Fungal and Plant Phenylalanine Ammonia-lyase

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L-Phenylalanine is one of the essential amino acids that cannot be synthesized in mammals in adequate amounts to meet the requirements for protein synthesis. Fungi and plants are able to synthesize phenylalanine via the shikimic acid pathway. L-Phenylalanine, derived from the shikimic acid pathway, is used directly for protein synthesis in plants or metabolized through the phenylpropanoid pathway. This phenylpropanoid metabolism leads to the biosynthesis of a wide array of phenylpropanoid secondary products. The first step in this metabolic sequence involves the action of phenylalanine ammonia-lyase (PAL). The discovery of PAL enzyme in fungi and the detection of $^{14}CO_2$ production from ^{14}C-ring-labeled phenylalanine and cinnamic acid demonstrated that certain fungi can degrade phenylalanine by a pathway involving an initial deamination to cinnamic acid, as happens in plants. In this review, we provide background information on PAL and a recent update on the presence of PAL genes in fungi.

Phenylalanine ammonia-lyase (PAL; E.C. 4.3.1.5) catalyzes the nonoxidative deamination of L-phenylalanine to form *trans*-cinnamic acid and a free ammonium ion (Fig. 1) [1]. The conversion of the amino acid phenylalanine to *trans*-cinnamic acid is the entry step for the channeling of carbon from primary metabolism into phenylpropanoid secondary metabolism in plants. PAL has been extensively studied because of its role in plant development and its response to a wide variety of environmental stimuli. The importance of this enzyme in plant metabolism is demonstrated by the huge diversity and large quantities of phenylpropanoid products found in plant materials [2]. In fungi, there is no direct evidence for the significance of this enzyme except as a catabolic function [3].

Since its discovery [1], the presence of PAL has been reported in diverse plants [4, 5] including certain algae, including *Dunaliella m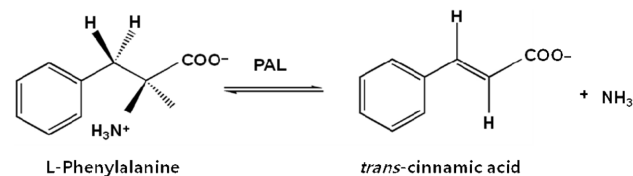arina* [6], fungi [7-10], and a few prokaryotic organisms, including *Streptomyces* [11, 12]. In plants, PAL activity has been detected in many species, representing monocots, dicots, gymnosperms, ferns, and lycopods [13]. In fungi, PAL activity has been detected only in a few basidiomycetes and deuteromycetes, and in one ascomycete, *Nectria cinnabarina* [7, 14]. There have been no reports of PAL in animals.

In this review, we provide background information on PAL and a recent update on the presence of PAL genes in fungi.

Structural Properties of PAL Proteins

PAL has been isolated and characterized from a number of plant species, some fungi and few bacterial sources. Source tissues used for PAL isolation are diverse. They include seedlings [15], shoots [1], leaf-sheath [16], cell culture [17-19], fruit [20], mycelium [21, 22], and prokaryotic cells [12]. Most known PAL sources for enzyme isolation and its properties are well tabulated and documented in several reviews [4, 23, 24]. Difficulties in purification are often encountered, partly resulting from the low abundance of PAL in cells and changes in size and properties that occur during purification. Although an apparently homogeneous protein preparation can often be obtained in non-denaturing conditions, additional polypeptide bands

Fig. 1. Deamination of L-phenylalanine by L-phenylalanine ammonia-lyase (PAL).

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are usually detected in analytical polyacrylamide gel electrophoresis gels under denaturing conditions. This can create confusion in the estimation of PAL subunit sizes.

