

Expressed sequence tag analysis of the soybean rust pathogen *Phakopsora pachyrhizi*

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Abstract

Soybean rust is caused by the obligate fungal pathogen *Phakopsora pachyrhizi* Sydow. A unidirectional cDNA library was constructed using mRNA isolated from germinating *P. pachyrhizi* urediniospores to identify genes expressed at this physiological stage. Single pass sequence analysis of 908 clones revealed 488 unique expressed sequence tags (ESTs, unigenes) of which 107 appeared as multiple copies. BLASTX analysis identified 189 unigenes with significant similarities ($E_{\text{value}} < 10^{-5}$) to sequences deposited in the NCBI non-redundant protein database. A search against the NCBI dbEST using the BLASTN algorithm revealed 32 ESTs with high or moderate similarities to plant and fungal sequences. Using the Expressed Gene Anatomy Classification, 31.7% of these ESTs were involved in primary metabolism, 14.3% in gene/protein expression, 7.4% in cell structure and growth, 6.9% in cell division, 4.8% in cell signaling/cell communication, and 4.8% in cell/organism defense. Approximately 29.6% of the identities were to hypothetical proteins and proteins with unknown function.

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Keywords: *Phakopsora pachyrhizi*; Genome analysis; cDNA sequencing; Expressed sequence tags; Gene expression

1. Introduction

Soybean rust causes significant yield loss to soybean crops in Asia, Africa, Australia, and nearly all tropical countries in the Eastern Hemisphere where soybeans are grown have reported its occurrence (AVRDC, 1987, 1992). Recent findings of soybean rust in Hawaii in 1994 (Killgore and Heu, 1994), Zimbabwe in 1998 (Levy, 2003), Nigeria in 1999 (Akinsanmi and Ladipo, 2001), South Africa in 2001 (Pretorius et al., 2001), Paraguay in 2001 (Morel, 2001), Brazil and Argentina in 2002 (Rossi, 2003; Yorinori et al., 2002), and Bolivia in 2003 (Yorinori et al., 2005) demonstrate that *Phakopsora pachyrhizi*

is spreading to new geographic regions. Rust is considered to be a major threat to soybean production in the United States (Sinclair, 1989), especially with the identification of *P. pachyrhizi* in Louisiana in November 2004 (Schneider et al., 2005). In Brazil, this disease was estimated to cost growers approximately \$1.2 billion (USD) in 2003 alone: \$500 million in direct yield losses to the disease and \$700 million resulting from inappropriate use of fungicides (Yorinori et al., 2005). If *P. pachyrhizi* becomes established in the continental US, serious yield losses are likely to occur. It has been estimated that yield losses could exceed 10% in most of the United States with up to 50% yield loss in the Mississippi Delta and southeastern states (Yang et al., 1991).

Four single resistance genes, *Rpp1–4* (for resistance to *P. pachyrhizi*), have been described that impart resistance to some isolates of *P. pachyrhizi* (Bromfield and Hartwig, 1980; Hartwig, 1986; Hartwig and Bromfield, 1983; McLean and Byth, 1980). However, no

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soybean lines have been found with broad-spectrum resistance to all isolates of *P. pachyrhizi*, and all of the commercial soybean cultivars currently grown in the US are susceptible to soybean rust. In countries where rust has become problematic to commercial production, control strategies have relied on the use of fungicides; however, most growers in the US currently do not apply fungicides to soybeans. The increased costs associated with multiple applications of fungicides might be prohibitive for some growers in the US, and there are concerns about the potential negative effects to the environment if fungicides are applied to such large production acreage.

Soybean rust is caused by two closely related species of fungi, *P. pachyrhizi* Sydow and *P. meibomiae* (Arthur) Arthur, which are differentiated based upon morphological characteristics of the telia (Ono et al., 1992). Sequence analysis of the internal transcribed spacer region of the ribosomal RNA genes revealed approximately 80% similarity between these two *Phakopsora* species; however, only a few nucleotide differences were observed among isolates of *P. pachyrhizi* or *P. meibomiae* (Frederick et al., 2002). Unlike most other rust pathogens, both *Phakopsora* species infect and produce disease symptoms on a wide range of host plants. *P. pachyrhizi* naturally infects 31 species in 17 genera of *Leguminosae*, and it has been found to infect 60 species in other genera under controlled conditions (Rytter et al., 1984; Sinclair and Hartman, 1996). Similarly, *P. meibomiae* infects 42 species in 19 genera of *Leguminosae*, and it can infect 18 species in another 12 genera following artificial inoculation (Sinclair and Hartman, 1996). On soybeans, *P. pachyrhizi* is the more aggressive pathogen and causes considerably more yield loss compared to *P. meibomiae*.

Phakopsora pachyrhizi produces three types of spores. The urediniospore is the most common spore type and is found throughout the growing season on soybeans and other legume hosts. Urediniospores are produced in large quantities, easily wind disseminated, and multiple spore cycles occur throughout the growing season. Telia and teliospores have been observed on infected plants late in the season in Asia as well as in greenhouse studies (Bromfield, 1984; Yeh et al., 1981). Teliospore germination and the subsequent production of basidiospores have been reported, but only under laboratory conditions (Saksirirat and Hoppe, 1991). As no alternate host has been identified, there has been no further characterization of the life cycle.

Most of the published research on soybean rust has focused on monitoring disease development, evaluating yield losses, modeling epidemics, host range studies, developing risk assessment models, and screening for sources of resistance. In addition, there have been several reports on the basic biology of the fungus, including histological studies using susceptible lines and those con-

taining single resistance genes (Bonde et al., 1976; Hartwig and Bromfield, 1983; Sinclair and Hartman, 1996). The infection process employed by *P. pachyrhizi* consists of several distinct steps: attachment of the spore to the host surface, spore germination, formation of the appressorium, penetration through the cuticle, and invasive growth within the host plant (Bonde et al., 1976). Understanding these processes at both the biochemical and molecular levels is essential for developing new methods of disease management.

Here, we report the first assessment of gene expression at a critical stage of the *P. pachyrhizi* life cycle: urediniospore germination. This study identifies transcripts present in germinating urediniospores and provides insight into the biochemical processes that occur at this developmental stage. Some of the genes expressed display a high degree of similarity to genes described in other fungi and plants, but the majority corresponded to unclassified genes or genes of unknown function. A preliminary report of this work has been given (Posada and Frederick, 2002).

2. Materials and methods

2.1. Fungal isolate and growth conditions

The *P. pachyrhizi* isolate Taiwan 72-1 (TW 72-1) used in this study was maintained at the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU) Plant Pathogen Biosafety Level 3 Containment Facility at Ft. Detrick, MD (Melching et al., 1983) under the appropriate USDA Animal and Plant Health Inspection Service (APHIS) permit. TW 72-1 was propagated by spray inoculation onto soybean plants, and urediniospores were harvested from infected leaves 10–14 days following inoculation and at subsequent intervals using a mechanical harvester (Cherry and Peet, 1966). Urediniospores were maintained under liquid nitrogen. Frozen urediniospores were heat shocked at 42 °C for 5 min, and 300 mg of spores was germinated in 300 ml distilled water in a sterile 13 in. × 9 in. Pyrex baking dish for 16 h at room temperature. The fungal tissue was collected using a spatula, frozen in liquid nitrogen, and used for RNA extractions.

2.2. cDNA library construction

Total RNA was isolated from germinating spores of *P. pachyrhizi* isolate TW 72-1 using the ToTally RNA kit (Ambion, Austin, TX, USA), and the poly(A)⁺ mRNA was purified using an OLIGOTEX mRNA purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A unidirectional cDNA library was constructed in the plasmid pSPORT1 using the Superscript Plasmid System for cDNA synthesis and

Cloning (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The titer of the library was approximately 20,000 colonies, and 5000 individual colonies were transferred to 96-well microtiter plates containing Luria broth with 15% (v/v) sterile glycerol. The plates were archived by storing in a freezer at -80°C , and 908 clones were sent for sequencing.

2.3. DNA sequencing

Prior to sequencing, all colonies were checked for the presence of an insert by colony-PCR using the SP6 and T7 primers. The PCR products were separated by electrophoresis using 1.5% agarose gels. DNA was prepared for sequencing reactions using a Qiagen BioRobot 9600 and a Beckman Biomek 2000. Purified plasmid DNA was sequenced from the 5' end with the M13 reverse primer using an Applied Biosystems (ABI) PRISM big dye terminator kit (Perkin-Elmer) and an ABI Applied Biosystems 3700 DNA analyzer at the USDA Agricultural Research Service, Eastern Regional Research Center, Nucleic Acids Facility (ARS-ERRC-NAF) in Wyndmoor, PA.

2.4. Data handling

Raw sequence data were retrieved electronically from the USDA-ARS-ERRC-NAF using the file transfer protocol (ftp) for subsequent processing and analysis. The sequence data were imported into the computer software package Chromas 2.13 (Technelysium Pty, Helensvale, Australia) and manually trimmed of vector sequences. Ambiguous base calls were corrected by manually inspecting the sequence electropherograms, and the edited sequences were used in similarity searches.

Each cDNA sequence was queried against the current non-redundant (nr) protein database at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) using the BLASTX algorithm and the NCBI EST database using the BLASTN algorithm (Altschul et al., 1997). In both cases, the default BLAST parameters were used. The redundancy of the 908 cDNA sequences was determined by comparing all sequences with one another using the program FastA (Wisconsin Package, Genetic Computer Group, Madison, WI, USA).

3. Results

3.1. EST analysis

The cDNA clones were checked by PCR, and 99% were found to contain inserts ranging in size from 350 to 3000 bp. A total of 908 clones were sequenced from the 5' end of the cDNA inserts. The single pass sequencing

runs generated an average of 650 nucleotides of readable sequences after manual editing.

