REVIEW

Genomics Reveals Traces of Fungal Phenylpropanoid-flavonoid Metabolic Pathway in the Filamentous Fungus *Aspergillus oryzae*

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Fungal secondary metabolites constitute a wide variety of compounds which either play a vital role in agricultural, pharmaceutical and industrial contexts, or have devastating effects on agriculture, animal and human affairs by virtue of their toxigenicity. Owing to their beneficial and deleterious characteristics, these complex compounds and the genes responsible for their synthesis have been the subjects of extensive investigation by microbiologists and pharmacologists. A majority of the fungal secondary metabolic genes are classified as type I polyketide synthases (PKS) which are often clustered with other secondary metabolism related genes. In this review we discuss on the significance of our recent discovery of chalcone synthase (CHS) genes belonging to the type III PKS superfamily in an industrially important fungus, *Aspergillus oryzae*. CHS genes are known to play a vital role in the biosynthesis of flavonoids in plants. A comparative genome analyses revealed the unique character of *A. oryzae* with four CHS-like genes (*csyA*, *csyB*, *csyC* and *csyD*) amongst other Aspergilli (*Aspergillus nidulans* and *Aspergillus fumigatus*) which contained none of the CHS-like genes. Some other fungi such as *Neurospora crassa*, *Fusarium graminearum*, *Magnaporthe grisea*, *Podospora anserina* and *Phanerochaete chrysosporium* also contained putative type III PKSs, with a phylogenic distinction from bacteria and plants. The enzymatically active nature of these newly discovered homologues is expected owing to the conservation in the catalytic residues across the different species of plants and fungi, and also by the fact that a majority of these genes (*csyA*, *csyB* and *csyD*) were expressed in *A. oryzae*. While this finding brings filamentous fungi closer to plants and bacteria which until recently were the only ones considered to possess the type III PKSs, the presence of putative genes encoding other principal enzymes involved in the phenylpropanoid and flavonoid biosynthesis (*viz.*, phenylalanine ammonia-lyase, cinnamic acid hydroxylase and *p*-coumarate CoA ligase) in the *A. oryzae* genome undoubtedly prove the extent of its metabolic diversity. Since many of these genes have not been identified earlier, knowledge on their corresponding products or activities remain undeciphered. In future, it is anticipated that these enzymes may be reasonable targets for metabolic engineering in fungi to produce agriculturally and nutritionally important metabolites.

Key words: *Aspergillus oryzae*, chalcone synthase, flavonoids, fungal secondary metabolism, phenylpropanoids, type III polyketide synthase

Polyketide synthases play vital role in the synthesis of secondary metabolites

Living organisms ranging from the microbes to plants synthesize a wealth of complex biologically active metabolites using a coordinated sequential group of enzyme activities known as "polyketide synthases" (PKSs). PKSs with their multienzyme protein architecture build complex compounds through step wise reactions by using the simple 2-, 3-, 4-carbon units *viz.*, acetyl CoA, propionyl CoA,

butyryl CoA or their derivatives, malonyl, methylmalonyl and ethylmalonyl CoAs (Liou and Khosla, 2003). In general, PKSs contain various active domains termed as (i) an acyl-transferase domain required for the loading of starter, extender and intermediate acyl units, (ii) an acyl carrier domain for holding the growing macrolide as a thiol ester, (iii) a β-ketoacyl synthase domain for catalyzing the chain extension, (iv) a β-keto reductase domain responsible for the reduction, (v) a dehydratase domain for the formation of unsaturated thiol ester, (vi) an enoyl reductase domain for catalyzing the saturation, and finally (vii) a thiol esterase domain for releasing the macrolide and facilitat-

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ing its cyclization. Based on the presence or absence of these domains, three kinds of PKSs have been characterized until now and are grouped as the type I, type II and type III PKSs. While the type I PKSs are multifunctional enzymes with multiple active domains organized into modules, the type II PKSs contain active sites in several small monofunctional polypeptides and catalyze the formation of compounds that only require aromatization and cyclization, but not extensive reduction or reduction/dehydration cycles. On the other hand, the type III PKSs are comparatively small proteins with a single polypeptide chain, and thus far well characterized only in plants and bacteria (Austin and Noel, 2003). They aid in the synthesis of either chalcones and stilbenes in plants or polyhydroxy phenols in bacteria. Unlike all other PKSs, these proteins do not have a phosphopantetheinyl arm on which the growing polyketide chains are tethered.

Enzymes involved in the phenylpropanoid-flavonoid metabolism

The well known representatives of type III polyketide synthase (PKS) superfamily are the chalcone synthases (CHS) that are traditionally supposed to be plant and bacterial specific (Ferrer *et al.*, 1999; Funa *et al.*, 1999; Moore and Hopke, 2001; Saxena *et al.*, 2003). These enzymes have received significant attention in plants and bacteria by virtue of their products with agricultural and pharmaceutical values (Forkmann and Martens, 2001; Nijveldt *et al.*, 2001; Dixon *et al.*, 2002; Austin and Noel, 2003). Plant CHSs are known to catalyze the first committed step in the phenylpropanoid pathway leading to the biosynthesis of flavonoids, isoflavonoids and anthocyanins, by the condensation of 4-hydroxycinnamoyl-CoA and three malonyl-CoA molecules to form the chalcone derivative, naringenin chalcone (Ferrer *et al.*, 1999). Essentially CHSs form a part of the downstream pathway
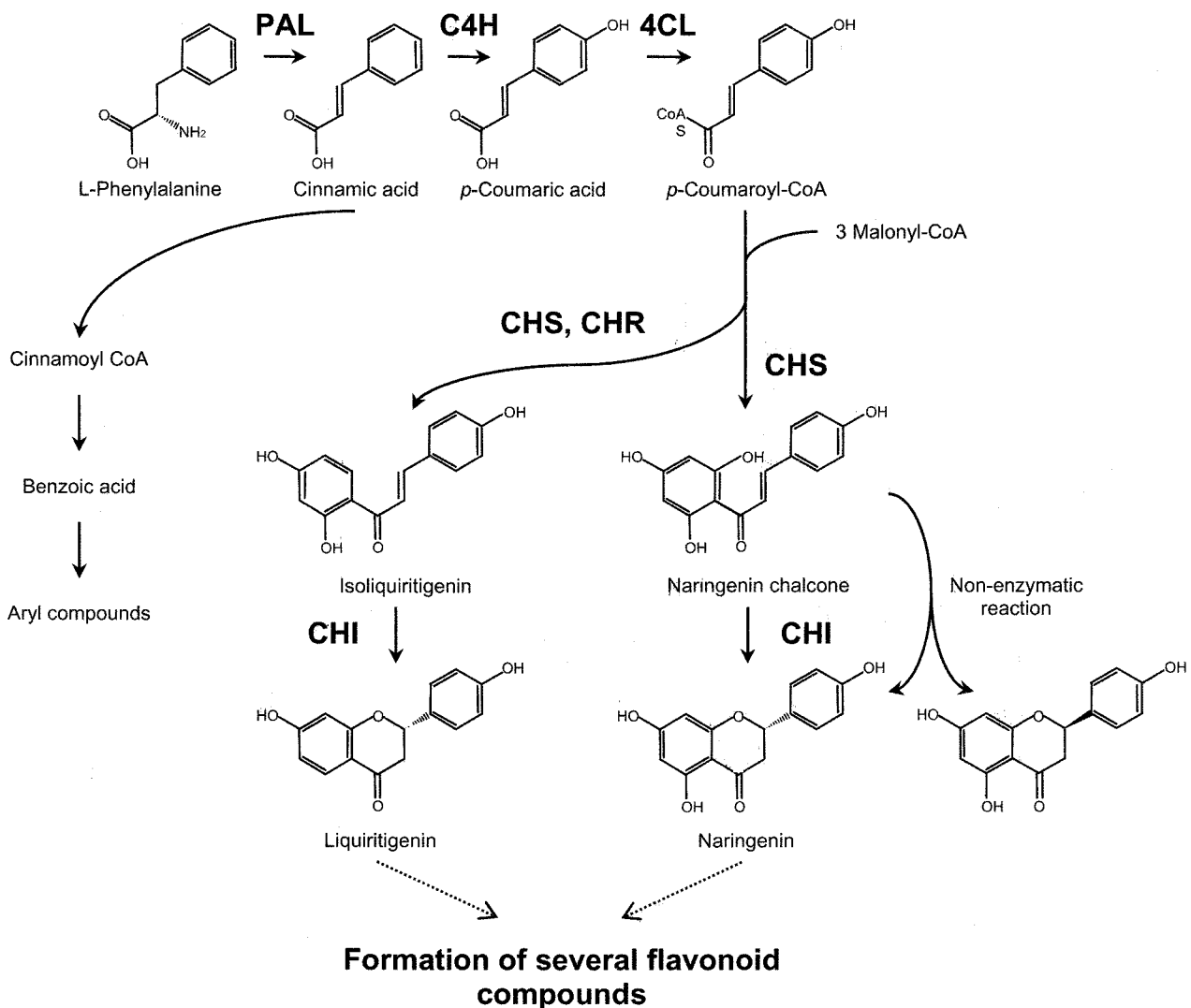

