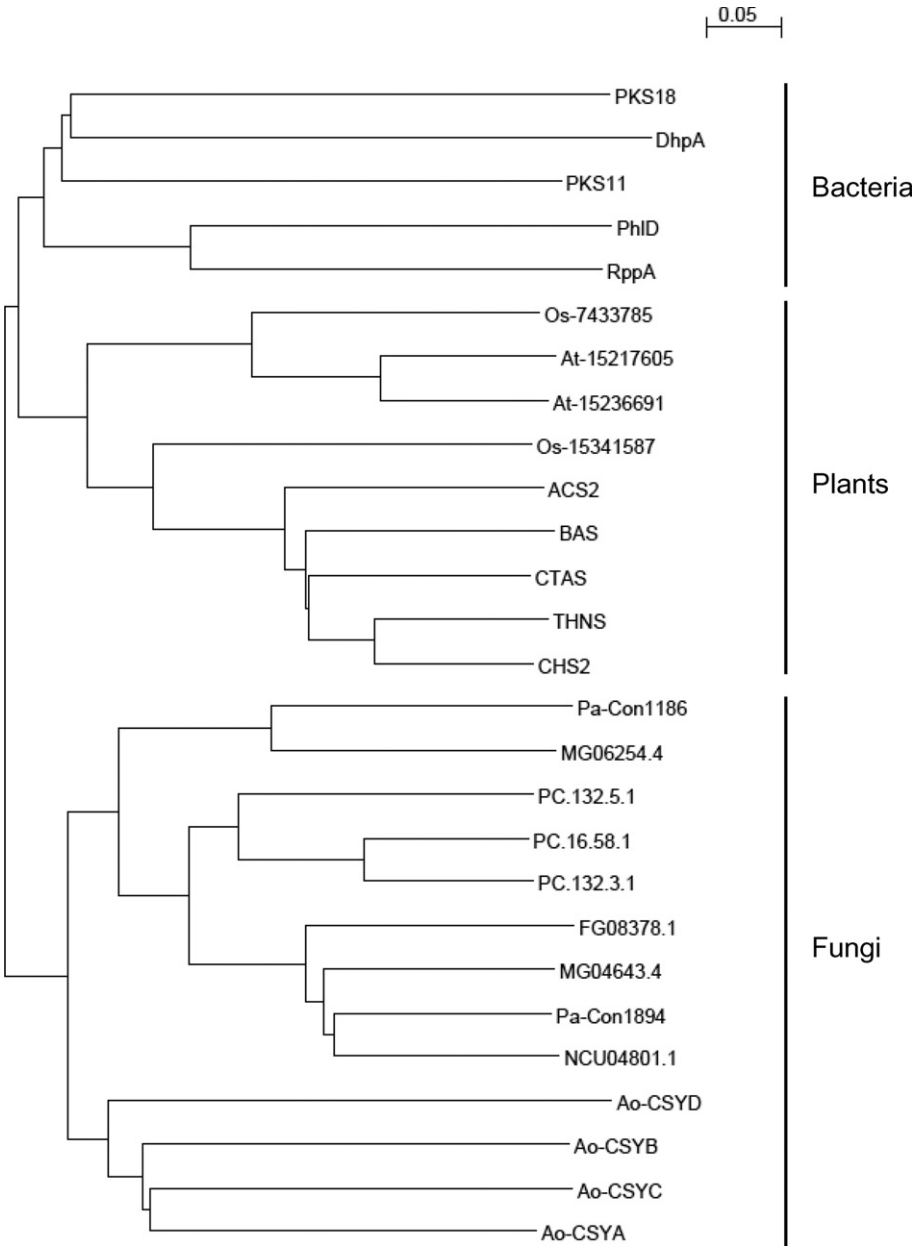


Fig. 3. Phylogenetic tree for type III PKS sequences from bacteria, plants, and fungi. The dendrogram was generated for bacterial, plant, and putative fungal type III PKSs using chalcone synthase and stilbene synthase domains from PFAM searches. Sequences are designated according to species abbreviation. Scale bar represents 0.05 amino acid substitutions per site. (Refer to Table 1 for gi numbers of putative fungal CHSs; see Materials and methods for description on accession numbers of plant CHSs; refer to Table 1 for *A. oryzae* GenBank accession numbers.)

demethylases, trichothecene hydroxylases, and isotrichodermin hydroxylases) reminiscent of plant pathogenic character [16]. This is supported by the fact that we could not find putative CHS homologs in the genomes of other Aspergilli (*A. nidulans* or *A. fumigatus*) that are not considered as plant pathogens.

