Genomewide Analysis of Phospholipid Signaling Genes in *Phytophthora* spp.: Novelties and a Missing Link

Harold J. G. Meijer, and Francine Govers

Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven 5, NL-6709 PD Wageningen, The Netherlands.

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Phospholipids are cellular membrane components in eukaryotic cells that execute many important roles in signaling. Genes encoding enzymes required for phospholipid signaling and metabolism have been characterized in several organisms, but only a few have been described for oomycetes. In this study, the genome sequences of *Phytophthora sojae* and *P. ramorum* were explored to construct a comprehensive genomewide inventory of genes involved in the most universal phospholipid signaling pathways. Several genes and gene families were annotated, including those encoding phosphatidylinositol synthase (PIS), phosphatidylinositol (phosphate) kinase (PI[PK]), diacylglycerol kinase (DAG), and phospholipase D (PLD). The most obvious missing link is a gene encoding phospholipase C (PLC). In all eukaryotic genomes sequenced to date, PLC genes are annotated based on certain conserved features; however, these genes seem to be absent in *Phytophthora* spp. Analysis of the structural and regulatory domains and domain organization of the predicted isoforms of PIS, PIK, PIPK, DAG, and PLD revealed many novel features compared with characterized representatives in other eukaryotes. Examples are transmembrane proteins with a C-terminal catalytic PLD domain, secreted PLD-like proteins, and PIPKs that have an N-terminal G-protein-coupled receptor-transmembrane signature. Compared with other sequenced eukaryotes, the genus *Phytophthora* clearly has several exceptional features in its phospholipid-modifying enzymes.

Additional keywords: signal transduction.

Phospholipid-based signaling is an important component of the signal transduction network in eukaryotes. Well-known examples of phospholipids are the polyphosphoinositides (PPIs) that constitute only a minor fraction of the membrane but execute many important roles. They modulate the activity of membrane-bound proteins, act as anchors to recruit and translocate proteins containing phospholipid-binding modules, play a role in membrane trafficking, and, above all, act as signaling molecules (Behnia and Munro 2005; Lemmon 2003; Meijer and Munnik 2003). Phospholipid signaling and metabolism is studied in a wide variety of organisms ranging from higher eukaryotes such as plants and mammals to microorganisms,

including pathogens. This study is focused on identifying phospholipid modifying enzymes in the genus *Phytophthora*. Genus *Phytophthora* belongs to the oomycetes, a unique group of fungal-like eukaryotes that includes significant pathogens of plants, insects, and animals, and in which phospholipid signaling hardly has been investigated.

One important class of phospholipids comprises the PPIs and includes phosphorylated derivatives of phosphatidylinositol (PI). PI is the starting point for several kinase activities. Its headgroup, inositol, can be phosphorylated to PI mono-, bis-, and trisphosphates, ultimately resulting in seven different phosphorylated products. PI is synthesized from CDP-diacylglycerol and *myo*-inositol by PI-synthase (PIS) and the phosphorylation is accomplished by PI kinases (PIKs) and PI-phosphate kinases (PIPKs) to generate PI-phosphates (PIPs) and -bisphosphates (PIP₂s), respectively (Fig. 1). Of the latter, PI(4,5)P₂ is well known as a second messenger (Doughman et al. 2003; McLaughlin and Murray 2005). PIP₂ can be either phosphorylated, resulting in PI(3,4,5)P₃, or hydrolyzed by a phosphoinositide-specific phospholipase C (PLC) to generate inositol (1,4,5)-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG). PI(3,4,5)P₃, IP₃, and DAG all are known to function as key second messengers. DAG can be phosphorylated by diacylglycerol kinase (DGK) to generate phosphatidic acid (PA), a phospholipid that not only is an intermediate in lipid biosynthesis but also is emerging as one of the most prominent lipid second messengers in eukaryotes (Testerink and Munnik 2005). PA is known to act as a docking site that recruits proteins and, as a result, alters the subcellular localization of these proteins and modifies their activities. In plants, yeast, and mammals, many PA targets have been identified, including protein kinases, protein phosphatases, PIPKs, and transcription factors (Fernandez-Murray and McMaster 2006; Ktistakis et al. 2003; Loewen et al. 2004; Testerink et al. 2004). PA can also be produced via hydrolysis of a structural phospholipid, such as phosphatidylcholine, a reaction catalyzed by phospholipase D (PLD). PA levels are downregulated by a PA-phosphohydrolase that dephosphorylates PA, resulting in DAG, or by the hydrolyzing activity of a PA-specific phospholipase A₂, resulting in the signaling molecule lyso-phosphatidic acid (Meijer et al. 2001). PA also can be phosphorylated by PA-kinase, leading to the formation of diacylglycerol pyrophosphate (DGPP). This phospholipid is found in plants and yeast as a response to various stress stimuli and is another potential signaling molecule (Meijer and Munnik 2003; van Schooten et al. 2006). The tight network of enzymes illustrates the delicacy to control balanced levels of these phospholipid second messengers.