Formation of several flavonoid compounds

Fig. 1. Schematic diagram of the biosynthetic pathway of phenylpropanoids and other branched pathway resulting in the formation of aryl-related metabolites. Also shown are the enzymes involved in the pathway conversions.

in the phenylpropanoid-flavonoid metabolic pathway, that includes the upstream participants *viz.*, phenylalanine ammonia-lyase (PAL), cinnamic acid hydroxylase (C4H), and *p*-coumarate:CoA ligase (4CL) (Jorgensen *et al.*, 2005). The activity of these respective enzymes facilitates the conversion of the precursor amino acid, L-phenylalanine, via cinnamic acid and *p*-coumaric acid to the key intermediate, *p*-coumaroyl-CoA, which is then channelled into diverse pathways leading to the synthesis of a wide variety of metabolites through the activity of CHSs, and other downstream enzymes such as chalcone reductase (CHR) and chalcone-flavonone isomerase (CHI) (Fig. 1). Although PAL is known to catalyze the primary step in phenylpropanoid synthesis it also regulates the overall metabolic flux into the pathway, meaning that any inhibition in its acitivity causes a complete block in the phenylpropanoid pathway (Ro and Douglas, 2004). With the exception of only CHI, an enzyme responsible for the cyclization of chalcone into flavanone, the activities of all the other enzymes involved in the formation of the C_6-C_3 phenylpropane skeleton in plants vary in a coordinated fashion (Scheel and Hahlbrock 1989; Gensheimer and Mushegian, 2004). Moreover, apart from the phenylpropanoid pathway intermediates regulating the CHS gene expression, a similar trend in the transcriptional induction and activities of both PALs and CHSs was noted in responses to different elicitors or microbial compounds in cultured plant cells (Ebel *et al.*, 1984; Loake *et al.*, 1992) indicating the intricate interconnection between the central phenylpropanoid pathway and its branching points.

While the occurrence of phenylpropanoids and flavonoids in plants and their relevance for antimicrobial activity, pigmentation, and as protectants from the hazardous effects of UV light have been investigated over the past several years (Christensen *et al.*, 1998; Winkel-Shirley, 2002), the recent reports on the existence of type III PKS pathway even in microorganisms were very significant which in a way brought bacteria closer to plants (Funa *et al.*, 1999; Moore and Hopke, 2001; Saxena *et al.*, 2003). Interest in these genes gained further impetus with the more recent report on the presence of the CHIs in the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the filamentous fungus *Neurospora crassa* (Gensheimer and Mushegian, 2004). Since the CHIs are known to act on the products of CHSs the above finding provided a preliminary clue for the existence of a flavonoid/isoflavonoid-like pathway even in fungi. Although the genes encoding type I PKSs and their role in pigmentation and the synthesis of some secondary metabolites (such as melanins, gibberellins, fumonisins, aflatoxins, cercosporins, etc.) have been well documented in filamentous fungi (Calvo *et al.*, 2002; Varga *et al.*, 2003; Adrio and Demain, 2003; Fujii *et al.*, 2004; Graziani *et al.*, 2004; Choquer *et al.*, 2005), the fungal type III PKSs have never been known or received attention

until our recent finding on the existence of genes encoding these enzymes in an industrially exploited fungus, *Aspergillus oryzae*, and some other fungal species as well (Seshime *et al.*, 2005a).

In this review we summarize the current knowledge on the *A. oryzae* type III PKS genes with focus on the sequences homology, conservation in the catalytic residues and specific signature domains, and their expression patterns. Apart from these an account on the identification of the upstream phenylpropanoid pathway genes is also presented and discussed in relation to the downstream flavonoid pathway genes.

Type III polyketide synthase genes in *A. oryzae* and other fungal species

Filamentous fungi are used as sources of important metabolites that find wide usage in agriculture, industry, medicine and nutrition. 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 (Calvo *et al.*, 2002). Little is known about their genetic architecture and physiological role despite the fact that some fungi have been exploited industrially for over several decades. While the CHS encoding genes belonging to type III PKS superfamily and their products have been previously well studied in plants, only type I PKSs and their relevance for secondary metabolic activity have been investigated to a large extent in fungi. One of the well characterized fungal secondary metabolic pathway is the aflatoxin biosynthetic pathway in *Aspergillus flavus* and *Aspergillus parasiticus*, and includes transcriptional activators, other genes and genes encoding type I PKSs, that are arranged as a cluster in the genome (Yu *et al.*, 2004). While the *koji* mold, *A. oryzae*, is well recognized as a source of many useful enzymes and proteins in Japanese fermentation industry (Kitamoto *et al.*, 1991; Kitamoto, 2002), 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 (Machida *et al.*, 2005). 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 (Kusumoto *et al.*, 2000), the presence of putative type III PKSs in this fungus or any other fungi has never been examined.

In order to verify the presence of type III PKS encoding genes in the *A. oryzae* genome the *Medicago sativa* CHS2 amino acid sequence was utilized which revealed 4 CHS-like type III PKS sequences that were designated as *csyA*, *csyB*, *csyC* and *csyD*, respectively (Fig. 2) (Seshime *et al.*, 2005a). Using these *A. oryzae* putative type III PKS sequences, the other available fungal genome databases were searched for the presence of CHS-like type III PKS

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Ao-CSYA    1   --------------MAPLIHGTSPPEIRNHSDDSLSKRA...
Ao-CSYC    1   --------------------------MAPPV...
Ao-CSYB    1   -----------------MIEPLPTEDIPKQS...
Ao-CSYD    1   MQFLHLQQKYHAVRLELITRWRICKQACRQIFRKDEL SSPS...
Ms-CHS2    1   ---------------MVSVAEIRQAQRAEGPATIMATGTANP NCVEQSTYPDFYFKITN...KVELKEKFQRMC