As shown in Fig. 3, a phylogenic tree constructed by using the conserved chalcone and stilbene synthase domains revealed three major branches corresponding to the plant, bacterial, and fungal PKSs, respectively. While the fungal type III PKSs whose functions are yet to be deciphered were present in a separate branch in the dendrogram, *A. oryzae* putative type III PKSs belonged to a different cluster. Similar distinction in cluster may be observed between the bacterial and plant type III PKSs. Based on this phylogenic analysis it may be considered that the fungal type III PKSs are distinct from those of bacteria and plants. While it is too early to confirm on the nature of products produced by these putative type III PKSs, it may be hypothesized that plant flavonoid-like metabolites are produced in certain fungi.

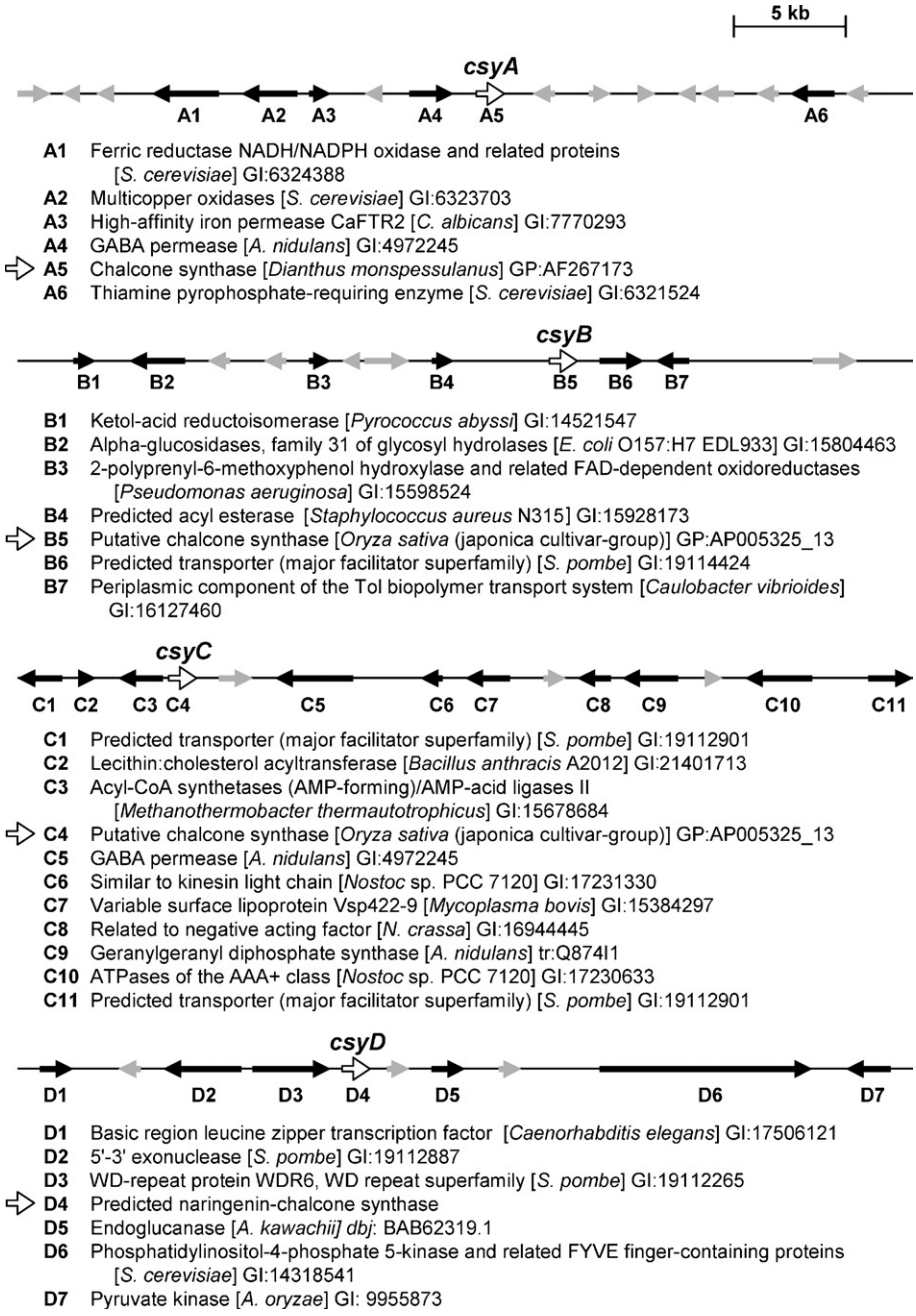


Fig. 4. Genomic organization of *A. oryzae* type III PKSs. Arrows in black represent putative ORFs and arrows in grey are genes with unknown function. The respective *csy* genes are shown as white arrows.

*Genomic organization and expression pattern of chalcone synthase genes in A. oryzae*

Since several fungal secondary metabolic genes in common are clustered [14,25], we sought to examine the genomic organization of the CHS-like type III PKS genes in *A. oryzae*. As shown in Fig. 4, an examination of ~40 kb region surrounding the *csy* genes revealed some interesting data on other genes present close to each of these candidate genes. Importantly, the presence of genes encoding ferric reductase, copper oxidase, and GABA permease close to the *csyA* gene (Fig. 4A), and acyl-CoA synthase, GABA permease, geranylgeranyl diphosphate synthase, and predicted transporter near the *csyC* gene (Fig. 4C), implicated some of these genes participating in coordination with the *csy* genes during the synthesis of complex secondary products. However, no chalcone–flavanone isomerase gene was detected in these gene clusters. Interestingly, plant stress