All ESTs were assembled into a database and compared using the FastA program (Wisconsin Package, Genetic Computer Group, Madison, WI, USA) to identify redundant clones. A total of 488 unique ESTs were identified of which 381 appeared only once and 107 were represented by multiple clones at frequencies ranging from 2 to 142. The frequency of redundant ESTs is shown in Fig. 1. The sequences of the *P. pachyrhizi* EST clones were submitted to NCBI as dbEST IDs 28583523–28584357 and GenBank Accession Nos. DN739461–DN740295.

The BLASTX algorithm (Altschul et al., 1997) was used to translate each edited EST into the six possible reading frames for comparison with data in the current nr protein database at the NCBI. A total of 431 ESTs displayed significant similarity to sequences in the NCBI database, while 477 ESTs did not exhibit significant similarity to the database entries. ESTs with similarity scores of $E_{\text{value}} < 10^{-5}$ were grouped according to their putative function (Table 1), according to the Expressed Gene Anatomy Database (EGAD) categories developed by The Institute for Genomic Research (TIGR, Rockville, MD, USA).

The best BLASTX score is reported for redundant clones (Table 1). A total of 189 putative genes were identified, of which 28.6% shared similarity to proteins from yeast, 50.8% to protein sequences from other fungi, while the rest exhibited similarity to proteins from a wide variety of organisms including bacteria, plants, mammals, insects, nematodes, and other invertebrates. The *P. pachyrhizi* cDNA library contained a broad range of genes, predominantly encoding putative proteins involved in primary metabolism, gene/protein expression, and cell structure (Table 1; Fig. 2). The ESTs with significant similarity to hypothetical proteins or proteins with unknown function were placed into the unclassified proteins category (Table 1; Fig. 2). The EST sequences with significant similarities ($E_{\text{value}} \leq 10^{-15}$) to fungal and plant ESTs are shown in Table 2. Two different homologs of gEgh16, a protein expressed by *Blumeria graminis* f. sp. *hordei* during appressorium formation, were the

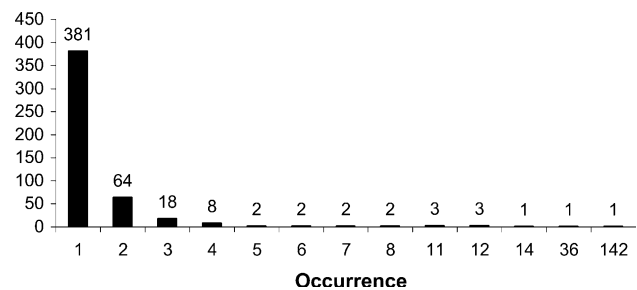


Fig. 1. The frequency of occurrence of EST clones derived from germinating *P. pachyrhizi* urediniospores. The number of EST clones is shown above each of the number of occurrences.

Table 1

EST clones displaying similarity (BLASTX, $E_{\text{value}} < 1\text{E}-05$) to proteins in the non-redundant protein NCBI database, grouped into functional categories according to expressed gene anatomy database

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
1. Cell division						
1.1. DNA synthesis/replication						
Pp0906	NP_595357	Checkpoint rad 3	<i>Schizosaccharomyces pombe</i>	9.00E-88	1	Yeast
Pp1817	T41457	DNA repair protein rad 18	<i>S. pombe</i>	4.00E-17	1	Yeast
Pp0244	NP_593482	Exonuclease II	<i>S. pombe</i>	2.5E-44	1	Yeast
Pp2018	CAB91747	Related to syntaxin 12	<i>Neurospora crassa</i>	4.00E-14	1	Filamentous fungus
1.2. Apoptosis						
Pp0322	AF316601	Metacaspase	<i>S. pombe</i>	9.00E-50	1	Yeast
1.3. Cell cycle						
Pp1417	AAA34617	G1 cyclin	<i>S. cerevisiae</i>	8.00E-12	1	Yeast
Pp1017	AJ272133	Cyclin	<i>A. nidulans</i>	7.00E-06	3	Filamentous fungus
1.4. Chromosome structure						
Pp0437	P62792	Histone H4	<i>Phanerochaete chrysosporium</i>	3.70E-42	1	Filamentous fungus
Pp0729	P62792	Histone H4	<i>P. chrysosporium</i>	3.70E-45	1	Filamentous fungus
Pp1936	AAA35311	Histone H2A- α	<i>S. pombe</i>	6.00E-33	3	Yeast
Pp1709	PN0142	Histone H2B	<i>N. crassa</i>	5.00E-39	2	Filamentous fungus
Pp1628	A35072	Non-histone chromosomal protein NHP6A	<i>S. cerevisiae</i>	4.00E-19	1	Yeast
Pp1812	S78076	Non-histone chromosomal protein NHP6B	<i>S. cerevisiae</i>	2.00E-23	1	Yeast
2. Cell signaling/cell communication						
2.1. Cell adhesion						
Pp0813	Q28983	Zonadhesin	<i>Sus scrofa</i>	1.00E-09	2	Mammal
2.3. Effectors/modulators						
Pp0839	NP_593464	Calmodulin kinase I homolog	<i>S. pombe</i>	5.00E-34	2	Yeast
Pp2023	AAA21544	Casein kinase-1	<i>S. pombe</i>	7.00E-48	1	Yeast
Pp0948	T18359	Nik-1 protein (histidine kinase)	<i>N. crassa</i>	1.00E-39	1	Filamentous fungus
Pp0229	T45137	Phosphoprotein phosphatase catalytic chain 2B	<i>S. pombe</i>	2.00E-06	1	Yeast
Pp1003	D84555	Probable protein kinase	<i>Arabidopsis thaliana</i>	5.00E-27	1	Plant
Pp0424	T11657	RhoGDP dissociation inhibitor	<i>S. pombe</i>	3.00E-30	1	Yeast
Pp1001	NP_596024	RhoGAP GTPase activating protein	<i>S. pombe</i>	5.00E-12	1	Yeast
Pp1337	NP_594429	Probable phosphatidylinositol-4-phosphate kinase	<i>S. pombe</i>	1.00E-51	1	Yeast
3. Cell structure and growth						
3.1. Cytoskeletal						
Pp1318	CAC17476	α -Tubulin	<i>Ustilago maydis</i>	3.10E-87	1	Filamentous fungus
Pp1432	CAC83953	β -Tubulin	<i>Uromyces viciae-fabae</i>	3.00E-98	1	Filamentous fungus
Pp1440	Q90631	Kinectin	<i>Gallus gallus</i>	5.00E-07	1	Bird
Pp0920	AB018696	RanBPM	<i>Xenopus laevis</i>	6.00E-05	1	Amphibian
Pp0414	U92845	Kinesin motor protein	<i>U. maydis</i>	2.00E-37	1	Filamentous fungus
3.2. Growth and sporulation						
Pp0432	XP_330886	Conidiation-specific protein 6	<i>N. crassa</i>	8.60E-16	7	Filamentous fungus
Pp0926	AAA33573	Conidiation protein	<i>N. crassa</i>	1.00E-06	1	Filamentous fungus
Pp0122	CAD10036	Deacetylase	<i>Filobasidiella neoformans</i>	5.00E-39	3	Filamentous fungus
Pp1605	A59290	Csm1 (class V chitin synthase with a myosin motor-like domain)	<i>Magnaporthe grisea</i>	3.00E-07	1	Filamentous fungus
Pp1209	AAO49384	Class V chitin synthase	<i>Fusarium oxysporum</i>	5.70E-88	1	Filamentous fungus
3.3. Others						
Pp0941	EAA57250	Hypothetical protein MG08219.4	<i>M. grisea</i>	1.00E-08	2	Filamentous fungus
Pp0223	BAB13330	<i>N</i> -Acetylglucosaminidase	<i>Emericella nidulans</i>	4.00E-25	1	Filamentous fungus
Pp1112	NP_014463	Sortilin homolog	<i>S. cerevisiae</i>	1.00E-52	1	Yeast
Pp0811	NP_595238	Putative vacuolar protein; β -catenin family	<i>S. pombe</i>	2.00E-27	1	Yeast

Table 1 (continued)