Ao-CSYA   65
Ao-CSYC   44
Ao-CSYB   53
Ao-CSYD   80
Ms-CHS2   61

Ao-CSYA  144
Ao-CSYC  123
Ao-CSYB  132
Ao-CSYD  159
Ms-CHS2  141

Ao-CSYA  224
Ao-CSYC  203
Ao-CSYB  212
Ao-CSYD  200
Ms-CHS2  214

Ao-CSYA  300
Ao-CSYC  282
Ao-CSYB  292
Ao-CSYD  280
Ms-CHS2  290

Ao-CSYA  374
Ao-CSYC  357
Ao-CSYB  366
Ao-CSYD  357
Ms-CHS2  364
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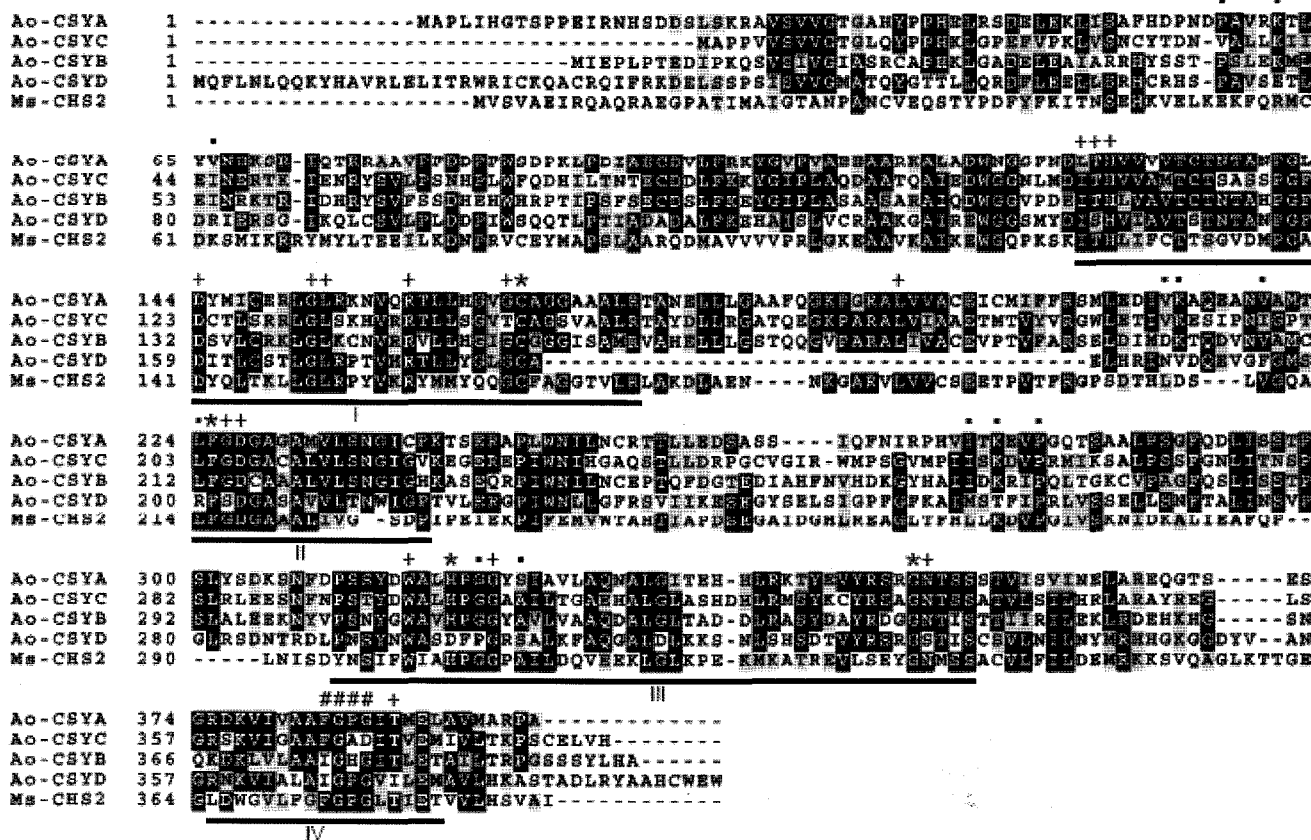
Fig. 2. Comparison of amino acid sequences of the four CHS-like type III PKSs from *A. oryzae* with CHS from *M. sativa* (CHS2 GenBank accession No. ALFCHS2A). Highly conserved residues in all the sequences are shaded in black. Domains showing conservation are designated as I, II, III and IV. The conserved catalytic triad residues, other highly conserved residues, CoA-binding sites and CHS-signature sequence residues are indicated as *, +, •, and # respectively.

genes. Putative type III PKSs could be identified in some fungi such as *N. crassa*, *Magnaporthe grisea*, *Phanerochaete chrysosporium*, *Fusarium graminearum* and *Podospora anserina* (Table 1). However, in contrast to 4 putative type III PKS sequences in *A. oryzae*, *N. crassa* and *F. graminearum* contained only a single homologue each, *M. grisea* and *P. anserina* each contained 2 homologues and *P. chrysosporium* had 3 homologues, respectively. Moreover, a predominant conservation in the catalytic residues and the CHS-family signature sequence was evident in all the fungal type III PKS sequences (Seshime *et al.*, 2005a). Surprisingly, the other closely related species of Aspergilli (*A. nidulans* and *A. fumigatus*) did not possess any of the CHS-like genes. Moreover, as shown in Table 1, a majority of the fungi which contained CHS-like sequences are plant pathogens. It is thus 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 considered as opportunistic plant pathogens (Wilson *et al.*, 2002). This aspect is significant because *A. oryzae* genome revealed

the presence of several other genes (encoding pisatin demethylases, trichothecene hydroxylases and isotrichodermin hydroxylases) reminiscent of plant pathogenic character (Machida *et al.*, 2005).

In order to verify if these putative CHS-like sequences contained catalytic site residues a comparison was performed using the *M. sativa* CHS2 sequence. As shown in Fig. 2, a conservation in the catalytic residues at positions 164, 215, 303 and 336, with Cys, Phe, His and Asn amino acids respectively, was noted (amino acid positions indicated by asterisks are with respect to *M. sativa* CHS2 sequence). Evidences to support the nature of these CHS-like type III PKS sequences was also obtained based on their conservation with other plant CHSs (Seshime *et al.*, 2005a). As shown in Fig. 2, *A. oryzae* type III PKSs contained 4 domains that were highly conserved. Among these the first domain in the N-terminal half contained the active Cys residue apart from at least 8 other residues (indicated by +) known to be highly conserved among the plant CHSs, and the second domain included the Phe active residue in addition to 2 other conserved residues, Asp and Gly. While the third conserved domain included

Table 1. Putative type III polyketide synthases in fungi

Fungi	Number	Gene	Database
Aspergillus oryzae	4	*csyA, csyB* *csyC, csyD*	AB206758*, AB206759* AB206760*, AB206761*
Aspergillus fumigatus	0		http://www.tigr.org/tdb/e2k1/aful/
Aspergillus nidulans	0		http://www.broad.mit.edu/annotation/fungi/aspergillus/
Fusarium graminearum	1	FG08378.1	http://www.broad.mit.edu/annotation/fungi/fusarium/
Magnaporthe grisea	2	MG04643.4 MG06254.4	http://www.broad.mit.edu/annotation/fungi/magnaporthe/
Neurospora crassa	1	NCU04801.1	http://www.broad.mit.edu/annotation/fungi/neurospora_crassa_7/index.html
Podospora anserina	2	Contig1186 Contig1894	http://podospora.igmors.u-psud.fr/index.html
Rhizopus oryzae	0		http://www.broad.mit.edu/annotation/fungi/rhizopus_oryzae/
Ustilago maydis	0		http://www.broad.mit.edu/annotation/fungi/ustilago_maydis/
Coprinus cinereus	0		http://www.broad.mit.edu/ annotation/fungi/coprinus_cinereus/
Phanerochaete chrysosporium	3	PC132.3.1 PC132.5.1 PC16.58.1	http://genome.jgi-psf.org/whiterot1/whiterot1. home.html,
Candida albicans	0		http://www.candidagenome.org/
Cryptococcus neoformans	0		http://www.broad.mit.edu/annotation/fungi/cryptococcus_neoformans/index.html
Saccharomyces cerevisiae	0		http://www.yeastgenome.org/
Schizosaccharomyces pombe	0		http://www.genedb.org/genedb/pombe/index.jsp

*The respective NCBI accession numbers for the *chs* genes of *A. oryzae*

the His and Asn active residues, along with 3 other conserved residues (Trp and 2 Gly residues), the fourth domain included the CHS-family signature sequence G(F/L)GPG at position 372-376 on the C-terminus with the 4 conserved residues (Phe, Pro and 2 Gly residues; indicated by #). In addition to these characteristics, the conservation in 16 (Fig. 2; indicated by +) of the 18 residues that are usually conserved in plant CHSs (Narozna et al., 2004) confirmed that these sequences encoded functional chalcone synthases. Further evidence to support the functionality of the *A. oryzae* putative type III PKSs was obtained by RT-PCR analysis (Seshime et al., 2005a). While the *csyA* and *csyD* genes expressed during 1 to 7 days of culturing *A. oryzae* in DPY medium and the *csyB* gene was expressed during 1-3 days, the *csyC* was not expressed. Although the expression analysis reveals a possibility of the *csy* genes functioning in *A. oryzae*, their participation in the synthesis of new products is yet to be investigated.

Phylogeny and genomic organization of the *A. oryzae* type III polyketide synthase genes

As shown in Fig. 3, a dendrogram constructed by using the conserved chalcone and stilbene synthase domains, revealed three main branches corresponding to the plant, bacterial and fungal PKSs, respectively. While the fungal type III PKSs branched out separately as shown in the dendrogram, *A. oryzae* putative type III PKSs seemed to belong to a different cluster. The bacterial and plant type

III PKSs also remained separated. From the phylogenic analysis it may be inferred that the fungal type III PKSs are distinct from those of bacteria and plants. Although the nature of products produced by these putative type III PKSs is yet unknown, it may be hypothesized that plant flavonoid-like metabolites are produced in some fungi.