responses relating to low nitrogen and iron availability depend on the phenyl propanoid pathways involving CHSs [26]. Other genes of interest were the keto-acid reductoisomerase, 2-polyprenyl-6-methoxyphenol hydroxylase, and a predicted acyl esterase surrounding the *csyB* gene (Fig. 4B), which resembled those from bacterial species. Filamentous fungi share a common trait with higher plants and certain prokaryotes in their ability to synthesize a wide variety of secondary metabolites [27]. For instance, gibberellins that are produced by all higher plants and antibiotics produced by some bacteria are also produced by filamentous fungi [25], however, the gene clusters responsible for the synthesis of these compounds reveal low level amino acid identity. Although it is largely believed, it is not yet clear if the secondary metabolic genes have been acquired by fungi through horizontal transfer either from plants or bacteria [27]. Considering higher homology of these CHS-like type III PKS sequences with those of plant CHSs, it seems reasonable to postulate a lateral gene transfer mechanism that led to some of these fungi acquiring the CHS genes from plants during evolution.

In order to assess the functionality of *A. oryzae* putative type III PKSs, their expression was verified by RT-PCR. While the *csyA* and *csyD* genes expressed during 1–7 days of culturing *A. oryzae* in DPY medium, the *csyB* gene was expressed during 1–3 days. However, the reason behind the absence in expression of *csyC* during all the days examined remains unknown (Fig. 5). In the case of *csyD* expression, two transcripts which could be the non-spliced and spliced forms were noted. Although the genomic organization and expression analysis reveal a possibility of the *csy* genes functioning in *A. oryzae*, their participation in the synthesis of new products is to be investigated. CHS genes express constitutively in a tissue-specific manner in pigmented flowers and roots, and are induced by environmental stresses,