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
4. Cell/organism defense						
4.1. Apoptosis						
Pp1737	I49285	Defender against death protein 1	<i>Mus musculus</i>	1.00E–26	1	Mammal
4.2. Stress response						
Pp0611	CAC20378	14-3-3-like protein	<i>Hypocrea jecorina</i>	7.00E–92	1	Filamentous fungus
Pp0528	AAK15159	Heat-induced catalase	<i>Pleurotus sajor-caju</i>	2.00E–82	4	Filamentous fungus
Pp1848	1908431A	Heat-shock protein	<i>A. thaliana</i>	1.00E–62	1	Plant
Pp1303	NP_596091	hsp16 (heat-shock protein 16)	<i>S. pombe</i>	1.00E–19	1	Yeast
Pp1121	AAN75572	Copper chaperone TahA <i>Trametes versicolor</i>	<i>Trametes versicolor</i>	3.00E–10	1	Filamentous fungus
Pp1929	CAD21425	Related to stress response protein rds1p	<i>N. crassa</i>	7.00E–34	3	Filamentous fungus
Pp2004	BAA77283	DyP (peroxidase)	<i>Galactomyces geotrichum</i>	3.00E–07	1	Filamentous fungus
Pp1616	T49477	Phenol hydroxylase related protein	<i>N. crassa</i>	2.00E–16	1	Filamentous fungus
5. Gene/protein expression						
5.1. RNA synthesis						
5.1.1. RNA polymerases						
Pp0946	P29035	Probable RNA-directed RNA polymerase (2Aprotein) (RNA replicase)	Tomato aspermy virus	3.00E–08	1	Virus
Pp2029	NP_049325	Replicase	<i>Pea early browning virus</i>	1.00E–07	1	Virus
5.1.2. RNA processing (e.g., spliceosomal, helicases)						
Pp0519	O42861	Probable helicase	<i>S. pombe</i>	4.00E–22	1	Yeast
Pp1810	S22646	Splicing factor U2AF homolog	<i>M. musculus</i>	9.00E–42	1	Mammal
Pp1327	AAF37551	RNA-binding motif protein 8	<i>Homo sapiens</i>	4.00E–25	1	Mammal
5.1.3. Transcription factors						
Pp1348	NP_010680	Transcription factor; Spt3p	<i>S. cerevisiae</i>	1.00E–25	1	Yeast
Pp1504	AAA79367	TATA-binding protein	<i>Pneumocystis carinii</i>	2.00E–95	1	Filamentous fungus
Pp0237	NP_011561	Transcription factor Tfc4p	<i>S. cerevisiae</i>	3.40E–17	1	Yeast
Pp2041	Q00659	Sulfur metabolite repression control protein	<i>E. nidulans</i>	1.00E–16	1	Filamentous fungus
5.2. Protein synthesis						
5.2.1. Post-translational modification/targeting						
Pp1724	S34655	Polyubiquitin 5	<i>P. chrysosporium</i>	9.00E–91	2	Filamentous fungus
Pp0936	T06053	Probable ubiquitin-dependent proteolytic protein	<i>A. thaliana</i>	2.00E–35	1	Plant
5.2.2. Post-translational modification/trafficking						
Pp1547	T39383	t-Complex protein 1, α -subunit	<i>S. pombe</i>	1.00E–44	1	Yeast
Pp0719	NP_596649	Putative cytochrome C oxidase copper chaperone protein	<i>S. pombe</i>	1.00E–12	2	Yeast
Pp0105	2113205A	DNA J-like protein	<i>S. pombe</i>	3.00E–19	1	Yeast
5.2.3. Protein turnover						
Pp0126	CAA09863	Putative tripeptidyl peptidase I	<i>M. musculus</i>	3.00E–06	1	Mammal
Pp1331	BAC56232	Tripeptidyl peptidase A	<i>A. oryzae</i>	3.70E–39	2	Filamentous fungus
Pp1031	CAC39600	Prolidase	<i>A. nidulans</i>	2.00E–38	1	Filamentous fungus
5.2.4. Ribosomal proteins						
Pp1147	XP_326286	40S ribosomal protein S22 (S15A) (YS24)	<i>N. crassa</i>	3.70E–74	1	Filamentous fungus
Pp1420	P05736	60S ribosomal protein L2 (YL6) (L5) (RP8)	<i>S. cerevisiae</i>	2.00E–68	1	Yeast
Pp2011	T40111	14p-like ribosomal protein	<i>S. pombe</i>	2.00E–12	1	Yeast
5.2.5. tRNA synthesis/metabolism						
Pp1213	P46655	Cytosolic glutamyl-tRNA synthetase	<i>S. cerevisiae</i>	6.00E–64	1	Yeast

(continued on next page)

Table 1 (continued)

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
5.2.6. Translation factors						
Pp2028	S43861	Translation elongation factor eEF-1 α -chain	<i>Podospora anserina</i>	1.00E–104	1	Filamentous fungus
Pp0835	NP_595367	eIF3 p48 subunit eIF3/signalosome component	<i>S. pombe</i>	2.60E–43	1	Yeast
Pp0206	T48731	Probable translation initiation factor	<i>N. crassa</i>	2.00E–100	1	Filamentous fungus
Pp0317	NP_015366	Tif5p	<i>S. pombe</i>	1.00E–34	1	Yeast
Pp1107	P32186	Elongation factor eEF-1 α -chain	<i>Puccinia graminis</i>	6.20E–82	1	Filamentous fungus
Pp2027	NP_502791	ADP-ribosylation factor-like protein (21.3 kDa) (4P563)	<i>Caenorhabditis elegans</i>	7.40E–15	2	Nematode
6. Metabolism						
6.1. Amino acid						
Pp0134	M10139	3-Dehydroshikimate dehydratase	<i>N. crassa</i>	9.00E–31	2	Filamentous fungus
Pp0425	AAN31488	DAHP synthase	<i>Phytophthora infestans</i>	1.00E–74	1	Oomycete
Pp1503	NP_289154	DAHP synthetase, tyrosine repressible	<i>Escherichia coli</i>	4.00E–40	1	Bacteria
Pp1336	NP_009808	DAHP synthase (is feedback-inhibited by tyrosine)	<i>S. cerevisiae</i>	1.00E–37	1	Yeast
Pp1502	NP_012612	Tryptophan 2,3-dioxygenase	<i>S. cerevisiae</i>	2.00E–12	1	Yeast
Pp0744	NP_592942	Phospho-2-dehydro-3-deoxyheptonate aldolase	<i>S. pombe</i>	7.70E–29	1	Yeast
Pp1343	O94225	Homocitrate synthase, mitochondrial precursor	<i>Penicillium chrysogenum</i>	3.00E–116	1	Filamentous fungus
Pp1727	T39244	Probable phospho-2-dehydro-3-deoxyheptonate aldolase	<i>S. pombe</i>	3.00E–25	4	Yeast
Pp0806	AAO27751	Monooxygenase	<i>Fusarium sporotrichioides</i>	1.00E–24	1	Filamentous fungus
6.2. Cofactor						
Pp1931	CAB85691	Riboflavin aldehyde-forming enzyme	<i>Agaricus bisporus</i>	2.00E–11	2	Filamentous fungus
6.3. Energy/TCA cycle						
Pp1004	P34728	ADP-ribosylation factor	<i>F. neoformans</i>	5.00E–108	1	Filamentous fungus
Pp2036	AAK18073	Aldehyde dehydrogenase ALDH15	<i>E. nidulans</i>	3.00E–38	1	Filamentous fungus
Pp1816	BAA09832	Isobutene-forming enzyme and benzoate 4-hydroxylase	<i>Rhodotorula minuta</i>	8.00E–37	1	Yeast
Pp0727	CAA67613	Mitochondrial carrier protein	<i>S. cerevisiae</i>	2.00E–13	1	Yeast
Pp1722	NP_035016	NADH dehydrogenase (ubiquinone) 1 α subcomplex 4	<i>M. musculus</i>	5.00E–08	2	Mammal
Pp1517	NP_594397	Putative isocitrate dehydrogenase (NADP+)	<i>S. pombe</i>	2.00E–15	1	Yeast
Pp2040	T50403	Probable succinate dehydrogenase membrane anchor subunit precursor	<i>S. pombe</i>	3.00E–24	1	Yeast
Pp1317	AAN74818	Fum1 5p	<i>Gibberella moniliformis</i>	1.30E–16	1	Filamentous fungus
Pp2033	NP_172773	Putative cytochrome P450 monooxygenase	<i>A. thaliana</i>	2.00E–16	1	Plant
Pp0837	NP_593578	Putative mitochondrial carrier	<i>S. pombe</i>	2.90E–11	1	Yeast
Pp0127	CAC81058	Mitochondrial F1 ATP synthase β -subunit	<i>A. thaliana</i>	1.30E–16	1	Plant
Pp0236	CAC81058	Mitochondrial F1 ATP synthase β -subunit	<i>A. thaliana</i>	9.90E–16	1	Plant
6.4. Lipid						
Pp1703	AAK26619	Acetyl-CoA acetyl transferase	<i>Laccaria bicolor</i>	2.00E–39	1	Filamentous fungus
Pp0546	AAK26620	Acetyl-CoA acetyltransferase	<i>L. bicolor</i>	7.00E–23	1	Filamentous fungus
Pp1511	CAB55552	Fox2 protein	<i>Glomus mosseae</i>	2.00E–28	1	Filamentous fungus
Pp0337	XP_325309	Glycerol-3-phosphate dehydrogenase precursor related protein	<i>N. crassa</i>	1.4E–92	1	Filamentous fungus

Table 1 (continued)