Since the typical characteristic of several fungal secondary metabolic genes is to remain clustered for jointly functioning in the production of complex compounds, we sought to examine the genomic organization of the type III PKS genes in *A. oryzae*. For this purpose an ~40 kb region flanking the *csy* genes was examined and this revealed some interesting aspects on other genes present close to each of these *csy* genes (Fig. 4). Importantly, genes encoding ferric reductase, copper oxidase, GABA permease close to the *csyA* gene and acyl-CoA synthase, GABA permease, geranylgeranyl diphosphate synthase and predicted transporter near the *csyC* gene, implicated that these genes might function in coordination with the *csy* genes during the synthesis of complex secondary products. Supporting this observation there is a report on plant overcoming stress responses (related to low nitrogen and iron sources) by the activity of CHSs (Dixon and Paiva, 1995). However, no CHI gene could be detected in any of these gene clusters. Other genes of interest were the keto-acid reductoisomerase, 2-polyprenyl-6-methoxyphenol hydroxylase and a predicted acyl esterase surrounding the *csyB* gene, which resembled those from bacterial species.
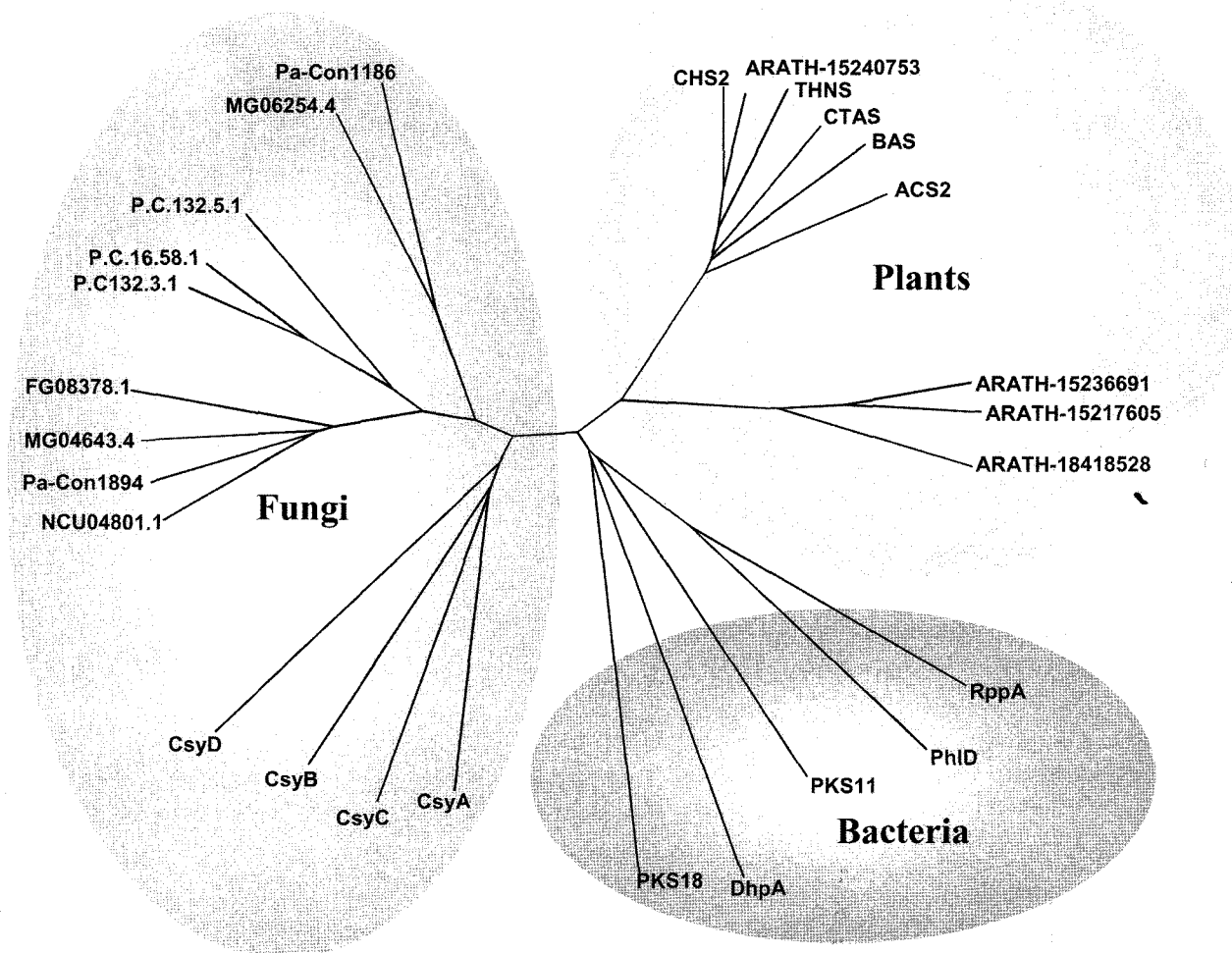
Fig. 3. Dendrogram was constructed for type III PKS sequences from bacteria, plants and fungi using chalcone synthase and stilbene synthase domains from PFAM searches. Sequences are named according to species abbreviation. Refer to Table 1 for GI numbers of putative fungal CHSs.

Although fungi synthesize some secondary metabolites in common to higher plants and certain prokaryotes (Walton, 2000), the gene clusters involved in their synthesis reveal low identity. For instance, gibberellins that are produced by all higher plants and antibiotics produced by some bacteria are also produced by filamentous fungi but the gene clusters do not show high identity (Tudzynski, 2005). Considering higher homology of these CHS-like type III PKS sequences with those of plant CHSs it seems reasonable to postulate a horizontal gene transfer mechanism that led to some of these fungi acquiring the CHS genes from plants during evolution.

Closing in on the phenylpropanoid metabolic pathway genes in A. oryzae
As mentioned earlier phenylpropanoids including flavonoids have often been considered as plant specific secondary metabolites or restricted to some bacteria. However, recent evidence on the presence of the enzyme,

CHI, in the yeasts and *N. crassa* (Gensheimer and Mushegian, 2004), and our own findings on the existence of the type III PKS genes in *A. oryzae* (Seshime *et al.*, 2005a) and few other fungi suggested a flavonoid-like metabolic activity in fungi. To more clearly understand the consequences on the existence of these flavonoid pathway genes and also considering the fact that synthesis of flavonoids requires the coordinated expression of genes encoding several enzymes in the phenylpropanoid biosynthetic pathway, we surveyed the *A. oryzae* genome for the upstream and downstream genes encoding various enzymes of the phenylpropanoid pathway. To our surprise we came across several genes encoding enzymes of the phenylpropanoid pathway in the *A. oryzae* genome (Seshime *et al.*, 2005b). *A. oryzae* alone contained 4 genes encoding PAL (Table 2) in parallel to the plant, *Arabidopsis thaliana*. It was noted that the closely related species of *A. nidulans* and *A. fumigatus* contained only 2 and 1 PAL encoding genes, respectively. While CHSs are con-

A1   Ferric reductase NADH/NADPH oxidase and related proteins [*S. cerevisiae*] GI:6324388
A2   Multicopper oxidases [*S. cerevisiae*] GI:6323703
A3   High-affinity iron permease CaFTR2 [*C. albicans*] GI:7770293
A4   GABA permease [*A. nidulans*] GI:4972245
⇨A5   Chalcone synthase [*Dianthus monspessulanus*] GP:AF267173
A6   Thiamine pyrophosphate-requiring enzyme [*S. cerevisiae*] GI:6321524

B1   Ketol-acid reductoisomerase [*Pyrococcus abyssi*] GI:14521547
B2   Alpha-glucosidases, family 31 of glycosyl hydrolases [*E. coli* O157:H7 EDL933] GI:15804463
B3   2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases
      [*Pseudomonas aeruginosa*] GI:15598524
B4   Predicted acyl esterases [*Staphylococcus aureus* N315] GI:15928173
⇨B5   Putative chalcone synthase [*Oryza sativa* (japonica cultivar-group)] GP:AP005325_13
B6   Predicted transporter (major facilitator superfamily) [*S. pombe*] GI:19114424
B7   Periplasmic component of the Tol biopolymer transport system [*Caulobacter vibrioides*]
      GI:16127460