Phospholipid signaling in oomycetes has been investigated only superficially and all studies to date are restricted to *Phy-*

Corresponding author: H. J. G. Meijer; Telephone.: +31 317 483 403; Fax: +31 317 483 412; E-mail: Harold.Meijer@wur.nl

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trophthora spp. Work by Zhang and associates (1992) revealed a role for PA in inducing encystment of zoospores in *Phytophthora palmivora*, and Latijnhouwers and associates (2002) reported a similar effect of PA and the G-protein activator mastoparan in *P. infestans*. In *P. infestans*, mastoparan also induced increased activity of PLD and, as a result, increased levels of PA and DGPP, thus suggesting a link between heterotrimeric G-protein signaling and phospholipid signaling (Latijnhouwers et al. 2002). PLC was implicated in *P. infestans* zoosporogenesis because inhibitors of calcium channels, IP₃-gated ion channels, and PLC blocked this process (Tani et al. 2004). In the same study, IP₃ levels were measured but no increase was found during sporangial cleavage. Recently, we described the cloning of a *P. infestans* gene encoding a novel membrane-associated PLD, homologs of which are present in *P. sojae* and *P. ramorum* (Meijer et al. 2005). The function of this gene is still unknown, and the relation between pathogenicity and phospholipid signaling in *Phytophthora* spp. also is a black box.

In several systems, the role of phospholipid signaling in pathogenicity has been studied (Songer 1997; Titball 1993, 1998). In mammalian systems, pathogenic bacteria recruit PPI_n while entering host cells in order to promote invasion, or they modify the intracellular host compartments for their own needs (Pizarro-Cerda and Cossart 2004). In *Mycobacterium tuberculosis*, PLCs play a role in virulence (Raynaud et al. 2002) and, in *Corynebacterium* spp., secreted PLDs are known to act as virulence determinants (McNamara et al. 1994, 1995). Also in pathogenic fungi, extracellular phospholipases are considered as potential virulence factors. One example is a *Cryptococcus* phospholipase B that hydrolyzes fatty acids from the phospholipid backbone and is located at the cell surface as a glycosylphosphatidylinositol-anchored protein in lipid rafts (Cox et al. 2001; Djordjevic et al. 2005; Siafakas et al. 2006). Other examples are extracellular phospholipase A, B, C, and D activities identified in *Aspergillus fumigatus* (Rementeria et al. 2005). Experimental proof for involvement of PLD in fungal pathogenicity comes from studies in *Candida albicans*, where deletion of the *CaPLD1* gene resulted in a major loss of virulence (Dolan et al. 2004; Hube et al. 2001).

The aim of this study was to make a comprehensive genome-wide inventory of genes involved in the most universal phospholipid signaling pathways in genus *Phytophthora*. Many of these genes have been cloned from a variety of organisms revealing several catalytic and regulatory domains. The release of genome sequences of *P. sojae* and *P. ramorum* (Tyler et al. 2006) and the presence of expressed sequence tag (EST) databases of *P. infestans* and *P. brassicae* enabled screening and identification of putative candidate genes. These included genes encoding PIS, PIK, PIPK, DGK, and PLD. In general,

Fig. 1. Schematic representation of some universal components in phospholipid signaling. Phosphatidylinositol (PI) is synthesized from the CDP-diacylglycerol (CDP-DAG) and *myo*-inositol catalyzed by phosphatidylinositol synthase (PIS). Subsequently, PI is phosphorylated by phosphatidylinositol kinase (PIK) and phosphatidylinositol phosphate kinase (PIPK), resulting in the formation of phosphatidylinositol (bis)phosphate (PIP and PIP₂). Phospholipase C (PLC) hydrolysis of PI(4,5)P₂ results in diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃) formation. Phosphorylation by diacylglycerol kinase (DGK) of DAG yields phosphatidic acid (PA); the hydrolysis of structural phospholipids (PS), such as phosphatidylcholine, by phospholipase D (PLD) also results in the formation of PA. All substrates shown here also can act as substrates for other enzymes not included in this figure. The enzymes are shown in boxes.

the predicted proteins are well conserved between the *Phytophthora* spp., whereas conservation with the corresponding enzymes from other species is mostly restricted to regulatory or catalytic domains. In many of the predicted proteins, novel domain compositions were identified and, in some cases, characteristic domains were lacking. Most remarkable was the apparent lack of any sequence encoding a PLC.