Most reported PALs range in size from 300 to 340 kDa in native molecular mass. Some examples of exceptions are reported masses of 152 kDa in *Ocimum basilicum* [25], 226 kDa in the bacterium, *Streptomyces* [11], 250 kDa in *Helianthus annuus* [5], 266 kDa in *Fragaria ananassa* [20], 320 kDa in *Ustilago maydis* [26], and 560 kDa in *Alternaria* [21]. PAL is normally a homo-tetrameric protein consisting of four identical subunits. Hetero-tetrameric PAL as a complex of two hetero-dimers has been reported from *H. annuus* (2×58 kDa and 2×68 kDa) [20] and *Rhizoctonia solani* (2×70 kDa and 2×90 kDa) [22]. Neumann and Schwemmle [27] reported that *Oenothera* seedlings have two PAL isoenzymes with four identical subunits each of 75.5 kDa and 79.2 kDa. *Rhodosporidium toruloides* PAL has been reported to be a dimer composed of two identical subunits with a mass of 80 kDa [28].

Iso-electric points (pIs) for PAL are usually in the acid range, from 2.5 [27] to 6.3 [26]. Isoforms with different pIs have been reported from some sources; three isoforms ranged between pI 4.8 and 5.4 in *Leptosphaeria maculans* fungus [29], several isoforms between pI 5.1 and 6.1 in alfalfa [17], and two isoforms between pI 4.8 and 5.4 in bean [30]. Interestingly, expression of a single cDNA of poplar PAL in a baculovirus expression system produced two isoforms with different pIs [31].

Most PALs are considered to be hydrophobic proteins. This property has led to the use of hydrophobic affinity column chromatography for the purification of PAL from cotton [32] and *Rhodotorula glutinis* [33]. Alfalfa PAL has been reported to be highly hydrophobic [17]. Consistent with this, the hydropathy profile of the protein sequence deduced from the cDNA sequence also predicted that alfalfa PAL would be hydrophobic [34].

The association of carbohydrate with PAL has been reported for the maize and potato enzymes [35, 36]. Through the analyses of PAL gene sequences, the presence of potential glycosylation sites has been reported from bean [37] and parsley [38], but the importance of glycosylation in PAL function has not been explored extensively. The production of active PAL in *Escherichia coli* cells transformed with PAL genes from the yeast *Rhodosporidium* [39] and from parsley [40, 41] suggests that PAL catalysis is not likely to be influenced by glycosylation. It has not been excluded that glycosylation is involved in enzyme stability and in localization of the enzyme within cells [35, 36].

The three-dimensional structure of the red yeast *R. toruloides* PAL has been described using X-ray crystallography [42]. This homotetrameric protein contains 716 residues per subunit with a molecular mass of 76.88 kDa. A seahorse-like shape of each subunit interlocks with two other subunits, thereby maximizing adjacent subunit interactions and resulting in tetramer formation.

PAL Active Site and Enzyme Mechanism

PAL is one of the few amino acid-transforming enzymes not containing the cofactor pyridoxal 5-phosphate. Instead, PAL contains the unusual prosthetic group dehydroalanine [9]. The role of this post-translationally modified amino acid in catalysis is assumed to be the activation of the amino group of phenylalanine to form a better leaving group than NH_3^+ [43]. Modification of an electrophilic center at the active site of PAL by electrophilic reagents such as borohydride, cyanide, bifulfite, or nitromethane results in the complete inactivation of the enzyme. The identity of [^{3}H]-alanine and [^{14}C]-aspartic acid released following acid hydrolysis of PAL enzyme inactivated with radiolabeled reagents, NaB^3H_4 and $^{14}CN^-$, provide evidence for the presence of dehydroalanine in the active site [9, 44]. Studies on the ability of substrates and substrate analogs of PAL to prevent inactivation by these reagents provide further evidence to support the idea that the active site contains dehydroalanine [9, 44]. An alternative model for the role of dehydroalanin in PAL catalysis has been also proposed [45].