C1   Predicted transporter (major facilitator superfamily) [*S. pombe*] GI:19112901
C2   Lecithin:cholesterol acyltransferase [*Bacillus anthracis* A2012] GI|21401713
C3   Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II
      [*Methanothermobacter thermautotrophicus*] GI:15678684
⇨C4   Putative chalcone synthase [*Oryza sativa* (japonica cultivar-group)] GP:AP005325_13
C5   GABA permease [*A. nidulans*] GI:4972245
C6   Similar to kinesin light chain [*Nostoc* sp. PCC 7120] GI:17231330
C7   Variable surface lipoprotein Vsp422-9 [*Mycoplasma bovis*] GI:15384297
C8   Related to negative acting factor [*N. crassa*] GI:16944445
C9   Geranylgeranyl diphosphate synthase [*A. nidulans*] tr:Q874I1
C10   ATPases of the AAA+ class [*Nostoc* sp. PCC 7120] GI:17230633
C11   Predicted transporter (major facilitator superfamily) [*S. pombe*] GI:19112901

D1   Basic region leucine zipper transcription factor [*Caenorhabditis elegans*]
      GI:17506121
D2   5'-3' exonuclease [*S. pombe*] GI:19112887
D3   WD-repeat protein WDR6, WD repeat superfamily [*S. pombe*] GI:19112265
⇨D4   Predicted naringenin-chalcone synthase
D5   Endoglucanase [*A. kawachii*] dbj: BAB62319.1
D6   Phosphatidylinositol-4-phosphate 5-kinase and related FYVE finger-containing
      proteins [*S. cerevisiae*] GI:14318541
D7   Pyruvate kinase [*A. oryzae*] GI: 9955873

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.

sidered as enzymes of the flavonoid glycoside pathway, PAL is grouped as an enzyme of general phenylpropanoid metabolism (Gardiner *et al.*, 1980). Moreover, similar degree of expression and activities of PAL and CHS were observed in cultured soybean cells in response to three diverse microbial compounds *viz.*, the glucan elicitor from *Phytophthora megasperma*, xanthan, an extracellular polysaccharide from *Xanthomonas campestris*, and endo-polygalacturonase from *Aspergillus niger* (Ebel *et al.*, 1984). Being the most prominent enzyme PAL catalyzes the starting reaction for a central pathway that generates hundreds of different phenylpropanoid metabolites and is universally present in higher plants. Gene encoding PAL has earlier been identified in the phytopathogenic fungus,

Table 2. Putative phenylpropanoid pathway genes in *Aspergillus oryzae*

Gene	Blast match* [species]	GI number	*E*-value
AO070248000002	phenylalanine ammonia-lyase [*Rhodosporidium toruloides*]- fungus	169746	1e-125
AO070339000251	phenylalanine ammonia-lyase [*R. toruloides*] - fungus	169746	1e-128
AO070340000119	phenylalanine ammonia-lyase [*R. toruloides*] - fungus	169746	1e-131
AO070286000079	phenylalanine ammonia-lyase [*R. toruloides*] - fungus	169746	1e-108
AO070248000004	CYP73A5** (cinnamic acid 4-hydroxylase) [*A. thaliana*] - plant	3915085	1e-35
AO070340000287	4-coumarate:CoA ligase [*Streptomyces coelicolor* A3(2)] - bacterium	8894733	1e-93
AO070290000036	4-coumarate:CoA ligase [*S. coelicolor* A3(2)] -bacterium	8894733	9e-85
AO070336000096	putative 4-coumarate-CoA ligase [*Arabidopsis thaliana*] - plant	15234634	2e-69
AO070343000578	putative 4-coumarate-CoA ligase [*A. thaliana*] - plant	15234634	5e-80
AO070318000050	4-coumarate:CoA ligase [*S. coelicolor* A3(2)] - bacterium	8894733	3e-63
AO070314000133	4-coumarate:CoA ligase [*S. coelicolor* A3(2)] - bacterium	8894733	9e-59
AO070290000065	putative 4-coumarate-CoA ligase [*A. thaliana*] - plant	15234634	8e-59
AO070297000068	4-coumarate:CoA ligase [*S. coelicolor* A3(2)] - bacterium	8894733	2e-53
AO070290000018	4-coumarate:CoA ligase [*Populus tremuloides*] - plant	3258636	1e-63
AO070336000084	4-coumarate:CoA ligase 1 [*Populus trichocarpa* x *P. deltoides*] - plant	2911798	2e-40
AO070333000069	4-coumarate:coA ligase 2 [*Rubus idaeus*] - plant	9651914	4e-29
csyA	chalcone synthase [*Betula pendula*] -plant	1834387	3e-34
csyB	2-pyrone synthase [*Gerbera* hybrid cultivar] - plant	13276209	8e-29
csyC	putative chalcone synthase [*Hordeum vulgare* subsp. *vulgare*] - plant	55276717	2e-30
csyD	coumaroyl triacetic acid synthase [*Hydrangea macrophylla*] - plant	3551033	2e-16

*Genes predicted as encoding hypothetical proteins have been excluded from the list.
**P450 Blast server at http://drnelson.utmem.edu/CytochromeP450.html was used for identification of CYP73 family.

Table 3. List of putative transcription factor binding sites on *chs* promoters

Transcription factor	Sequence*	Gene	Position	Source organism
PacC (pH stress)	GCCARG	*csyA*	-770, -973, -1024	*Aspergillus nidulans*
G-Box (Coumaric acid dependent)	CACGTG	*csyC*	-7	*Phaseolus vulgaris*
		csyD	-208	
H-Box (Coumaric acid dependent)	CCTACC(N)7CT	-		*Phaseolus vulgaris*
P-Box (White light dependent)	CCAMCWCC	*csyC*	-1363	*Pisum sativum*
L-Box (UV light dependent)	WCTMACCTAMCM	-		*Pisum sativum*
Box II (Light dependent)	GGTTAA	*csyC*	-1246	*Arabidopsis thaliana*
LeMYB1 (Light dependent)	GATAAG	*csyC*	-645	*Pisum sativum*
		csyD	-264	
	GATAA	*csyA*	-289	

*W-A/T; R-A/G; M-A/C; N-A/T/G/C

Ustilago maydis but its role remains unknown (Kim *et al.*, 2001). Considering the role of PAL in catalyzing the conversion of phenylalanine to cinnamic acid in plants, it is possible similar pathways operate in fungi, one leading to the conversion of cinnamic acid to coumaric acid by the activity of C4H and the other to cinnamoyl CoA by the activity of acyl CoA ligase, which subsequently leads to the formation of benzaldehyde, benzyl alcohol, and benzoic acid (Fig. 1). The formation of these aryl-related metabolites is already known in *A. oryzae* and other fungi (Murakami, 1980; Jensen *et al.*, 1994). It was observed