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
Pp0913	AAK63186	Probable acyl-CoA dehydrogenase	<i>G. intraradices</i>	1.00E–37	2	Filamentous fungus
Pp1714	AAK63186	Probable acyl-CoA dehydrogenase	<i>G. intraradices</i>	7.00E–33	3	Filamentous fungus
Pp0121	T40135	Probable involvement in ergosterol synthesis	<i>S. pombe</i>	3.00E–33	1	Yeast
Pp1910	AAQ72469	SCS7p (oxidoreductase)	<i>Pichia pastoris</i>	3.00E–09	3	Yeast
Pp0804	AAF27123	Putative glycerolkinase	<i>A. thaliana</i>	4.00E–33	2	Plant
6.5. Sugar/glycolysis						
Pp1827	AAA34858	6-Phosphofructo-2-kinase	<i>S. cerevisiae</i>	4.00E–21	2	Yeast
Pp1140	Q24319	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase	<i>Drosophila melanogaster</i>	1.00E–12	1	Insect
Pp1235	AAB22823	Fructose-2,6-biphosphatase	<i>S. cerevisiae</i>	2.70E–20	1	Yeast
Pp1735	CAC48025	Mutanase (α -1,3 glucanase)	<i>E. nidulans</i>	3.00E–23	1	Filamentous fungus
Pp1048	CAC48025	Mutanase (α -1,3 glucanase)	<i>E. nidulans</i>	2.00E–14	2	Filamentous fungus
Pp1033	XP_323561	Neutral trehalase	<i>N. crassa</i>	1.00E–83	1	Filamentous fungus
Pp0133	CAA20128	Phosphomannomutase (predicted)	<i>S. pombe</i>	6.00E–43	1	Yeast
Pp1501	ZP_00110197	COG0235: Ribulose-5-phosphate 4-epimerase, related epimerases and aldolases	<i>Nostoc punctiforme</i>	1.00E–40	1	Bacteria
Pp1306	AAC17104	Endo-1,3(4)- β -glucanase	<i>Phaffia rhodozyma</i>	1.00E–30	1	Filamentous fungus
6.6. Transport						
Pp0917	CAD21006	ABC transporter (ATP-binding cassette transporter)	<i>F. neoformans</i>	3.00E–68	1	Filamentous fungus
Pp1541	AAC08353	Calcium/proton exchanger	<i>N. crassa</i>	6.00E–10	1	Filamentous fungus
Pp1713	T40789	Clathrin light chain	<i>S. pombe</i>	1.00E–17	2	Yeast
Pp1340	CAA05841	Plasma membrane (H ⁺) ATPase	<i>U. viciae-fabae</i>	1.00E–105	1	Filamentous fungus
Pp1536	T38039	Probable nuclear transport factor 2	<i>S. pombe</i>	1.00E–26	1	Yeast
Pp1524	NP_594553	Putative membrane protein required for ER-Golgi transport	<i>S. pombe</i>	4.00E–10	1	Yeast
6.7. Nucleotide						
Pp2031	BAD00051	Ribonuclease T2	<i>A. bisporus</i>	2.00E–29	1	Filamentous fungus
Pp1845	XP_322797	Ribonucleoside-diphosphate reductase large chain	<i>N. crassa</i>	1.00E–90	1	Filamentous fungus
Pp0547	AAN73281	UPL-1	<i>Giardia intestinalis</i>	4.10E–06	1	Protozoa
6.8. Protein modification						
Pp0618	BAB56108	Carboxypeptidase	<i>Aspergillus nidulans</i>	4.00E–21	1	Filamentous fungus
Pp1631	NP_253798	Lactoylglutathione lyase	<i>Pseudomonas aeruginosa</i>	3.00E–13	1	Bacteria
Pp0643	AAA20876	Pepsinogen	<i>Aspergillus niger</i>	1.00E–77	3	Filamentous fungus
Pp1341	CAC28786	Related to UDP-acetylglucosamine-peptide <i>N</i> -glucosaminyltransferase	<i>N. crassa</i>	7.00E–44	1	Filamentous fungus
Pp1032	NP_035322	Proteasome activator subunit 3	<i>M. musculus</i>	1.00E–11	3	Mammal
Pp0747	AAG05190	ATP-dependent Clp protease proteolytic subunit	<i>P. aeruginosa</i>	5.00E–22	2	Bacteria
Pp0915	AAB19394	Aspartate aminotransferase	<i>S. cerevisiae</i>	2.00E–47	1	Yeast
6.9. Other metabolism						
Pp1221	CAD79489	Glyoxal oxidase 2	<i>Ustilago maydis</i>	8.20E–28	1	Filamentous fungus
Pp0235	CAD79489	Glyoxal oxidase 2	<i>Ustilago maydis</i>	2.00E–34	1	Filamentous fungus
Pp1324	AAF02494	Alcohol oxidase 1	<i>Pichia methanolica</i>	1.00E–23	1	Yeast
Pp0218	T46646	Pyridoxine (Vitamin B6) biosynthesis protein ppx1	<i>Cercospora nicotianae</i>	5.00E–26	1	Filamentous fungus

(continued on next page)

Table 1 (continued)

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
7. Transposon						
Pp0944	NP_921277	Transposase Tn10	<i>Oryza sativa</i>	7.00E–77	1	Plant
8. Unclassified						
Pp0116	T30954	Hypothetical protein C44E4.6	<i>C. elegans</i>	6.00E–15	1	Nematode
Pp0115	AAA65309	pB602L	African swine fever virus	1.00E–07	2	Virus
Pp1326	NP_780783	Hypothetical protein CTC00065	<i>Clostridium tetani</i>	5.10E–14	1	Bacteria
Pp0529	NP_754280	Transthyretin-like protein precursor	<i>E. coli</i>	2.00E–17	1	Bacteria
Pp0819	NP_267806	Hypothetical protein L98109	<i>Lactococcus lactis</i>	7.00E–07	1	Bacteria
Pp0308	CAB85694	Hypothetical protein	<i>A. bisporus</i>	7.60E–15	12	Filamentous fungus
Pp0103	AAK25792	Putative Egh16H1 precursor isoform A	<i>B. graminis</i> f. sp. <i>hordei</i>	7.00E–12	4	Filamentous fungus
Pp0104	JC4750	gEgh 16 protein	<i>B. graminis</i> f. sp. <i>hordei</i>	2.00E–32	36	Filamentous fungus
Pp0417	JC4750	gEgh 16 protein	<i>B. graminis</i> f. sp. <i>hordei</i>	3.00E–34	142	Filamentous fungus
Pp0730	AAK25793	Putative Egh16H1 precursor isoform B	<i>B. graminis</i> f. sp. <i>hordei</i>	5.00E–11	1	Filamentous fungus
Pp1039	JC4750	gEgh 16 protein	<i>B. graminis</i> f. sp. <i>hordei</i>	3.00E–26	1	Filamentous fungus
Pp1043	JC4750	gEgh 16 protein	<i>B. graminis</i> f. sp. <i>hordei</i>	3.00E–32	1	Filamentous fungus
Pp0326	CAD10781	Pentahydrophobin	<i>Claviceps purpurea</i>	7.90E–06	1	Filamentous fungus
Pp0927	NP_758766	Hypothetical protein	<i>Erwinia amylovora</i>	2.00E–24	1	Filamentous fungus
Pp1044	AAK52794	MAS3 protein	<i>M. grisea</i>	9.00E–09	1	Filamentous fungus
Pp1429	AF264035	MAS1 protein	<i>M. grisea</i>	4.00E–20	1	Filamentous fungus
Pp1610	AAK52794	MAS3 protein	<i>M. grisea</i>	5.00E–05	1	Filamentous fungus
Pp0119	EAA55479	Hypothetical protein MG09286.4	<i>M. grisea</i>	1.10E–10	1	Filamentous fungus
Pp0222	EAA49745	Hypothetical protein MG09736.4	<i>M. grisea</i>	4.00E–06	1	Filamentous fungus
Pp0612	EAA48468	Hypothetical protein MG00126.4	<i>M. grisea</i>	3.30E–16	1	Filamentous fungus
Pp1045	EAA53245	Hypothetical protein MG07522.4	<i>M. grisea</i>	1.40E–31	1	Filamentous fungus
Pp2038	EAA51058	Hypothetical protein MG04818.4	<i>M. grisea</i>	2.00E–25	2	Filamentous fungus
Pp0225	XP_330149	Hypothetical protein	<i>N. crassa</i>	3.40E–20	1	Filamentous fungus
Pp0534	XP_328580	Hypothetical protein	<i>N. crassa</i>	1.30E–07	1	Filamentous fungus
Pp0704	XP_322643	Predicted protein	<i>N. crassa</i>	1.10E–11	1	Filamentous fungus
Pp0809	XP_328793	Hypothetical protein	<i>N. crassa</i>	5.20E–10	1	Filamentous fungus
Pp0829	XP_328520	Hypothetical protein	<i>N. crassa</i>	9.60E–09	1	Filamentous fungus
Pp0925	XP_331047	Hypothetical protein	<i>N. crassa</i>	1.00E–35	1	Filamentous fungus
Pp1006	XP_322643	Predicted protein	<i>N. crassa</i>	1.00E–12	1	Filamentous fungus
Pp1013	XP_324202	Predicted protein	<i>N. crassa</i>	2.60E–09	2	Filamentous fungus
Pp1027	XP_327468	Hypothetical protein	<i>N. crassa</i>	2.00E–59	1	Filamentous fungus
Pp1325	XP_326398	Hypothetical protein	<i>N. crassa</i>	2.70E–29	1	Filamentous fungus
Pp1411	XP_328221	Hypothetical protein	<i>N. crassa</i>	1.70E–06	1	Filamentous fungus
Pp1615	XP_324693	Hypothetical protein	<i>N. crassa</i>	3.00E–30	1	Filamentous fungus
Pp1835	XP_327028	Hypothetical protein	<i>N. crassa</i>	5.00E–11	1	Filamentous fungus
Pp1925	CAD21504	conserved hypothetical protein	<i>N. crassa</i>	5.00E–09	1	Filamentous fungus
Pp2010	XP_324370	Predicted protein	<i>N. crassa</i>	1.00E–16	1	Filamentous fungus
Pp0334	NP_054890	Post-synaptic protein CRIPT; HSPC139 protein	<i>H. sapiens</i>	8.80E–16	1	Mammal
Pp0748	BAA91611	Unnamed protein product	<i>H. sapiens</i>	9.00E–09	1	Mammal
Pp0346	NP_001009405	PTPL1-associated RhoGAP 1	<i>Rattus norvegicus</i>	1.70E–06	1	Mammal
Pp1446	AAP78751	Ac1147	<i>R. norvegicus</i>	7.10E–34	1	Mammal
Pp1514	Q94480	VEG136 protein	<i>Dictyostelium discoideum</i>	2.00E–31	1	Mycetozoon
Pp0516	T23541	Hypothetical protein K09C8.4	<i>C. elegans</i>	1.00E–05	1	Nematode
Pp0826	NP_917657	P0410E01.14 (hypothetical protein)	<i>O. sativa</i>	7.00E–14	1	Plant
Pp1134	AAB49498	183 kDa protein	<i>Odontoglossum ringspot virus</i>	3.00E–05	1	Virus
Pp1439	XP_332134	Hypothetical protein	<i>N. crassa</i>	1.00E–39	1	Filamentous fungus
Pp0444	T37512	Hypothetical protein SPAC11D3.01c	<i>S. pombe</i>	6.00E–14	1	Yeast

Table 1 (continued)