The mechanism of formation of dehydroalanine has not been determined yet. In cases of other proteins containing dehydroalanine, such as subtilin [46], thyroglobulin [47], and pyruvoyl enzymes [48], a serine residue is considered to be the precursor of dehydroalanine. PAL amino acid sequences contain a serine residue that is completely conserved among different species [49], and is presumed to be associated with the active site of the enzyme. Recently, the precursor of the dehydroalanine residue has been identified as serine in parsley [45] and poplar PAL [31]. In fungal PAL, it is likely that a similar process would account for the formation of the active site dehydroalanine from serine, but a role for serine as the precursor of dehydroalanine has not been directly demonstrated yet. Expression of PAL in *E. coli* produced active PAL enzymes in cells in which PAL was not normally produced [40, 47]. The expressed PAL proteins showed similar enzyme properties compared to endogenous PAL from other sources. This suggests that the formation of dehydroalanine may be an autocatalytic process, although it cannot be ruled out that a widespread modifying enzyme is involved in the dehydroalanine formation.

Functional Properties of PAL

PAL enzymes from many sources, especially from monocots and certain fungi, have activity towards L-tyrosine and can produce *trans-p*-coumaric acid. This has been described as tyrosine ammonia-lyase (TAL) activity [4, 13, 50]. In most

PAL preparations, TAL activity is very low. The PAL : TAL ratio in PAL preparations varies from 1.35~5 in *Sporobolomyces pararoseus* fungus [51], from 4~20 in wheat [13], and from 0.6~1.3 in bean [52]. An even larger range in PAL : TAL ratios from several different plant species was reported [53]. No TAL enzyme without PAL activity has been purified. It has been demonstrated in *E. coli*-expressed maize PAL that PAL and TAL activities reside in the same polypeptide [54].

PAL preparations from a number of sources are reported to have only one Michaelis constant (K_m), but the kinetic properties of other preparations suggest that the enzyme is negatively cooperative with respect to substrate binding [55]. Two different K_m values for PAL have been reported from many sources [23]. Individual isoforms were highly purified and the kinetic analysis of each isoform revealed normal Michaelis-Menten saturation kinetics [17, 56]. The K_m values for L-phenylalanine have been reported to range from 0.011 mM [57] to 1.7 mM [1]. Most PALs show no metal ion requirement, although slight stimulation of PAL activity by metal ions such as Mg^{2+} and Ba^{2+} has been reported [5]. Inhibition of PAL activity can be induced by a wide range of compounds including carbonyl, sulfhydryl, and thiol reagents, phenolic acids, and heavy metal ions [24]. Most PALs tested are sensitive to synthetic PAL inhibitors such as (*S*)-2-aminooxy-3-phenylpropanoic acid (AOPP), (*R*)-(1-amino-2-phenylethyl)phosponic (APEP) acid, and 2-aminoindan-2-phosphonic acid (AIP). Thus, these inhibitors have often been used to block the biosynthesis of phenylpropanoid compounds in plant cells and tissues [23, 58].

The pH optimum for PAL is generally in the range from 8.2~9.0 [21, 23]. The temperature optimum for PAL has been reported to be 35°C in tobacco [57], 55°C in sunflower [5], and 44~46°C in *Rhizoctonia* [22]. Plant PAL enzymes are generally sensitive to repeated freezing and thawing and lose activity as the temperature approaches 60°C. In contrast, fungal PAL is more thermally stable too [22]. *Rhodotorula* PAL is apparently stable for at least 6 months when it is kept at −60°C [59].

Structural Properties of PAL-Encoding Genes

Following the isolation of PAL cDNA from bean [37], parsley [38] and sweet potato [60], PAL genes have been isolated from many sources. In most plants, PAL is encoded by a small gene family of 3~5 genes. Exceptions to this are the potato PAL gene family, which is made up of 40~50 genes [61], and the loblolly pine PAL, which has been reported to be encoded by a single gene [62]. Currently, either partial or full fungal genome data is available from more 50 species. When we searched through the DNA databases of the Broad Institute (Cambridge, MA, USA; http://www.broadinstitute.org) and National Center