that only *A. thaliana* and *A. oryzae* contained a single C4H enzyme (belonging to the CYP73 family) responsible for the conversion of cinnamic acid to *p*-coumaric acid (Table 2). *p*-coumarate or 4-hydroxycinnamate is a key molecule in plant stress response and has been shown to stimulate the expression of the *Phaseolus vulgaris* and *Medicago sativa* CHSs (Loake *et al.*, 1992). The role of phenylpropanoid pathway intermediates in regulating the expression of *chs* gene promoters has been reported (Loake *et al.*, 1991; Loake *et al.*, 1992). Essentially the cis elements designated as the G-box (CACGTG), H-box
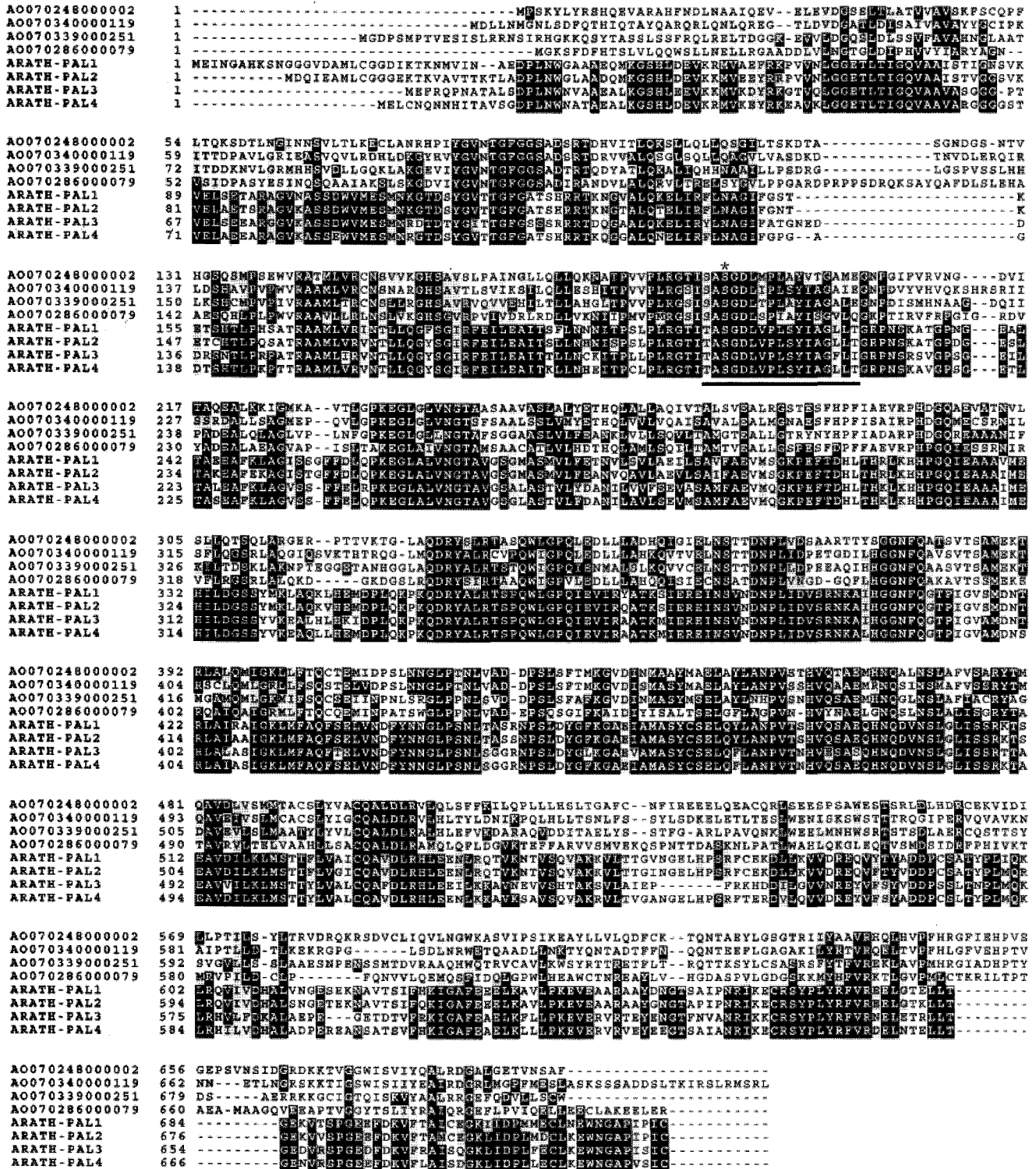
AO070248000002   1   ---------------------------------MDSKYLYRSHQEVARAHFNDLNAAIQEV--ELEVDGSSLLLATVVAVSKFSCQPF
AO070340000119   1   -------------------------MDLLNMGNLSDFQTHIQTAYQARQRLQNLQREG--TLDVDGAHDISAIVAVAYYSCIPK
AO070339000251   1   ----------------MGDPSMPTVESISLRRNSIRHGKKQSYTASSLSSFRQLRELTDGGK-EHVSDGQSEDLSSVFAVAHNGLAAT
AO070286000079   1   --------------------------MGKSFDFHTSLVLQQWSLLNELLRGAADDLVHNGTGEDHPHVVYIHRYAEN--
ARATH-PAL1   1   MEINGAHKSNGGGVDAMLCGGDIKTKNMVIN--AEDPLNWGAANEQMKGSHLDEVKRMVAEFRKPVVNLGGETLTIGQVAAISTIGNSVK
ARATH-PAL2   1   ----------MDQIEAMLCGGGEKTKVAVTTKTLADPLNWGLANDQMKGSHLDEVKKMVEEYRRPVVNLGGETLTIGQVAAISTVGGSVK
ARATH-PAL3   1   ------------------MEFRQPNATALSDPLNWNVAAEALKGSHLEEVKKMVKDYRKGTVQEGGETLTIGQVAAVANSGGC-PT
ARATH-PAL4   1   -------------------MELCNQNHHITAVSGDPLNWNATAEALKGSHLDEVKRMVKEYRKEAVKLGGETLTIGQVAAVARGGGST

AO070248000002   54 LTQKSDTLNGINNGVLTLKECLANRHPIFGVNTGFGGSADSRTDHVITLQKSLLQLLQSELLTSKDTA-----------SGNDGS-NTV
AO070340000119   59 ITTDPAVLGRIEAGVQVLRDHLDKHYRVTGVNTGFGGSADSRTDRVVALQSGLSQLLQAGVLVASDKD-----------TNVDLERQIR
AO070339000251   72 ITDDKNVLGRMHHSVGLLGQKLAKGEVIYGVNTGFGGSADTRIQDYATLQKALHQHHNAARLLPSDRG-----------LGSPVSSLHH
AO070286000079   52 NSIDPASYESINQSQAAIAKSLSKGDVIYGVNTGFGGSAITRANDVLALQEVLTRESSYGVLPPGARDPRPPSDRQKSAYQAFDLSLRHA
ARATH-PAL1   89 WTLSPTARACVNASSDWVMESMNKGTDSYGVTTGFGATSHRRTKNGVALQKELIRFLNAGIFGST----------------------K
ARATH-PAL2   81 VELASTSRACVKASSDWVMESMNKGTDSYGVTTGFGATSHRRTKNGTALQTELIRFLNAGIFGNT----------------------K
ARATH-PAL3   67 VELSEEARCGVKASSDWVMESMMRDTDTYGHTTGFGSSRRRTDQGAALQKELIRVLNAGIFATGNED--------------------D
ARATH-PAL4   71 VELADEARAGVKASSDWVMESMMRGTDSYGVTTGFGATSHRRTKQGGALQNELIRFLNAGIFGPG--A--------------------G

AO070248000002   131 HGEQSMDSEWVRATMLVRCNSVVKGHSAVSLPAINGLLQLLQKNAIPVVFLRGTISASGDLIPLAAVVTGAMEGNGGIPVRVNG----DVI
AO070340000119   137 LDSHAVIVEHVRAAMLVRCNSNARGHSAVTLSVIKSHLQLLESHITPVVPLRGSISASGDLIPLSYIAGAIEGNHDVYVHVQKSHRSRII
AO070339000251   150 LKSHCHPVVPIVRAAMLYRCNSERGHSAVEVQVVSHDLTLLAHGLTPVVPLRGSISASGDLIPLAYIAGALREGNPDISMHNAAG--DQII
AO070286000079   142 AESQHLPLSWVRAAVILRLNSLVKGHSEVPVTVDRLRDLLVKNIIPMVPERGSISASGDLSPTAYISGVLQGRPTIRVFRHGIG--RDV
ARATH-PAL1   155 NTSHTLPHSATRAAMLVRINTLLQGFSGIRFEILEAITSFLNNNITPSLPLRGTIASGDLVPLSYIAGLLTGRPNSKATGPNG---BAD
ARATH-PAL2   147 ETCHTLPQSATRAAMLVRVNTLLQGYSGIRFEILEAITSLLNNNISPSLPLRGTIASGDLVPLSYIAGLLTGRPNSKATGPDG---BSF
ARATH-PAL3   136 DRSNTLPRPATRAAMLVRVNTLLQGYSGIRFEILEAITLLNCKLTPLLPLRGTIASGDLVPLSYIAGLLIGRPNSRSVGPSG---BIF
ARATH-PAL4   138 DTSHTLPKPTTRAAMLVRVNTLLQGYSGIRFEILEAITKLLNHETTPCLPLRGTIASGDLVPLSYIAGLLTGRPNSKAVGPSG--BTB

AO070248000002   217 TAQSALRKIEMKA--VTDGPKEGLGLVNSTAASAAVASLALYETHQLALLAQIVTALSVSALRGSTESFHPFIAEVRPHDGQAEVATRVL
AO070340000119   227 SSRDRLLSRGMEP--QVLGPKEGLGLVNGTGFSAALSSLVMYETHQLWLVQAISAVALBALMGNABSFHPFISAIRPHDGQMECSRNIL
AO070339000251   238 PRDELQEAGLVP--LNFGPKEGLGLLNGTAFSGGANSLVIFBANKLVLLSQVLTAMGTBALLGTRYNYXHPFIADARPHDGQREAAANIF
AO070286000079   230 YADEALABAGVAP--ISWTAKEGLAIVNGTAMSAACSILVLHDTHQLAMLGSQILTAMTVBALLGSFBSFBPFFAEVRPHPGQEESSRNIR
ARATH-PAL1   242 TAESAFKLAGISGPFELQPKEGLALVNGTAVGSGMASMVLFETNVLSVLAEILSAVPAEVMSGKFEFTDHLTHKUKHHPGQIEAAAVMB
ARATH-PAL2   234 TAKEAFEKAGISTGFELQPKEGLALVNGTAVGSGMASMVLFEANVQAVLAEVLSAIPAEVMSGKFEFTDHLTHKUKHHPGQIEAAAIMB
ARATH-PAL3   223 TALEAFKLAGVSS-FFELPKEGLALVNGTASGSALASTVLFDANILAVPAEVMSGKFEFTDHLTHKUKHHPGQIEAAAIMB
ARATH-PAL4   225 TASEAFKLAGVSS-FFELQPKEGLALVNGTAVGSGLASTVLFDANILAVLSEVMSAMPAEVMGGKFEFTDHLTHKUKHHPGQIEAAAIMB