Clone	Accession No.	Description	Species	E_{value}	No. of clones	Organism
Pp0522	T38996	Hypothetical protein SPAC637.04	<i>S. pombe</i>	2.00E–07	1	Yeast
Pp0924	NP_596150	Hypothetical zinc finger protein	<i>S. pombe</i>	2.50E–06	1	Yeast
Pp0931	NP_595085	Hypothetical glycine-rich protein	<i>S. pombe</i>	6.80E–06	1	Yeast
Pp1019	NP_595449	Conserved hypothetical protein	<i>S. pombe</i>	8.00E–21	1	Yeast
Pp1106	NP_595642	Hypothetical protein	<i>S. pombe</i>	1.00E–07	2	Yeast
Pp1238	T41411	Hypothetical protein SPCC576.01c	<i>S. pombe</i>	1.20E–06	1	Yeast
Pp1704	EAA50939	Hypothetical protein MG04698.4	<i>M. grisea</i>	1.00E–16	1	Filamentous fungus
Pp0406	EAA55557	Hypothetical protein MG01208.4	<i>M. grisea</i>	2.50E–44	1	Filamentous fungus
Pp1626	EAA49354	Hypothetical protein MG01012.4	<i>M. grisea</i>	3.00E–41	1	Filamentous fungus

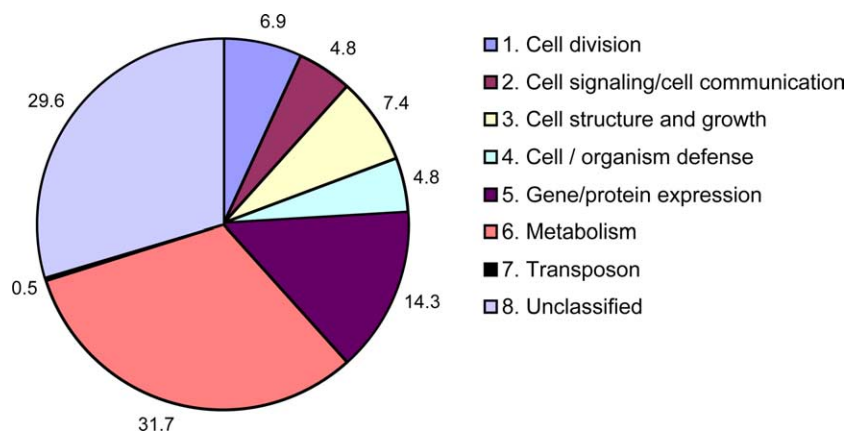


Fig. 2. Classification of the 189 unique *P. pachyrhizi* ESTs from the germinating urediniospore library. The ESTs with significant matches (BLASTX $E_{\text{value}} < E-5$) to the non-redundant database were classified into functional Expressed Gene Anatomy Database categories as described in Table 1. The percentage of ESTs in each of the eight categories is shown.

most abundant ESTs in the *P. pachyrhizi* EST library (Table 1).

3.2. Gene families

Among the 908 ESTs analyzed, 18 potential gene families were identified by sequence similarity. Predicted function of these gene families could be ascribed to four groups, whereas 14 of the putative gene families did not show any significant similarity to entries in the databases. Eleven families contained two members, and four of them had three members. The remaining three putative gene families consisted of four, five, and nine members, respectively. The latter gene family contained four distinct homologs of gEgh16, one for the putative gEgh16 precursor isoform A, and one for the putative gEgh16 precursor isoform B from *B. graminis* f. sp. *hordei*. In addition, this group had one homolog for MAS1 and two homologs for MAS3 from *Magnaporthe grisea*. Three gene families showed similarity to homologs for DAHP

synthase, conidiation protein 6 from *Neurospora crassa*, and the non-histone chromosomal proteins from *Saccharomyces cerevisiae*.

4. Discussion

Within the past decade, EST analyses have been conducted for several filamentous fungi and oomycetes such as: *Agaricus bisporus* (Ospina-Giraldo et al., 2000), *Aspergillus flavus* and *Aspergillus parasiticus* (O'Brien et al., 2003), *Aspergillus nidulans* (Sims et al., 2004), *B. graminis* (Thomas et al., 2001), *Cryphonectria parasitica* (Dawe et al., 2003), *Fusarium graminearum* (Trail et al., 2003), *Heterobasidium annosum* (Abu et al., 2004; Karlsson et al., 2003), *M. grisea* (Ebbole et al., 2004; Kim et al., 2001), *Mycosphaerella graminicola* (Keon et al., 2000), *N. crassa* (Nelson et al., 1997; Zhu et al., 2001), *Pleurotus ostreatus* (Lee et al., 2002), *Schizophyllum commune* (Guettler et al., 2003), *Sclerotinia sclerotiorum* (Li et al., 2004), *Trichoderma reesi* (Diener et al., 2004; Steen et al., 2003), *Ustilago*

Table 2

EST clones displaying similarity (BLASTN, $E_{\text{value}} < 1E-15$) to entries in the NCBI EST database

Clone	Accession No.	Description	E value	Organism
Pp0116	BI191959	l3h06fs.r1 <i>Fusarium sporotrichioides</i> Tri 10 overexpressed cDNA library <i>F. sporotrichioides</i> cDNA clone l3h06fs 5', mRNA sequence	1.10E-24	Filamentous fungus
Pp0206	AU011975	AU011975 <i>S. pombe</i> late log phase cDNA <i>S. pombe</i> cDNA clone spc06169, mRNA sequence	4.90E-16	Yeast
Pp0406	BU060702	Fgr-C_1_H20_T3 Carbon-starved mycelia <i>G. zeae</i> cDNA, mRNA sequence	6.00E-35	Filamentous fungus
Pp0437	CB12207	Lb12C03 mycelium of <i>L. bicolor</i> grown for 3 weeks <i>L. bicolor</i> cDNA 5', mRNA sequence	7.00E-32	Filamentous fungus
Pp1004	CF883485	Tric088xm20.b1 <i>Trichoderma reesei</i> mycelial culture, Version 6 October 2003	1.00E-51	Filamentous fungus
		<i>H. jecorina</i> cDNA clone tric088xm20, mRNA sequence.		
Pp1027	BU060160	Fgr-C_0_M05_T7 Carbon-starved mycelia <i>G. zeae</i> cDNA, mRNA sequence	0	Filamentous fungus
Pp1147	BG279541	b3h06np.r1 <i>N. crassa</i> sexual cDNA library, Uni-zap vector system <i>N. crassa</i> cDNA clone b3h06np 5', mRNA sequence	1.00E-152	Filamentous fungus
Pp1318	CF190146	k7i06j2.r1 <i>C. neoformans</i> strain B3501 <i>C. neoformans</i> var. <i>neoformans</i> cDNA clone k7i06j2 5', MRNA sequence	1.30E-21	Filamentous fungus
Pp1326	CF847171	psHB042xA02f USDA-IFAFS: expression of <i>P. sojae</i> genes during infection and propagation_sHB <i>P. sojae</i> cDNA clone sHB042A02 5', mRNA sequence	3.00E-121	Oomycete
Pp1420	BQ110457	VD0108A10 VD01 <i>Verticillium dahliae</i> cDNA, mRNA sequence	1.00E-114	Filamentous fungus
Pp1432	AW324553	Basidiome and primordium cDNA libraries <i>A. bisporus</i> cDNA 5' similar to β -tubulin, mRNA sequence	2.50E-46	Filamentous fungus
Pp1446	BU038322	LIT000228 root-induced cDNA library from <i>L. bicolor</i> <i>L. bicolor</i> cDNA, MRNA sequence	1.00E-124	Filamentous fungus
Pp1504	CF641217	D37_B10 Filamentous Forced Diploid <i>Ustilago maydis</i> cDNA 3', mRNA sequence	5.00E-42	Filamentous fungus
Pp1547	CB898049	tric013xf01 <i>Trichoderma reesei</i> mycelial culture, Version 3 April <i>H. jecorina</i> cDNA clone tric013xf01, mRNA sequence	2.30E-51	Filamentous fungus
Pp1709	CF639134	D11_G01 Filamentous Forced Diploid <i>U. maydis</i> cDNA 3', mRNA sequence	4.00E-27	Filamentous fungus
Pp1744	AI211414	p0b02a1.r1 <i>A. nidulans</i> 24 h asexual developmental and vegetative cDNA lambda zap library <i>E. nidulans</i> cDNA clone p0b02a1 5', mRNA sequence	3.20E-28	Filamentous fungus
Pp1811	AW333990	S29A2 AGS-1 <i>P. carinii</i> cDNA 3', mRNA sequence	4.80E-68	Filamentous fungus
Pp1843	CF644300	K19_B10 Filamentous Forced Diploid <i>U. maydis</i> cDNA 3', mRNA sequence	3.00E-116	Filamentous fungus
Pp0127	BQ800593	EST 7628 Veraison Grape berries SuperScript Plasmid Library <i>Vitis vinifera</i> cDNA clone PT011A12 3', mRNA sequence	6.90E-54	Plant
Pp0207	BQ464782	HU01102T HU <i>Hordeum vulgare</i> subsp. <i>vulgare</i> cDNA clone HU01102 5-PRIME, mRNA sequence	0	Plant
Pp0236	BQ907430	P006B08 <i>Oryza sativa</i> mature leaf library induced by <i>M. grisea</i> <i>O. sativa</i> cDNA clone P006B08, mRNA sequence	1.30E-53	Plant
Pp0404	CB643819	OSJNEb04L15.r OSJNEb <i>O. sativa</i> (japonica cultivar-group) cDNA clone OSJNEb04L15 3', mRNA sequence	0	Plant
Pp0611	CA522045	KS11039D12 KS11 <i>Capsicum annuum</i> cDNA, mRNA sequence	1.10E-32	Plant
Pp0630	CD879728	AZO4.106C24F011012 AZO4 <i>Triticum aestivum</i> cDNA clone AZO4106C24, mRNA sequence	1.40E-15	Plant
Pp0713	CD879049	AZO4.104E06F010929 AZO4 <i>T. aestivum</i> cDNA clone AZO4104E06, mRNA sequence	2.10E-17	Plant
Pp0729	BI123652	I026P65P <i>Populus</i> leaf cDNA library <i>Populus tremula</i> x <i>Populus tremuloides</i> cDNA, mRNA sequence	7.50E-39	Plant
Pp0910	BQ908773	T015B01 <i>Oryza sativa</i> mature leaf library induced by <i>M. grisea</i> <i>O. sativa</i> cDNA clone T015B01, MRNA sequence	2.80E-45	Plant
Pp1219	CD878534	AZO4.102P17F011002 AZO4 <i>T. aestivum</i> cDNA clone AZO4102P17, mRNA sequence	3.00E-17	Plant
Pp1724	CA126740	SCVPLR1006B09.g LR1 <i>Saccharum officinarum</i> cDNA clone SCVPLR1006B09 5', mRNA sequence	1.00E-80	Plant
Pp1729	CA253801	SCRLFL4105G02.g FL4 <i>S. officinarum</i> cDNA clone SCRLFL4105G02 5', mRNA sequence	2.80E-91	Plant
Pp1848	CF811551	NA760 cDNA non-acclimated Bluecrop library <i>Vaccinium corymbosum</i> cDNA 5', mRNA sequence	7.20E-24	Plant
Pp1924	BU672690	TR51 Leaf rust-infected wheat <i>T. aestivum</i> /P. <i>tritricina</i> mixed EST library cDNA clone TR51, mRNA sequence	8.00E-35	Plant