for Biotechnology Information (Bethesda, MD, USA) for the PAL motif [GS]-[STG]-[LIVM]-[STG]-[SAC]-S-G-[DH]-L-x-[PN]-L-[SA]-x(2,3)-[SAGVTL], 45 potent PAL sequences were found in 28 fungal species (Table 1). In red yeasts such as *Rhodosporium* spp., PAL is generally encoded by a single gene [63]. In filamentous fungi, PAL is encoded by a single, two, three, and four genes (Table 1). The *Aspergillus nidulan* genome contains four PAL genes. The presence of introns has been reported in both plant and fungal PAL genes. Plant PAL genes generally contain only one intron, while yeast PAL genes have five [63] or six introns [64]. Two introns have been found in the *Arabidopsis* PAL gene [65], while no introns occur in jack pine and loblolly pine and *U. maydis* PAL genes [66-68]. The analysis of 45 PAL gene sequences from the fungi listed in Table 1 show that the number of introns varies among species. The number of introns in PAL gene also differs within a species such as *A. flavus* and *A. oryzae*. In ascomycota, the intron number ranges from none ito six introns, while in basidiomycota, intron number ranges from none ito 13 introns. The highest intron number is present in the PAL genes of rust fungus *Puccinia* (Table 1). The inferred PAL protein length varies in fun ranging from 595 to 750 amino acids, except for *Botrytis cinerea*, which has 1,131 amino acids. The position of the PAL motif on the PAL protein sequence shows little variation among species. The fungal PAL motif positions mostly between 123 and 244 amino acids, except for *Neurospora* and *Neosartorya* sequences, which position near the C-terminus and N-terminus, respectively. The active site serine residue that is bolded in the PAL motif in Table 1 is very well-conserved both in ascomycota and basidiomycota.

Phylogenetic analysis of the 45 PAL sequences showed that PAL could be divided into three major groups: ascomycota I, ascomycota II, and basdiomycota (Fig. 2). PAL of ascomycota I is more closely related to PAL of basidiomycota than PAL of ascomycota II. The cladogram revealed the variation in the PAL protein sequence among fungi. Comparison of the inferred protein sequences of PAL from diverse fungal species showed a 33~77% protein sequence identity among the species (Table 2). The highest identity among ascomycota PALs was 65% while the highest identify among basidiomycota PALs was 97%. The highest identity between ascomycota and basidiomycota PAL found to date is 41%. Overall, the genome information on many fungi has revealed that many species have PAL gene(s) and the structural properties of the PAL gene vary within a species and among species.

PAL in Fungi

While the metabolism of phenylalanine in vascular plants and animals has been well documented, much less is