AO070248000002   305 SLLQTEQLARGER--PTTVKTG-LAQDRVGRTASQWLGPGLBDLLLLADHQHGIRLNSTTDNPLVDSAARTTYSGGNEQAISVTSAMEKT
AO070340000119   315 SFLQGSRLAQGIBGVKTHTRQG-LMQDRYALRCVPQHSGPQLBDLLAHKQVTVRLNSTTDNPLIDPETGDILHGGNFQAVSVTSAMEKT
AO070339000251   326 KNLTDBKLAKNPTEGGBTANHGGLAQDRYALRTSTQWIGPQINMALSLAQVVCRLNSTTDNPLEDPREAQIHHGGNFQAASVTSAMEKT
AO070286000079   318 VFLPRSRLALQKD-----GKDGSLAQDRYSIRTAAQWIGPQEDSISCNSATDNPLVHGD-GQFLHGGNFQAKAVTSBAMEK
ARATH-PAL1   332 HILDGSSYMDLAQKLHEMDPLQKPKQDRYALRTSPQWLGPQIEVIRYATKSIEREINSVNDNPLIDVSRNKAIHGGNPQGTSIGVSMDNT
ARATH-PAL2   324 HILDGSSYMDLAQKVHEMDPLQKPKQDRYALRTSPQWLGPQIEVIRQATKSIEREINSVNDNPLIDVSRNKAIHGGNPQGTSIGVSMDNT
ARATH-PAL3   312 HILDGSSYVKBALHLEKIDPLQKPKQDRYALRTSPQWLGPQIEVIRAATKMIEREINSVNDNPLIDVSRNKAIHGGNPQGTSIGVSMDNS
ARATH-PAL4   314 HILDGSSYVKBAQLLHEMDPLQKPKQDRYALRTSPQWLGPQIEVIRAATKMIEREINSVNDNPLIDVSRNKAIHGGNPQGTSIGVSMDNS

AO070248000002   392 RLALQMIGKLLETQCTBMIDPSLNNGLPTNLVAD-DPSISFTMKGVDBNKAAYMASBAYLANPVSTBVQTASMHNGALNSLAFVSARYDM
AO070340000119   404 RSCLQMIGRLLPSQSTBLVDPSLNNGLPTNLVAD-DPSLSFTMKGVDISMASYMAELAYLANPVSSHVQBAEMRNQSINSLAFVSSRYTM
AO070339000251   416 MGRAAQMLGRILPSQSOCSEELINPNLSFGLPPNLSVD-DPSLSFAMKGVDBNMASYMSELAYLNHPVSNBVQSAEMHNQGLNSLAFLACRYAG
AO070286000079   402 BQATQALGRMLETQCQBMINPATSWGLPPNLVAD-EPSQSGIFNAIDSYISALTSELGFLAGPVN-SVYNAELGNCSLNSLAAISGRYTA
ARATH-PAL1   422 RLAIRAIGKLMFAQFSELVNDFYNNGLPSNLTASBNPSLDYGFKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTS
ARATH-PAL2   414 RLAIAAIGKLMFAQFSELVNDFYNNGLPSNLTASBNPSLDYGFKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTS
ARATH-PAL3   402 RLALASIGKLMFAQFSELVNDFYNNGLPSNLSGGRNBSLDYGLKGAEIAMASYCSELQLFLANPVTMHVBSASQHNQDVNSLGLISSRKTA
ARATH-PAL4   404 RLAIASIGKLMFAQFSELVNDFYNNGLPSNLBGGRNBSLDYGFKGAEIAMASYCSELQFLANPVTNHVBSASQHNQDVNSLGLISSRKTA

AO070248000002   481 QRAVDLLVSMGTACSELYVAGQALDLRLVBQLSFFKILQPLLLHSLTGAFC--NFIREEELQEACQRESEESPSAWESBSRLBLHDBCEKVIDI
AO070340000119   493 QNVBFTVSBLCACSLYIGGQALDLRVBHLTYLDNISPQLHLLTSNLFS--SYLSDKELETLTESBWENISKSWSTBTRQGIPERVQVAVKN
AO070339000251   505 DAVBVVSLMAABYLYVLBQALDLRALHLEPVBDARAQQDDITAELYS--STFG-ARLPAVQNKBWEELMNHWSRBSTSBLAEBCQSTTSY
AO070286000079   490 TAVRVBTELVAAHLLSAGQALDLRAMQLQPLDGBRTEFFARVVSMVEKQSPNTTDABKNLPATBWAHLQKGLEQVSMDSIDBFPHIVKT
ARATH-PAL1   512 SAVDILKLMSTTFLVAICQAVDLRHLEENBRQTVKNTVSQVAKKVITTGVNGELHPSRFCEKBLIKVVDREVQVYTYADDPCSABYBLIPK
ARATH-PAL2   504 BAVDILKLMSTTFLVGICQAVDLRHLEENBRQTVKNTVSQVAKKVITTGINGELHPSRFCEKBLIKVVDREVQVYTYVDDPCSABYBLMQR
ARATH-PAL3   492 BAVTILKLMSTTTYLVALCQAFDLRHLEEIBKKAVNEVVVSHTAKSVLAIEP-------PRKHBDDBGVVNREVYVFSTVDDPSSLBLMLMQK
ARATH-PAL4   494 BAVDILKLMSTTHYLVALCQAVDLRHLEENBRKAVKSAVSQVAKRVITVGANGELHPSRFTERBVBQVVDREYVFSBADDPCSLBYBLMQK

AO070248000002   569 BLPTIBS-YBTRVDRQKRSDVCLIQVLHGWKASVIPSIKEAYLLVLQDFCK--TQNTAERYLGSGTRIIBAAVBHQBHVBFHRGFIEHPVS
AO070340000119   581 AIPTLBB-TBKEKRGPG-----LSDLNRWBTQAADLBNKTYQNTADTFFB--QQNTEEFLGAGAKIBYHTVRQBLTVBFHLGFVEHPTV
AO070339000251   592 SVGWLBS-SBAAESNPEBSSMTDVRAAQHWQTRVCAVBKWSYRTTBETFLT--RQTTKSYLCSABRSFYBVREKBAVPMHRGIADHPTY
AO070286000079   580 MBVFBLB-CBP-------FQNVVLQEMQSBIDQBGPWBHEAWCTNBREAYLV--HGDASPVLGDGBKMYBHVRKTBGVPMBCTRRILTPT
ARATH-PAL1   602 BRQWIVBHALVNGBSEKBAVTSIBBHIGAFBEBLKAVLPKEVEAARAAYDNGTSAIPNRIKECRSYPLYRFVRBELGTELLT--------
ARATH-PAL2   594 BRQWIVBHALSNGBTEKBAVTSIBQKIGAFBEBLKAVLPKEVEAARAAKGNGTSAPIPNRIKECRSYPLYRFVRBELGTKLLT--------
ARATH-PAL3   575 DRHWLVBHALBAEPB---GETDTVBBKIGAFBAEBLKFLUPKEVRVBRVTEYBNGTSFNVANRIKKCRSYPLYRFVRNELBTRLLT-----
ARATH-PAL4   584 BRHILVBHALADPBRRASBATSVBBKIGAFBAELKLLLPKEVERVBVEYBBGTSAIANRIKKCRSYPLYRFVRBBLNTELLT-------

AO070248000002   656 GEPSVNSIDBRDKKTVBGWISVIYQBLRDBABGETVNSAF--------------------
AO070340000119   662 NM---ETLNBRSKKTIBSWISIIYEABRDGBLMGBFMBSBASKSSSADDSLTKIRSLRMSRL
AO070339000251   679 DS------AERRKKGCIBTQISBYYABLRRBEFQDVBLSGW--------------------
AO070286000079   660 AEA-MAAGQVBEAPTVBGYTSLIYRABHQRBEFLPVIQBLBBCLAKEEBAR-----------
ARATH-PAL1   684 ---------SEKGTSBPEBBDKVBTABCBKOBLIDPLMBBLKBWNGAPIPIC----------
ARATH-PAL2   676 ---------GEKVVSBPSBBBDKVBTABCBKLIDPLMBBLKBWNGAPIPIC-----------
ARATH-PAL3   654 ---------GEDVBBFPGEDBDKVBTABSQBKLIDPLFBBLKBWNGAPISIC----------
ARATH-PAL4   666 ---------GENVBBSPGBBFDKVFLAISDBKLIDPLLBCLKBWNGAPVSIC----------

Fig. 5. Comparison of amino acid sequences of the four PALs from *A. oryzae* with the four PALs from *A. thaliana*. Active site residues are indicated as *. The conserved PAL-signature sequence in all the sequences is underlined.