maydis (Austin et al., 2004; Nugent et al., 2004), *Verticillium dahliae* (Neuman and Dobinson, 2003), and *Phytophthora infestans* (Kamoun et al., 1999; Qutob et al., 2000; Randall et al., 2005). In addition to the fungal ESTs available in the dbEST database at the NCBI, another EST database exists with sequences from 14 different phytopathogenic fungi and oomycetes (Soanes

et al., 2002). In this study, we investigate the molecular genetics in the obligate soybean rust pathogen *P. pachyrhizi*. A total of 908 randomly chosen EST clones were sequenced and analyzed to identify which genes are expressed in germinating urediniospores. A relatively low level of redundancy was found among the *P. pachyrhizi* EST clones, similar to what has been

observed in EST analyses from other filamentous fungi (Keon et al., 2000; Lee et al., 2002; Ospina-Giraldo et al., 2000; Thomas et al., 2001; Trail et al., 2003). More than 52% of the EST clones showed no significant similarity to the entries in the public protein databases, which highlights the paucity in our knowledge of gene expression in filamentous fungi. The 432 *P. pachyrhizi* sequences that showed significant matches to sequences in the databases were classified into eight functional categories following the EGAD. Although proteins with unknown function or hypothetical proteins were the most prevalent, proteins involved in metabolism and in protein and gene expression were highly represented (Table 1).

Among the 908 cDNA clones, 488 unique ESTs were identified. These unigenes represent approximately 4–5% of the total 8000–12,000 expressed genes that are estimated in filamentous fungi (Kupfer et al., 1997; Martinez et al., 2004). The remaining 420 sequences correspond to redundant cDNAs that form clusters ranging from 2 to 142 ESTs. Some *P. pachyrhizi* genes appear to be highly expressed during urediniospore germination, especially the two EST clones Pp0104 and Pp 0417, which share similarity to gEgh16 from *B. graminis* and appeared 36 and 142 times, respectively, among the clones sequenced in the library (Table 1). The function of gEgh16 is unknown (Justesen et al., 1996).

When the *P. pachyrhizi* ESTs were queried against the dbEST at NCBI, only 18 ESTs showed significant similarity ($E_{\text{value}} \leq 10^{-15}$) to fungal or yeast entries, while 14 ESTs showed significant similarity to plant entries (Table 2). The low number of *P. pachyrhizi* ESTs with similarity to other fungi is due to the lack of gene expression studies that have been conducted in fungi. Two *P. pachyrhizi* EST clones, Pp1147 and Pp1420, have significant similarity to ribosomal proteins from *N. crassa* and *S. cerevisiae*, respectively, and these two ESTs also have high similarity to ESTs from other filamentous fungi. The EST Pp1027 shows significant similarity to a hypothetical protein from *N. crassa* and *A. nidulans* ($E_{\text{value}} < 10^{-30}$) and 93% identity ($E_{\text{value}} = 0$) to an EST from *Gibberella zeae*, which suggests that it is a conserved gene.

Spore germination is an essential developmental stage in the life cycle of all filamentous fungi. It is a highly regulated process that responds to environmental stimuli via signaling cascades that are amenable to genetic and biochemical inquiry (Oshero and May, 2000, 2001). Three important steps can be distinguished during spore germination. First, the dormancy is broken in response to appropriate environmental conditions. Second, isotropic growth occurs, involving water uptake and the resumption of numerous metabolic activities. Third, polarized growth takes place and a germ tube is formed from which new mycelium originates (d'Enfert, 1997). Unlike most filamentous fungi in which low-molecular

mass nutrients such as sugars, amino acids, and inorganic salts are required for conidial germination (Oshero and May, 2001), *P. pachyrhizi* urediniospores are capable of germinating on the surface of water. For some fungi, contact with a solid surface is required for conidial germination (Thomas et al., 2001). It is interesting to note that two ESTs identified in this analysis, Pp1527 and Pp0839, share very high similarity to Ca^{2+} /calmodulin-dependent protein kinase and calmodulin kinase I, respectively. The expression of calmodulin is induced by contact with a hard surface in both *Colletotrichum gloeosporioides* and *M. grisea* (Kim et al., 1998, 2000; Liu and Kolattukudy, 1999). The expression of these calmodulin kinase homologs suggests that a similar calcium-signaling pathway may regulate urediniospore germination in *P. pachyrhizi*.

In fungi, the cell wall undergoes significant modification during spore germination. Three *P. pachyrhizi* ESTs showed similarity to enzymes involved in the dissolution and formation of the cell wall. EST clones Pp0122, Pp0922, and Pp1605 share similarity to chitin deacetylase, acetylxyloxyesterase, and chitin synthase (*csml*), respectively, from *M. grisea*. The *csml* gene product contains a myosin motor-like domain (Park et al., 1999). In *A. nidulans*, its homolog CsmA has an important role in polarized cell wall synthesis and maintenance of cell wall integrity, and the myosin motor-like domain has been shown to be required for these functions (Horiuchi et al., 1999).

DNA and RNA synthesis do not appear to be necessary during the early stages of spore germination, whereas protein synthesis is required (Oshero and May, 2001). They suggest that dormant conidia contain a pre-existing pool of mRNA and ribosomes that are primed for rapid activation and translation in the presence of nutrients. Our results indicate that increased protein synthesis activity occurs during spore germination in *P. pachyrhizi*. Three different homologs for translation initiation factors and two homologs for elongation factors were identified, as well as several genes involved in post-translational modification, protein modification, and metabolism of amino acids (Table 1). In their model of spore germination, DNA and RNA synthesis are required in the later stages of spore germination for hyphal development (Oshero and May, 2001). As the germination of the *P. pachyrhizi* urediniospores was asynchronous in our experiment, sequences similar to genes involved in both the early and later spore germination processes were found among the *P. pachyrhizi* ESTs.

Several putative gene families were identified among the ESTs analyzed in this study. The main group consists of nine different ESTs: four ESTs, Pp0104, Pp0417, Pp1033, and Pp1039, are homologs of *gEgh16*; Pp0103 is a homolog of the putative *gEgh16* precursor isoform A; Pp0730 is a homolog of the putative *gEgh16* precursor

isoform B from *B. graminis* f. sp. *hordei*; Pp1429 is a homolog for MAS1; and two ESTs, Pp1044 and Pp1610, are homologs for MAS3 from *M. grisea*. The EST clones Pp0104 and Pp0417, which are similar to *gEgh16*, are highly redundant in the *P. pachyrhizi* library suggesting that they are highly expressed during urediniospore germination. The function of the *gEgh16* protein has not been determined in *B. graminis* f. sp. *hordei*, but it is highly expressed during germ tube formation and hyphal growth. There is evidence that *gEgh16* is a member of a gene family in *B. graminis* f. sp. *hordei* (Justesen et al., 1996). Although the function of MAS1 and MAS3 are unknown in *M. grisea*, the genes encoding for these proteins are expressed during appressorium formation (Choi and Dean, 2000).

Another potential gene family in *P. pachyrhizi* is comprised of five ESTs similar to DAHP synthase (Pp0323, Pp0425, Pp0744, Pp1336, and Pp1503). DAHP synthase catalyzes the first step in the shikimate pathway that leads to the biosynthesis of aromatic amino acids. In *N. crassa* and *Escherichia coli*, three isozymes of DAHP synthase have been characterized and each one is regulated by the three aromatic amino acids. In *A. nidulans* and *S. cerevisiae*, two DAHP synthase encoding genes have been described, and the enzymes are differentially regulated by tyrosine and phenylalanine (Hartmann et al., 2001; Künzler et al., 1992). In addition to the DAHP synthase homologs, a *P. pachyrhizi* EST clone (Pp0134) was found to share similarity to dehydroshikimate dehydrogenase, which is also part of the shikimate pathway. It has been shown that quinate and shikimate, two metabolic intermediates of the shikimate pathway, can be metabolized by a variety of fungi as alternative carbon sources (Keller and Hohn, 1997).

Two ESTs (Pp1628 and Pp1812) share similarity to the non-histone chromosomal proteins NHP6A and NHP6B, respectively, from *S. cerevisiae*. NHP6A and NHP6B are high mobility group proteins, which are members of a family of heterogeneous chromatin-associated DNA-binding proteins in eukaryotic cells (Masse et al., 2002; Yen et al., 1998). NHP6A is a member of the subclass HMG1/2 proteins that contain the HMG DNA-binding domain and are present at approximately 1 molecule per 2–3 nucleosomes (Kuehl et al., 1984). These proteins have been implicated in chromatin remodeling, DNA replication, transcription, and recombination (Giavara et al., 2005), and it will be interesting to determine their role in *P. pachyrhizi* ediniopore germination.