Table 1. Fungal species having PAL motif sequences properties

Fungal species	Gene name	PAL motif	Position of motif	Length of protein	No. of introns
Ascomycota					
Aspergillus clavatus NRRL 1	ACLA_080920	GSISASGDLIPLSYIAA	123-139	664	1
A. flavus NRRL 3357	AFL2G_05505	GSISASGDLIPLSYIAG	189-205	721	2
A. flavus NRRL 3357	AFL2G_00533	GSISASGDLTPLAYIAG	202-218	714	1
A. flavus NRRL 3357	AFL2G_06214	GSISASGDLSPIAYISG	155-171	671	3
A. fumigatus Af293	Afu2g09110	GSISASGDLMPLSYIAG	181-197	728	2
A. nidulans FGSC A4	ANID_03897	GSISASGDLTPLAYIAG	182-198	687	1
A. nidulans FGSC A4	ANID_06075	GSISASGDLMPLSYIAG	187-203	702	2
A. niger ATCC 1015	e_gw1_15.39	GSISASGDLTPLAYIAG	194-210	719	1
A. niger ATCC 1015	e_gw1_3.237	GSISASGDLSPLSYIGG	181-197	720	2
A. niger ATCC 1015	fge1_pm_C_13000016	GTISASGDLMPLAYVVG	184-200	704	2
A. oryzae RIB 40	AO090005000532	GSISASGDLTPLAYIAG	202-218	714	1
A. oryzae RIB 40	AO090011000788	GSISASGDLIPLSYIAG	189-205	721	2
A. oryzae RIB 40	AO090026000586	GTISASGDLMPLAYVTG	183-199	696	2
A. oryzae RIB 40	AO090701000601	GSISASGDLSPIAYISG	228-244	744	4
A. terreus NIH 2624	ATEG_09127	GSISASGDLTPLAYIAG	177-193	695	0
A. terreus NIH 2624	ATEG_10006.1	GSVSASGDLMPLSYIAG	181-197	698	2
Botrytis cinerea B05.10	BC1G_05296.1	GSISASGDLSPLSYIGG	240-256	1131	6
Chaetomium globosum CBS 148.51	CHGG_02399.1	GSISASGDLSTLSYIAG	190-206	707	3
Fusarium. graminearum NRRL 31084	FGSG_09311	GTISASGDLMPMSYIAG	180-196	721	0
F. oxysporium f. sp. *lycopersici* 4287	FOXG_05927	GTISASGDLMPLSYIAG	180-196	750	0
F. verticillioides 7600	FVEG_03798	GTISASGDLMPLSYIAG	180-196	724	0
F. verticillioides 7600	FVEG_10552	GSISASGDLIPLSYIAG	196-212	706	1
Gaeumannomyces graminis R3-111a-1	GGTG_00837.1	GSISASGDLSPLSYIAG	204-220	743	2
Magnaporthe oryzae 70-15 (MG8)	MGG_10036.7	GSISASGDLSALAWIAA	207-223	627	0
M. poae ATCC 64411	MAPG_07598.1	GSISASGDLSPLSYIAG	198-214	730	2
Neurospora crassa OR74A (NC10)	NCU09391.5	GSISASGDLSTLSYIAG	610-623	763	1
Neosartorya fischeri NRRL 181	NFIA_084640	GSISASGDLMPLSYIAG	48-64	595	0
Stagonospora nodorum SN15	SNOG_08528.1	GSISASGDLSPLSYICG	190-206	700	4
S. nodorum SN15	SNOG_09914.1	GSISASGDLSALAWIGA	179-195	610	0
S. nodorum SN15	SNOG_16362.1	GSISASGDLSPLSYVGG	191-207	772	2
Uncinocarpus reesii 1704	UREG_04219.1	GTISASGDLMPLAYIVG	185-201	710	2
Verticillium albo-atrum VaMs.102	VDBG_08166.1	GTISASGDLMPLSYIAG	181-197	676	1
V. dahliae VdLs.17	VDAG_100581.1	GTISASGDLMPLSYIAG	181-197	696	0
V. dahliae VdLs.17	VDAG_05831.1	GSISASGDLSALAWICA	218-234	645	0
Basidiomycota					
Coprinus cinerea okayama7#130	CC1G_06838.3	TSISASGDLSPLSYIAG	212-228	734	9
C. cinerea okayama7#130	CC1G_14161.3	GSISASGDLSPLSYIAG	252-268	770	6
Laccaria bicolor S238N-H82	LACBIDRAFT_291120	GTISASGDLAPLSYIAG	163-179	688	5
L. bicolor S238N-H82	LACBIDRAFT_184628	GSISASGDLSPLSYIAG	201-217	731	11
Puccinia triticina 1-1 BBBD Race 1	PTTG_02413.1	GSISASGDLMPLSYVAA	175-191	653	13
P. graminis tritici CRL 75-36-700-3	PGTG_12283.2	GSISASGDLMPLSYVAA	190-206	691	13
Rhodosporidium toruloides	AAA33883	GTISASGDLSPLSYIAA	207-213	693	6
R. toruloides CBS 14	P11544	GTISASGDLSPLSYIAA	184-200	716	6
Rhodotorula graminis WP1	CAD23828	GSISASGDLSPLSYIAG	213-229	713	1
R. mucilaginosa NRRLY-15597	CAA31486	GTISASGDLSPLSYIAA	213-229	720	5
Ustilago maydis 521	UM00078	SSISASGDLSPLSYVAG	201-217	724	0