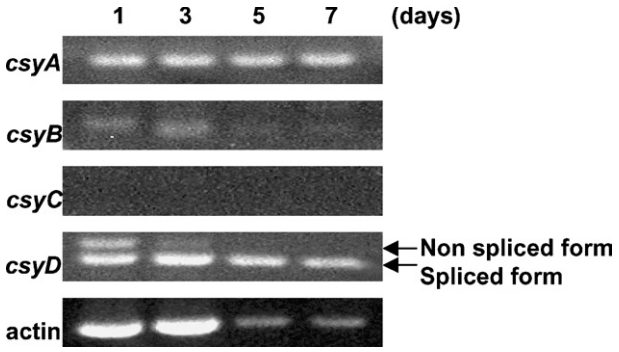


Fig. 5. Expression analysis of *A. oryzae* putative chalcone synthase genes. Conidia of *A. oryzae* (RIB40) strain were cultured in DPY medium for 1, 3, 5, and 7 days. RT-PCR was performed after total RNA extraction as mentioned under Materials and methods.

infection by microbial pathogens, UV light, wounding, and treatment with elicitors [28]. In this regard, MYB like proteins apart from other endogenous regulators (stress-induced transcription activators or repressors) up-regulate flavonoid gene expression in plants [29]. Although we have not examined the expression of *A. oryzae csy* genes under stress conditions, their promoters revealed putative binding sites for transcription factors that are usually present in plant CHS-encoding gene promoters [29] (data not shown).

*Aspergillus* species exhibit a clearly distinguishable genetic diversity with regard to being either pathogenic (*A. fumigatus*, *A. flavus*, *A. parasiticus*), or non-pathogenic (*A. nidulans*) or industrially useful (*A. oryzae*, *A. niger*). Hence, during the course of evolution, microbes of the same genus may tend to develop and maintain newer genotypic traits. Although we have been able to access the untapped metabolic diversity of *A. oryzae* and other fungi via genomic sequence analyses and herein reported the presence of type III PKS homologs, elucidating the role of *csy* genes in fungal stress response pathways and synthesis of flavonoid-like metabolites remains a challenging area of research. In particular, the discovery of novel type III PKS family members in *A. oryzae*, which is well recognized for its industrial applicability, demonstrates the possibility of exploiting its inherent metabolic potential and directing rational approaches for the expression of these genes, detection and purification of new bioactive natural products.

## Acknowledgments

The authors thank the *Aspergillus* genome consortia for permitting access to genome data of *A. oryzae*, *A. nidulans*, and *A. fumigatus*. This work was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (Japan).

## References


[1] J.L. Ferrer, J.M. Jez, M.E. Bowman, R.A. Dixon, J.P. Noel, Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis, Nat. Struct. Biol. 6 (1999) 775–784.

[2] R.A. Dixon, L. Achnine, P. Kota, C.J. Liu, M.S.S. Reddy, L.J. Wang, The phenylpropanoid pathway and plant defence—a genomics perspective, Mol. Plant Pathol. 3 (2002) 371–390.

[3] M.B. Austin, J.P. Noel, The chalcone synthase superfamily of type III polyketide synthases, Nat. Prod. Rep. 20 (2003) 79–110.

[4] A.B. Christensen, P.L. Gregersen, J. Schröder, D.B. Collinge, A chalcone synthase with an unusual substrate preference is expressed in barley leaves in response to UV light and pathogen attack, Plant Mol. Biol. 37 (1998) 849–857.

[5] N. Funa, Y. Ohnishi, I. Fujii, M. Shibuya, Y. Ebizuka, S. Horinouchi, A new pathway for polyketide synthesis in microorganisms, Nature 400 (1999) 897–899.

[6] B.S. Moore, J.N. Hopke, Discovery of a new bacterial polyketide biosynthetic pathway, Chembiochem 2 (2001) 35–38.

[7] P. Saxena, G. Yadav, D. Mohanty, R.S. Gokhale, A new family of type III polyketide synthases in *Mycobacterium tuberculosis*, J. Biol. Chem. 278 (2003) 44780–44790.

[8] M. Gensheimer, A. Mushegian, Chalcone isomerase family and fold: no longer unique to plants, Protein Sci. 13 (2004) 540–544.

[9] R.J. Cole, B.B. Jarvis, M.A. Schweikert, Handbook of Secondary Fungal Metabolites, Academic Press, UK, 2003, p. 2544.