In this study, approximately 39% of the unique ESTs appeared to be related to previously characterized genes. This highlights the scarcity of genomic information available from pathogenic fungi. The EST projects have been shown to be a valid and fast way to gain information on components that regulate vital processes in path-

ogenic fungi and the interaction with their hosts. In 2002, a *Phakopsora* genome sequencing project, funded by the U.S. Department of Agriculture-Agricultural Research Service and the Department of Energy (DOE), was initiated at the DOE-Joint Genome Institute to generate draft quality sequence of *P. pachyrhizi* and *P. meibomia*. The ESTs identified in this study, along with the analyses of the cDNA libraries from *P. pachyrhizi* infected soybeans, will aid in the annotation of genes from the *Phakopsora* genome project. These data will facilitate our understanding of the biology and the evolution of obligate fungal pathogens and will also advance our efforts to develop effective means for soybean rust control.

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References

- Abu, S.M., Li, G., Asiegbu, F.O., 2004. Identification of *Heterobasidion annosum* (S-type) genes expressed during initial stages of conidiospore germination and under varying culture conditions. FEMS Microbiol. Lett. 233 (2), 205–213.
- Akinsanmi, O.A., Ladipo, J.L., 2001. First report of soybean rust (*Phakopsora pachyrhizi*) in Nigeria. Plant Dis. 85, 97.
- Altschul, S.F., Madden, T.L., Schaffer, A.A., Jinghui Zhang, Zheng Zhang, Webb Miller, Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
- Asian Vegetable Research and Development Center, 1987. Bibliography of soybean rust, 1985–1986. AVRDC Asian Veg. Res. Dev. Cent. Library Bibliography Series 4. Publication No. 87-277.
- Asian Vegetable Research and Development Center, 1992. Annotated bibliography of soybean rust (*Phakopsora pachyrhizi* Sydow). AVRDC (Asian Veg. Res. Dev. Cent.) Library Bibliography Series 4-1. Publication No. 92-372.
- Austin, R., Provart, N.J., Sacadura, N.T., Nugent, K.G., Babu, M., Saville, B.J., 2004. A comparative genomic analysis of ESTs from *Ustilago maydis*. Funct. Integr. Genomics 4 (4), 201–218.
- Bonde, M.R., Melching, J.S., Bromfield, K.R., 1976. Histology of the suspect-pathogen relationship between *Glycine max* and *Phakopsora pachyrhizi*, the cause of soybean rust. Phytopathology 66, 1290–1294.
- Bromfield, K.R., 1984. Soybean rust, Monograph (American Phytopathological Society), No. 11. American Phytopathological Society, St. Paul, MN.
- Bromfield, K.R., Hartwig, E.E., 1980. Resistance to soybean rust (*Phakopsora pachyrhizi*) and mode of inheritance. Crop Sci. 20, 254–255.
- Cherry, E., Peet, C.E., 1966. An efficient device for the rapid collection of fungal spores from infected plants. Phytopathology 56, 1102–1103.

- Choi, W., Dean, R.A., 2000. Sequence analysis and characterization of genes expressed during appressorium formation in rice blast fungus, *Magnaporthe grisea*. Unpublished. MAS1 Accession No. AF264035.1. MAS3 Accession No. AF3630651.
- Dawe, A.L., McMains, V.C., Panglao, M., Kasahara, S., Chen, B., Nuss, D.L., 2003. An ordered collection of expressed sequences from *Cryphonectria parasitica* and evidence of genomic microsynteny with *Neurospora crassa* and *Magnaporthe grisea*. *Microbiology* 149 (9), 2373–2384.
- Diener, S.E., Dunn-Coleman, N., Foreman, P., Houfek, T.D., Teunissen, P.J., van Solingen, P., Dankmeyer, L., Mitchell, T.K., Ward, M., Dean, R.A., 2004. Characterization of the protein processing and secretion pathways in a comprehensive set of expressed sequence tags from *Trichoderma reesi*. *FEMS Microbiol. Lett.* 230 (2), 275–282.
- d'Enfert, C., 1997. Fungal spore germination: insights from the molecular genetics of *Aspergillus nidulans* and *Neurospora crassa*. *Fungal Genet. Biol.* 21 (2), 163–172.
- Ebbole, D.J., Jin, Y., Thon, M., Pan, H., Bhattarai, E., Thomas, T., Dean, R., 2004. Gene discovery and gene expression in the rice blast fungus, *Magnaporthe grisea*: analysis of expressed sequence tags. *Mol. Plant Microbe Interact.* 17 (12), 1337–1347.
- Frederick, R.D., Snyder, C.L., Peterson, G.L., Bonde, M.R., 2002. Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomia*. *Phytopathology* 92, 217–227.
- Giavara, S., Kosmidou, E., Prakash Hanfe, M., Bianchi, M.E., Morgan, A., d'Adda di Fagnana, F., Jackson, S.P., 2005. Yeast Nhp6A/B and mammalian Mmgb1 facilitate the maintenance of genome stability. *Curr. Biol.* 15 (1), 68–72.
- Guettler, S., Jackson, E.N., Lucchese, S.A., Honaas, L., Green, A., Hittinger, C.T., Tian, Y., Lilly, W.W., Gathman, A.C., 2003. ESTs from the basidiomycete *Schizophyllum commune* grown on nitrogen-replete and nitrogen-limited media. *Fungal Genet. Biol.* 39 (2), 191–198.
- Hartmann, M., Heinrich, G., Braus, G.H., 2001. Regulative fine-tuning of the two novel DAHP isoenzymes aroFp and aroGp of the filamentous fungus *Aspergillus nidulans*. *Arch. Microbiol.* 175 (2), 112–121.
- Hartwig, E.E., 1986. Identification of a fourth major gene conferring resistance to soybean rust. *Crop Sci.* 26, 1135–1136.
- Hartwig, E.E., Bromfield, K.R., 1983. Relationships among three genes conferring specific resistance to rust in soybeans. *Crop Sci.* 23, 237–239.
- Horiuchi, H., Fujiwara, M., Yamashita, S., Ohta, A., Takagi, M., 1999. Proliferation of intrahyphal hyphae caused by disruption of *esmA*, which encodes a class V chitin synthase with a myosin motor-like domain in *Aspergillus nidulans*. *J. Bacteriol.* 181 (12), 3721–3729.
- Justesen, A., Somerville, S., Christiansen, S., Giese, H., 1996. Isolation and characterization of two novel genes expressed in germinating conidia of the obligate biotroph *Erysiphe graminis* f. sp. *hordei*. *Gene* 170 (1), 131–135.
- Kamoun, S., Hraber, P., Sobral, B., Nuss, D., Govers, F., 1999. Initial assessment of gene diversity for the oomycete pathogen *Phytophthora infestans* based on expressed sequences. *Fungal Genet. Biol.* 28 (2), 94–106.
- Karlsson, M., Olson, A., Stenlid, J., 2003. Expressed sequences from the basidiomycetous tree pathogen *Heterobasidion annosum* during early infection of scots pine. *Fungal Genet. Biol.* 39 (1), 51–59.
- Keller, N.P., Hohn, T.M., 1997. Metabolic pathway gene clusters in filamentous fungi. *Fungal Genet. Biol.* 21 (2), 17–29.
- Keon, J., Bailey, A., Hargreaves, J.A., 2000. A group of expressed cDNA sequences from the wheat fungal leaf blotch pathogen, *Mycosphaerella graminicola* (*Septoria tritici*). *Fungal Genet. Biol.* 29 (2), 118–133.
- Killgore, E., Heu, R., 1994. First report of soybean rust in Hawaii. *Plant Dis.* 78, 1216.
- Kim, Y.K., Li, D., Kolattukudy, P.E., 1998. Induction of Ca²⁺-calmodulin signaling by hard-surface contact primes *Colletotrichum gloeosporioides* conidia to germinate and form appressoria. *J. Bacteriol.* 180 (19), 5144–5150.
- Kim, Y.K., Liu, Z.M., Li, D., Kolattukudy, P.E., 2000. Two novel genes induced by hard-surface contact of *Colletotrichum gloeosporioides* conidia. *J. Bacteriol.* 182 (17), 4688–4695.
- Kim, S., Ahn, I.-P., Lee, Y.-H., 2001. Analysis of genes expressed during rice–*Magnaporthe grisea* interactions. *Mol. Plant Microbe Interact.* 14 (11), 1340–1346.
- Kuehl, L., Salmond, B., Tran, L., 1984. Concentrations of high-mobility-group proteins in the nucleus and cytoplasm of several rat tissues. *J. Cell Biol.* 99 (2), 648–654.
- Künzler, M., Paravicini, G., Egli, C.M., Irniger, S., Braus, G.H., 1992. Cloning, primary structure and regulation of the ARO4 gene, encoding the tyrosine-inhibited 3-deoxy-L-arabino-heptulosonate-7-phosphate synthase from *Saccharomyces cerevisiae*. *Gene* 113 (1), 67–74.
- Kupfer, D.M., Reece, C.A., Clifton, S.W., Prade, R.A., 1997. Multicellular ascomycetous fungal genomes contain more than 8000 genes. *Fungal Genet. Biol.* 21 (3), 364–372.
- Lee, S.H., Kim, B.G., Kim, K.J., Lee, J.S., Yun, D.W., Hahn, J.H., Kim, G.H., Lee, K.H., Suh, D.S., Kwon, S.T., Lee, C.S., Yoo, Y.B., 2002. Comparative analysis of sequences expressed during the liquid-cultured mycelia and fruit body stages of *Pleurotus ostreatus*. *Fungal Genet. Biol.* 35 (2), 115–134.
- Levy, C., 2003. Measures to control soybean rust in Southern Africa and an initial investigation of the meteorological factors that favor its development. *Phytopathology* 93, S103.
- Li, R., Rimmer, R., Buchwaldt, L., Sharpe, A.G., Squin-Swartz, G., Coutu, C., Hegedus, D.D., 2004. Interaction of *Sclerotinia sclerotiorum* with a resistant *Brassica napus* cultivar: expressed sequence tag analysis identifies genes associated with fungal pathogenesis. *Fungal Genet. Biol.* 41 (8), 735–753.
- Liu, Z.M., Kolattukudy, P.E., 1999. Early expression of the calmodulin gene, which precedes appressorium formation in *Magnaporthe grisea*, is inhibited by self-inhibitors and requires surface attachment. *J. Bacteriol.* 181 (11), 3571–3577.
- Martinez, D., Larrondo, L.F., Putnam, N., Gelpke, M.D., Huang, K., Chapman, J., Helfenbein, K.G., Ramaiya, P., Detter, J.C., Larimer, F., Coutinho, P.M., Henrissat, B., Berka, R., Cullen, D., Rokhsar, D., 2004. Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nat. Biotechnol.* 22 (6), 695–700.
- Masse, J.E., Wong, B., Yen, Y.M., Allain, F.H., Johnson, R.C., Feigon, J., 2002. The *Saccharomyces cerevisiae* architectural HMGB protein NHP6A complexed with DNA: DNA and protein conformational changes upon binding. *J. Mol. Biol.* 323 (2), 263–284.
- McLean, R.J., Byth, D., 1980. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybean. *Aust. J. Agric. Res.* 31, 951–956.
- Melching, J.S., Bromfield, K.R., Kingsolver, C.H., 1983. The plant pathogen containment facility at Frederick, Maryland. *Plant Dis.* 67, 717–722.
- Morel, W.P., 2001. Roya de la soja. Ministerio de Agricultura y Ganadería, Subsecretaría de Agricultura, Dirección de Investigación Agrícola, Centro Regional de Investigación Agrícola-CRIA, Capitan Miranda, Itapua, Paraguay. Comunicado Técnico-Report Oficial, Serie Fitopatología, 1, Junio de 2001.
- Nelson, M.A., Kang, S., Braun, E.L., Crawford, M.E., Dolan, P.L., Leonard, P.M., Mitchell, J., Armijo, A.M., Bean, L., Blueyes, E., Cushing, T., Errett, A., Fleharty, M., Gorman, M., Judson, K., Miller, R., Ortega, J., Pavlova, I., Perea, J., Todisco, S., Trujillo, R., Valentine, J., Wells, A., Werner-Washburne, M., Yazzie, S., Natvig, D.O., 1997. Expressed sequences from conidial, mycelial, and sexual stages of *Neurospora crassa*. *Fungal Genet. Biol.* 21 (3), 348–363.
- Neuman, M.J., Dobinson, K.F., 2003. Sequence tag analysis of gene expression during pathogenic growth and microsclerotia development