PAL, phenylalanine ammonia-lyase; NRRL, Northern Regional Research Laboratory; FGSC, Fungal Genetics Stock Center; ATCC, American Type Culture Collection; RIB, Research Institute of Brewing; NIH, National Institutes of Health; CBS, Centraalbureau voor Schimmelcultures.

known about the fungal degradation of phenylalanine. Some of the known pathways of animal and plant metabolism of phenylalanine are also used in microorganisms. In some microorganisms, phenylalanine is converted to homogentisic acid through the intermediary formation of phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid by transamination and hydroxylation, as in the case of animals [69].

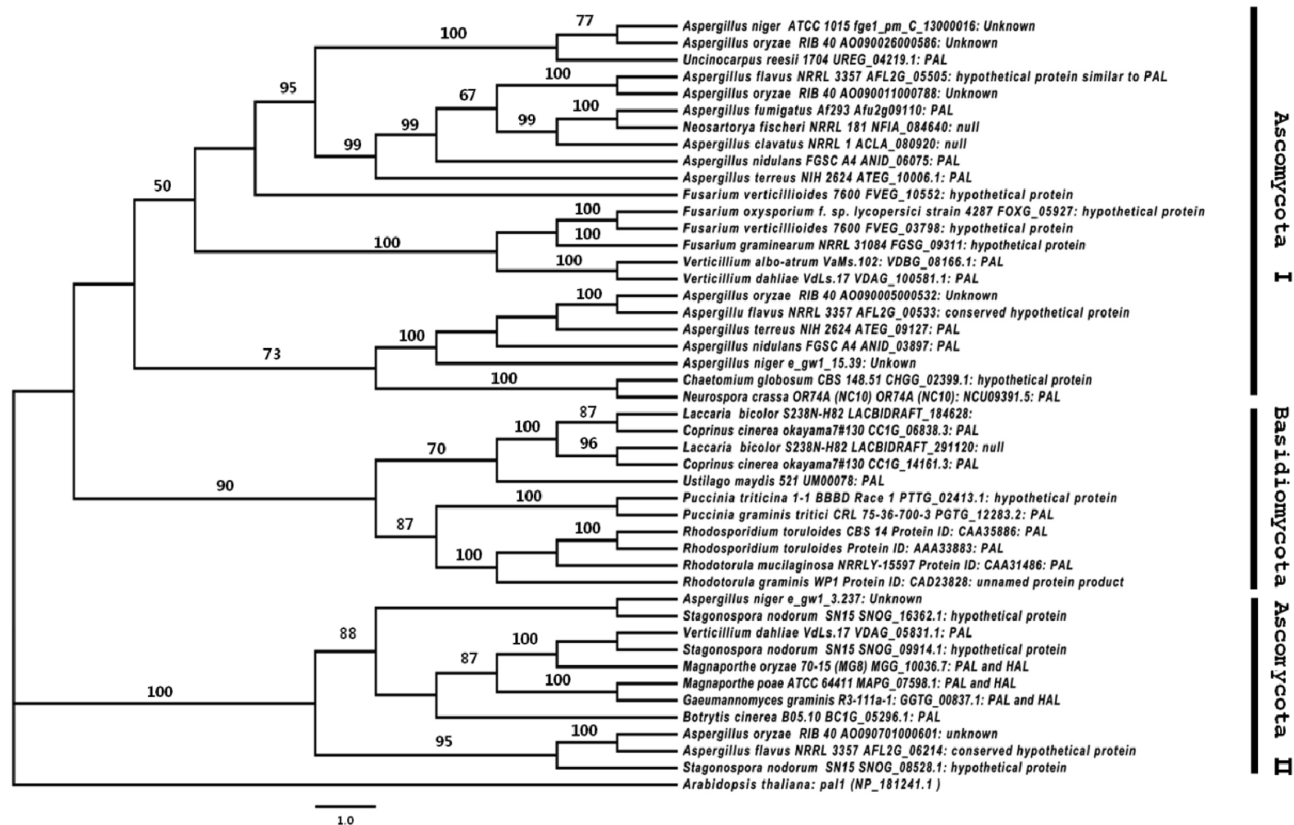The discovery of a PAL enzyme in fungi [70] and