[10] J.L. Adrio, A.L. Demain, Fungal biotechnology, Int. Microbiol. 6 (2003) 191–199.

[11] A.M. Calvo, R.A. Wilson, J.W. Bok, N.P. Keller, Relationship between secondary metabolism and fungal development, Microbiol. Mol. Biol. Rev. 66 (2002) 447–459.

[12] J. Varga, K. Rigo, S. Kocsube, B. Farkas, K. Pal, Diversity of polyketide synthase gene sequences in *Aspergillus* species, Res. Microbiol. 154 (2003) 593–600.

[13] K. Kitamoto, Molecular biology of the Koji molds, Adv. Appl. Microbiol. 51 (2002) 129–153.

[14] J. Yu, D. Bhatnagar, T.E. Cleveland, Completed sequence of aflatoxin pathway gene cluster in *Aspergillus parasiticus*, FEBS Lett. 564 (2004) 126–130.

[15] K. Kitamoto, K. Gomi, K. Goto, S. Hara, Genetic transfer applied to traditional sake brewing, Biotechnol. Genet. Eng. Rev. 9 (1991) 89–125.

[16] M. Machida et al., Characterization of *Aspergillus oryzae* genome structure, XXIII Fungal Genetics Conference, Asilamor, USA, Abstract-182, 2005, p. 82.

[17] K. Kusumoto, Y. Nogata, H. Ohta, Directed deletions in the aflatoxin biosynthesis gene homolog cluster of *Aspergillus oryzae*, Curr. Genet. 37 (2000) 104–111.

[18] G. Forkmann, S. Martens, Metabolic engineering and applications of flavonoids, Curr. Opin. Biotechnol. 12 (2001) 155–160.

[19] R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren, P.A. van Leeuwen, Flavonoids: a review of probable mechanisms of action and potential applications, Am. J. Clin. Nutr. 74 (2001) 418–425.

[20] S. Graziani, C. Vasnier, M.J. Daboussi, Novel polyketide synthase from *Nectria haematococca*, Appl. Environ. Microbiol. 70 (2004) 2984–2988.

[21] I. Fujii, A. Watanabe, Y. Ebizuka, More functions for multifunctional polyketide synthases, in: J.S. Tkacz, L. Lange (Eds.), Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine, Kluwer Academic/Plenum Publishers, 2004, pp. 97–125.

[22] J. Fliegmann, G. Schröder, S. Schanz, L. Britsch, J. Schröder, Molecular analysis of chalcone and dihydropinosylvin synthase from Scots pine (*Pinus sylvestris*), and differential regulation of these and related enzyme activities in stressed plants, Plant Mol. Biol. 18 (1992) 489–503.

[23] D. Narozna, J. Pas, J. Schneider, C.J. Madrzak, Two sequences encoding chalcone synthase in yellow lupin (*Lupinus luteus l.*) may have evolved by gene duplication, Cell. Mol. Biol. Lett. 9 (2004) 95–105.

[24] D.M. Wilson, W. Mubatanhema, Z. Jurjevic, Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns, Adv. Exp. Med. Biol. 504 (2002) 3–17.

[25] B. Tudzynski, Gibberellin biosynthesis in fungi: genes, enzymes, evolution, and impact on biotechnology, Appl. Microbiol. Biotechnol. 66 (2005) 597–611.

[26] R.A. Dixon, N.L. Paiva, Stress-induced phenylpropanoid metabolism, Plant Cell 7 (1995) 1085–1097.

[27] J.D. Walton, Horizontal gene transfer and the evolution of secondary metabolite gene clusters in fungi: an hypothesis, Fungal Genet. Biol. 30 (2000) 167–171.

[28] B. Winkel-Shirley, Biosynthesis of flavonoids and effects of stress, Curr. Opin. Plant Biol. 5 (2002) 218–223.

[29] E.G. Schijlen, C.H. Ric de Vos, A.J. van Tunen, A.G. Bovy, Modification of flavonoid biosynthesis in crop plants, Phytochemistry 65 (2004) 2631–2648.