- in the vascular wilt pathogen *Verticillium dahliae*. Fungal Genet. Biol. 38 (1), 54–62.
- Nugent, K.G., Choffe, K., Saville, B.J., 2004. Gene expression during *Ustilago maydis* filamentous growth: EST library creation and analysis. Fungal Genet. Biol. 41 (3), 349–360.
- O'Brien, G.R., Fakhoury, A.M., Payne, G.A., 2003. Identification of genes differentially expressed during aflatoxin biosynthesis in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet. Biol. 39 (2), 118–127.
- Ono, Y., Buritica, P., Hennen, J.F., 1992. Delimitation of *Phakopsora*, *Phytopella*, and *Cerotelium* and their species on leguminosae. Mycol. Res. 96, 825–850.
- Oshero, N., May, G.S., 2000. Conidial germination in *Aspergillus nidulans* requires RAS signaling and protein synthesis. Genetics 155 (2), 647–656.
- Oshero, N., May, G.S., 2001. The molecular mechanisms of conidial germination. FEMS Microbiol. Lett. 199 (2), 153–160.
- Ospina-Giraldo, M.D., Collopy, P.D., Romaine, C.P., Royse, D.J., 2000. Classification of sequences expressed during the primordial and basidiome stages of the cultivated mushroom *Agaricus bisporus*. Fungal Genet. Biol. 29 (2), 81–94.
- Park, I.C., Horiuchi, H., Hwang, C.W., Yeh, W.H., Ohta, A., Ryu, J.C., Takagi, M., 1999. Isolation of *csml* encoding a class V chitin synthase with a myosin motor-like domain from the rice blast fungus, *Pyricularia oryzae*. FEMS Microbiol. Lett. 170 (1), 131–139.
- Posada, M.L., Frederick, R.D., 2002. Expressed sequence tags analysis from germinating urediniospores of the plant pathogen *Phakopsora pachyrhizi*. Phytopathology 92, S66.
- Pretorius, Z.A., Kloppers, R.J., Frederick, R.D., 2001. First report of soybean rust in South Africa. Plant Dis. 85, 1288.
- Qutob, D., Hraber, P.T., Sobral, B.W.S., Gijzen, M., 2000. Comparative analysis of expressed sequences in *Phytophthora infestans*. Plant Physiol. 123, 243–253.
- Randall, T.A., Dwyer, R.A., Huitema, E., Beyer, K., Cvitanich, C., Kelkar, H., Ah Fong, A.M.V., Gates, K., Roberts, S., Yatzkan, E., Gaffney, T., Law, M., Testa, A., Torto-Alalibo, T., Zhang, M., Zheng, L., Mueller, E., Windass, J., Binder, A., Birch, P.R.J., Gisi, U., Govers, F., Gow, N.A., Mauch, F., van West, P., Waugh, M.E., Yu, J., Boller, T., Kamoun, S., Lam, S.T., Judelson, H.S., 2005. Large-scale gene discovery in the Oomycete *Phytophthora infestans* reveals likely components of phytopathogenicity shared with true fungi. Mol. Plant Microbe Interact. 18 (3), 229–243.
- Rossi, R.L., 2003. First report of *Phakopsora pachyrhizi*, the causal organism of soybean rust in the province of Misiones, Argentina. Plant Dis. 87, 102.
- Rytter, J.L., Dowler, W.M., Bromfield, K.R., 1984. Additional alternative hosts of *Phakopsora pachyrhizi*, causal agent of soybean rust. Plant Dis. 68, 818–819.
- Saksirat, W., Hoppe, H.H., 1991. Teliospore germination of soybean rust fungus (*Phakopsora pachyrhizi* Syd.). J. Phytopathol. 132, 339–342.
- Schneider, R.W., Hollier, C.A., Whitam, H.K., Palm, M.E., McKemy, J.M., Hernandez, J.R., Levy, L., DeVries-Paterson, R., 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis. 89, 773.
- Sims, A.H., Robson, G.D., Hoyle, D.C., Oliver, S.G., Prade, R.A., Russell, H.H., Dunn-Coleman, N.S., Gent, M.E., 2004. Use of expressed sequence tag analysis and microarrays of the filamentous fungus *Aspergillus nidulans*. Fungal Genet. Biol. 41 (2), 199–212.
- Sinclair, J.B., 1989. Threats to soybean production in the tropics: red leaf blotch and leaf rust. Plant Dis. 73 (7), 604–606.
- Sinclair, J.B., Hartman, G.L. (Eds.), 1996. Proceedings of the Soybean Rust Workshop (1995). National Soybean Research Laboratory, Publication No. 1.
- Soanes, D.M., Skinner, W., Keon, J., Hargreaves, J., Talbot, N.J., 2002. Genomics of phytopathogenic fungi and the development of bioinformatic resources. Mol. Plant Microbe Interact. 15 (5), 421–427.
- Steen, B.R., Zuyderduyn, S., Toffaletti, D.L., Marra, M., Jones, S.J., Perfect, J.R., Kronstad, J., 2003. *Cryptococcus neoformans* gene expression during experimental cryptococcal meningitis. Eukaryot. Cell 2 (6), 1336–1349.
- Thomas, S.W., Rasmussen, S.W., Glaring, M.A., Rouster, J.A., Christiansen, S.K., Oliver, R.P., 2001. Gene identification in the obligate fungal pathogen *Blumeria graminis* by expressed sequence tag analysis. Fungal Genet. Biol. 33 (3), 195–211.
- Trail, F., Xu, J.R., San Miguel, P., Halgren, R.G., Kistler, H.C., 2003. Analysis of expressed sequence tags from *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genet. Biol. 38 (2), 187–197.
- Yang, X.B., Dowler, W.M., Royer, M.H., 1991. Assessing the risk and potential impact of an exotic plant disease. Plant Dis. 75, 976–982.
- Yeh, C.C., Tschanz, A.T., Sinclair, J.B., 1981. Induced teliospore formation by *Phakopsora pachyrhizi* on soybeans and other hosts. Phytopathology 71, 1111–1112.
- Yen, Y.M., Wong, B., Johnson, R.C., 1998. Determinants of DNA binding and bending by the *Saccharomyces cerevisiae* high mobility group protein NHP6A that are important for its biological activities. Role of the unique N terminus and putative intercalating methionine. J. Biol. Chem. 273 (8), 4424–4435.
- Yorinori, J.T., Paiva, W.M., Frederick, R.D., Fernandez, F.T.P., 2002. Ferrugem da soja (*Phakopsora pachyrhizi*) no Brasil e no Paraguai, nas safras 2000/01 e 2001/02, p. 94. In: Resumos Da II Congresso Brasileiro de Soja e Mercosoja 2002. Embrapa Soja, Londrina, 2002, 393 p. (Documentos/Embrapa soja; n. 181. Resumo 094).
- Yorinori, J.T., Paiva, W.M., Frederick, R.D., Costamilan, L.M., Bertagnoli, P.F., Hartman, G.L., Godoy, C.V., Nunes, J.J., 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. Plant Dis. 89, 675–677.
- Zhu, H., Nowrousian, M., Kupfer, D., Colot, H.V., Berrocal-Tito, G., Lai, H., Bell-Pedersen, D., Roe, B.A., Loros, J.J., Dunlap, J.C., 2001. Analysis of expressed sequence tags from two starvation, time-of-day-specific libraries of *Neurospora crassa* reveals novel clock-controlled genes. Genetics 157, 1057–1065.