Fig. 2. Cladogram of the inferred amino acid sequences of L-phenylalanine ammonia-lyase (PAL) from diverse fungal species. Cladogram was constructed by the neighbor-joining method using PAUP v.4.0b10. *Arabidopsis thaliana* PAL sequence was used as an out-group.

the detection of $^{14}CO_2$ production from ^{14}C-ring-labeled phenylalanine, cinnamic acid, and benzoic acid [71] have demonstrated that certain fungi can degrade phenylalanine by a pathway involving an initial deamination to cinnamic acid, as happens in plants. A metabolic pathway for the metabolism of phenylalanine via cinnamic, benzoic, *p*-hydroxybenzoic, and protocatechuic acids has been reported in several basidiomycete fungi, including *Rhodotorula* [72], *Ustilago hordei* [71], *Schizophyllum commune* [71], and *Sporobolomyces roseus* [8]. *S. commune* can also metabolize phenylalanine through phenylpyruvic acid, phenylacetic acid, and *o*-hydroxyphenylacetic acid [71]. Interestingly, it has been reported that another basidiomycete, *Lentinus lepideus*, forms phenylpropanoid compounds (e.g., *p*-coumaric acid, caffeic acid, isoferulic acid, phloretic acid, and *p*-methoxycinnamic acid) via cinnamic acid derived from phenylalanine [73]. In this fungus, a number of these compounds accumulate in the medium as methyl esters, but the physiological significance of these compounds is not known. The conversion of phenylalanine to benzoic acid derivatives through cinnamic acid has also been reported in Deuteromycete fungi such as *Alternaria* [74], *R. solani* [75], and *Penicillium brevicompactum* [76]. The fungus *Gliocladium* produces gliotoxin, an antibiotic and antiviral

cyclic peptide, derived in part from phenylalanine and modified by the addition of sulfur across the peptide ring [77].

Commercial and Medical Potential of PAL

The therapeutic potential of using PAL enzyme against neoplasms has been suggested because of its selectivity for phenylalanine [78]. PAL substantially inhibited neoplastic cell growth *in vitro* [79], and produced cures in some mice that were inoculated with a lymphoblastic leukemia [80]. However, PAL is of special interest to clinicians primarily due to its potential as a treatment for the inherited metabolic disorder, phenylketonurea. A treatment involving the oral ingestion of PAL [81] were proposed to patients to consume a normal diet. Preclinical evaluation of multiple species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria has been performed with mouse model [82]. In 2011, BioMarin Pharmaceutical has announced PEG-PAL (PEGylated recombinant phenylalanine ammonia lyase) is currently in Phase II clinical development for the treatment of PKU.

With the increasing consumption of the aspartic acid-phenylalanine dipeptide artificial sweetener, aspartame, the commercial demand for L-phenylalanine has led to mass

Table 2. Comparison of the inferred protein sequences of PAL from diverse fungal species