(CCTACC(N)7CT), L-box (a/tCTa/cACCTAa/cCa/c) and P-box (CCAa/cCa/tCC) with specific consensus sequences were noted to be required for the stimulation of the *chs* gene promoter by either the intermediates of the phenyl-propanoid pathway (elicitor-induction) or by UV and white light, respectively (Loake *et al.*, 1992; Whitbred and Schuler, 2000). CHS encoding genes express constitutively in a tissue-specific manner in pigmented flowers and roots, and are induced by environmental stresses, infection by microbial pathogens, UV light, wounding and treatment

with elicitors (Winkel-Shirley, 2002). In this regard MYB like proteins apart from other endogenous regulators (stress-induced transcription activators or repressors) up-regulate flavonoid gene expression in plants (Schijlen et al., 2004). 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 (Schijlen et al., 2004), as shown in Table 3. As shown in Fig. 5, comparative alignments of the 4 PAL genes from A. thaliana and A. oryzae demonstrate the conservation in the PAL-signature sequence (Fig. 5; underlined), respectively. The perfectly conserved serine residue in the PAL-signature domain (indicated by asterisk) is the active site of the enzyme (Schuster and Retey, 1994). Interestingly, the Pal-box (AC-rich motif), a cis-acting element known to regulate the expression of phenylpropanoid biosynthesis genes (Boudet, 1998) also seemed to be conserved among the A. oryzae PAL and CHS encoding genes (data not shown).

The enzyme 4CL plays a key role in channelling carbon flow into diverse branch pathways of phenylpropanoid metabolism which serve important functions in plant growth and adaptation to environmental perturbations (Ehlting et al., 1999). Being the last enzyme of the general phenylpropanoid pathway, it provides precursors for the biosynthesis of a wide variety of plant natural products, by catalyzing the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates. Therefore, the existence of downstream genes encoding 4CL and CHSs in A. oryzae may aid in the formation of unknown flavonoid derivatives. An overall 11 genes encoding putative 4CL enzymes showing homology to bacterial and plant 4CLs could be found in A. oryzae genome (Table 2). While a similar number of 4CL genes could be found in the A. thaliana genome, A. nidulans and A. fumigatus showed only 8 and 2 putative 4CL encoding genes, respectively (Seshime et al., 2005b).

Moreover, although a true homolog could not be identified, at least 13 genes belonging to the aldo/keto reductase family in A. oryzae exhibited similarity to 3 CHRs (563540, 563536, and 563538) from M. sativa. A similar trend was observed in the case of A. thaliana which showed only 2 hypothetical proteins having similarity (~50%) to the CHR gene from M. sativa. Surprisingly, as mentioned earlier no CHI homolog could be detected in the A. oryzae genome. Since it is known that CHIs generally act on chalcones, the products of CHSs, this observation was quite unexpected. Support for clarifying such ambiguity could be derived from the fact that some bacteria containing a CHS ortholog and producing a chalcone-like compound did not necessarily contain a CHI (Gensheimer and Mushegian, 2004). In addition, the fact that the conversion of chalcone into flavanone can proceed non-enzymatically (Jez et al., 2000), the absence of a CHI homolog in the A. oryzae genome does not limit its ability to produce flavonoid-like compounds.

In general, isoflavones have drawn much attention because of their benefits to human health. These compounds are produced almost exclusively in legumes and have natural roles in plant defense and root nodulation (Dixon et al., 1996). Since, isoflavone synthase catalyzes the first committed step of isoflavone biosynthesis, a branch of the phenylpropanoid pathway, the genes encoding flavone synthase and enzymes acting downstream (such as isoflavone reductase, dihydroflavonol reductase, flavonol synthase, anthocyanin synthase etc.,) were searched in the A. oryzae genome. Despite their low homology observed (20-30%), the identification of these genes responsible for the downstream conversions would be possible if flavonoid-like metabolites in this filamentous fungus were characterized in the near future.

Another interesting feature noted in the A. oryzae genome was our observation of a large number of genes encoding cytochrome P450 superfamily in comparsion to the other Aspergilli (A. nidulans and A. fumigatus) (Machida et al., 2005). In plants it is known that more than 15 cytochrome P450-mediated oxidative reactions are involved in the biosynthesis of phenylpropanoids, phytoalexins, terpenes, and alkaloids that participate in defence against pathogens and UV light (Werck-Reichhart, 1995). Among these the sequences of two enzymes belonging to different P450 families, CYP73 and CYP75, are determined until now (Werck-Reichhart, 1995). Of these 2 enzymes, C4H is the most extensively studied plant P450 and is regarded as essential regulator of the whole pathway. Moreover, PAL, C4H, and cytochrome P450 reductase (CPR; a C4H redox partner) are important for the allocation of carbon flow from phenylalanine into phenylpropanoid biosynthesis. In this regard the multienzyme complexes containing PAL and C4H are thought to function at entry points into phenylpropanoid metabolism (Ro and Douglas, 2004). We therefore expect that some of the A. oryzae cytochrome P450s would be involved in the conversions of the phenylpropanoid-flavonoid metabolism.

Finally, although we show the existence of a major set of genes responsible for the phenylpropanoid-flavonoid metabolism, the principal question on the functioning of these genes as part of the secondary metabolic machinery in A. oryzae remains to be addressed. Since phenylpropanoids synthesized by plants contribute in resistance to pests, pathogens and physical barriers (UV light, low temperature etc.,) (Dixon et al., 2002), and are involved in interactions with beneficial microorganisms (Dixon et al., 1996), we presume fungal phenylpropanoids also might contribute to disease limitation and likely reflect the evolutionary significance. Some examples for such a character may be derived from the fact that A. oryzae conidia spread on steamed rice in open air during koji preparation for sake making remain uncontaminated, and some indus-

trial strains of *A. oryzae* (RIB128, RIB177, RIB609 and RIB647) exhibit enhanced tolerance to UV light and freeze-thaw treatment (Ishi *et al.*, 2005). These characteristics could probably be due to some flavonoid-like pigments produced by these strains.

Future perspectives

In order to elucidate the possible functions of the CHS-like genes in *A. oryzae*, we are pursuing the overexpression of the respective genes using different regulatable promoters and analysing the metabolites produced. Efforts to obtain the strains disrupted for the CHS-like genes are also underway. Since *A. oryzae* is a well established fungus for use in food and fermentation industry, the identification and an in-depth characterization of the phenylpropanoid biosynthesis pathway genes and intermediates will not only unravel the secondary metabolic secrets of *A. oryzae* but also opens up the possibility of engineering *A. oryzae* for improved production of important and novel metabolites.

Concluding remarks

Secondary metabolites are synthesized by bacteria, algae, fungi, plants, corals, sponges and several other lower organisms and many secondary pathways may be of relatively ancient origin but have arisen infrequently. Evidence is now being obtained that secondary metabolism might have evolved during the course of evolution either by modification of existing metabolic reactions or through gene transfer between organisms. The increasing amount of data on genome sequences from different fungal species is changing our view of how widespread or unique some of these pathways are in the fungal kingdom, bringing us to the question 'if *A. oryzae* can be a potential medicinal fungus?' Fungal genomics is opening a molecular avenue to the study of fungal secondary metabolism which will enable us to study the genes coding for the key metabolic enzymes, their molecular and physiological roles as well as the evolutionary significance of the pathway involved. This connection between genomics and natural products will allow the exploration of a wide range of fungi for biotechnological purposes. While species of Aspergilli 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*), the discovery of novel type III PKS family members in *A. oryzae*, 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

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