Fungal PAL	Ascomycota														Basidiomycota						
	Af*	An1	An2	At1	At2	Bc	Gg	Mo	Mp	Nc	Ur	Va	Vd1	Vd2	Cc1	Cc2	Pg	Rm	Rt1	Rt2	Um
Af																					
An1	65																				
An2	44	43																			
At1	44	43	57																		
At2	62	57	41	41																	
Bc	37	38	37	37	39																
Gg	36	38	39	38	40	45															
Mo	38	39	37	38	40	50	55														
Mp	37	37	39	37	40	45	85	54													
Nc	43	44	45	45	45	35	38	41	38												
Ur	53	51	43	45	51	40	39	41	39	41											
Va	43	42	36	38	45	41	36	37	37	40	41										
Vd 1	43	43	37	38	45	41	35	38	36	40	43	89									
Vd 2	38	37	37	36	39	39	55	74	54	41	40	36	36								
Cc 1	39	40	39	39	40	37	36	40	36	39	38	38	37	39							
Cc 2	35	37	38	37	37	31	34	39	35	33	37	35	33	37	57						
Pg	37	37	37	35	38	37	37	37	36	37	34	36	35	36	40	39					
Rm	36	38	38	37	40	34	34	37	35	35	36	35	35	35	42	41	44				
Rt1	38	39	40	38	41	34	35	37	36	36	37	36	36	37	42	42	46	77			
Rt2	39	39	40	38	41	35	36	38	37	37	38	36	36	37	44	43	46	76	97		
Um	38	37	36	36	41	38	37	41	39	35	39	36	36	39	44	43	39	39	41	42	

Numbers indicate the percentage of protein sequence identity.
PAL, phenylalanine ammonia-lyase; Af, *Aspergillus fumigatus* Af293 PAL; An1, *Aspergillus nidulans* FGSC A4 PAL1; An2, *Aspergillus nidulans* FGSC A4 PAL2; At1, *Aspergillus terreus* NIH 2624 PAL1; At2, *Aspergillus terreus* NIH 2624 PAL2; Bc, *Botrytis cinerea* B05.10 PAL; Gg, *Gaeumannomyces graminis* R3-111a-1 PAL; Mo, *Magnaporthe oryzae* 70-15 (MG8) PAL; Mp, *Magnaporthe poae* ATCC 64411 PAL; Nc, *Neurospora crassa* OR74A (NC10) PAL; Ur, *Uncinocarpus reesii* 1704 PAL; Va, *Verticillium albo-atrum* VaMs.102 PAL; Vd1, *Verticillium dahliae* VdLs.17 PAL1; Vd2, *Verticillium dahliae* VdLs.17 PAL2; Cc1, *Coprinus cinerea* okayama 7#130 PAL1; Cc2, *Coprinus cinerea* okayama 7#130 PAL2; Pg, *Puccinia graminis tritici* CRL 75-36-700-3 PAL; Rm, *Rhodotorula mucilaginosa* NRRLY-15597 PAL; Rt, *Rhodosporidium toruloides* CBS 14 PAL; Um, *Ustilago maydis* 521 PAL; ATCC, American Type Culture Collection; FGSC, Fungal Genetics Stock Center; NIH, National Institutes of Health; NRRL, Northern Regional Research Laboratory; CBS, Centraalbureau voor Schimmelcultures.

production of this amino acid [83]. Since the reaction is reversible, PAL can be used in a large-scale bio-conversion to produce L-phenylalanine from *trans*-cinnamic acid and ammonium salts acid [84]. Commercial production of PAL is available from *R. glutinis* (Sigma-Aldrich, St. Louis, MO, USA).

Conclusions

While a huge amount of information has accumulated on the structure, expression, and function of PAL in plants, the biological role of PAL in fungi has not been established, and, in general, information on fungal PAL is very limited. Most commonly, a catabolic function for fungal PAL has been suggested, in which the enzyme is used to obtain carbon and nitrogen from external supplies of amino acids. However, fungi can also obtain carbon and nitrogen from L-phenylalanine through phenylalanine aminotransferase or amino acid oxidase. What selective advantage does PAL offer that has led to its retention in this group of organisms? It appears that the ability to synthesize cinnamic acid is important in the life cycle of

fungi. Now with the full sequencing of fungal genome(s) in diverse fungal species including human and plant pathogens, saprophytes, and mushrooms, it is possible to compare and predict the potent pathways for phenylalanine degradation among different fungal species. Molecular genetic studies such as gene replacement should reveal whether PAL is essential in fungal physiology and/or especially reveal links, if any, between PAL activity and pathogenesis, development, and secondary metabolic activities. Further work to develop knowledge and tools that would enable us to rationalize the existence of PAL in certain fungi is needed.

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