RESULTS AND DISCUSSION

PIS.

PIS (CDP-diacylglycerol: *myo*-inositol 3-phosphatidyl-transferase; EC 2.7.8.11) catalyzes the last step in the de novo synthesis of PI via condensation of CDP-diacylglycerol and *myo*-inositol. Genes encoding PIS have been cloned from various organisms, including yeast (Nikawa and Yamashita 1997) rat, human, and *Arabidopsis* (Collin et al. 1999). All PIS proteins contain a conserved CDP-alcohol phosphotransferase signature, D-x(2)-DG-x(2)-AR-x(8)-G-x(3)-D-x(3)-D, embedding the active site and most encode one or more transmembrane domains (Collin et al. 1999).

P. sojae and *P. ramorum* each have one putative PIS gene that both contain two introns at the same positions. The encoded proteins are of similar size, 218 and 219 amino acids (aa), respectively, and have four predicted transmembrane domains. At the nucleotide level, the homology is 67% and, at the protein level, the two share 94% homology and 89% identity. In the *P. sojae* EST database, no PIS ESTs were found; however, the *P. infestans* EST database contains five PIS ESTs, all derived from one gene that is highly homologous to the *P. sojae* and *P. ramorum* PIS gene. Also, *P. brassicae* has a homologous PIS gene that is expressed. The origin of the ESTs in *P. infestans* suggests expression of the PIS gene in various developmental stages.

Phytophthora PIS is highly similar to PIS representatives from other organisms. The alignment of the conserved phosphotransferase signature and the phylogenetic tree of PIS proteins is shown in Figure 2.

Proteins with a PIK domain.

PIKs phosphorylate various forms of PPI_n at their 3-, 4-, or 5-hydroxyl position and are named after their catalytic activity. All eukaryotes have different types of PIKs and many PI 3-kinase (PI3K; EC 2.7.1.137) and PI 4-kinase (PI4K; EC 2.7.1.67) genes have been identified and characterized. In contrast, no specific PI 5-kinase genes have been identified. All PI3Ks and PI4Ks contain a conserved PI kinase domain (IPR000403), and most have various additional domains that are involved in protein-protein or phospholipid-protein interactions. PI3Ks are classified as type I PIKs, whereas type II and type III PIKs are PI4Ks (Whitman et al. 1988).

In *P. sojae*, 23 genes were identified that encode proteins containing PI kinase domains, and *P. ramorum* has the corresponding 23 homologues. In a phylogenetic analysis, the counterparts in *P. sojae* and *P. ramorum* cluster together (not shown). They are highly similar with only short stretches of dissimilarity, possibly in regions linking the various domains or in potential introns. Comparison of the coding sequences with ESTs identified in *P. sojae* and *P. infestans* suggested that some of the genes have introns, although the open reading frame was not disrupted. The catalytic domain of the majority of the 23 predicted proteins is highly homologous to other PI kinases; however, outside the catalytic domain, significant homology is lacking with only a few exceptions.

For 18 of the 23 predicted proteins, the highest homology was found with PI kinases from *Dictyostelium* (*n* = 10) and plants (*n* = 8), and is localized mainly in the catalytic domains. The do-

main organization of the various putative PI kinases in *Phytophthora* spp. is shown in Figure 3. In most cases, the catalytic domain is located at the C-terminal end. Based on homology and domain organization, 12 distinct groups of PI kinases can be distinguished, four of which are predicted to act as a true PI3K and seven as PI4K. Many of the *Phytophthora* PI kinases, however, have atypical combinations of domains or a novel domain organization which makes it difficult to place the PI kinases in a conventional classification system as used previously for *Arabidopsis* (Mueller-Roeber and Pical 2002). Therefore, the classification of the *Phytophthora* PIKs is preliminary. Below, the PI3Ks and PI4Ks are described in more detail.

PI3Ks.

Type I PI3Ks are subdivided into three classes of true PI3Ks named I, II, and III and a distantly related class named IV. In class I to III, the catalytic domain is preceded by a phosphoinositide 3-kinase accessory domain (PI3Ka; IPR001263) and a C2 domain (IPR000008) that originally was identified in protein kinase C (Sutton et al. 1995). A common feature of mammalian class I and II PI3Ks is the presence of a ras-binding domain (IPR000341) at the N-terminus.

One true class III PI3Ks homologue is present in the *Phytophthora* genome (Fig. 3, group A). The encoded protein has a characteristic PI3K C2 domain (PI3K_C2; IPR002420) and both accessory and catalytic domain are present. It has overall homology with Vps34p and its homologs. Vsp34p (IPR008290) is the only PI3K present in yeast and required for the formation of PI(3,4,5)P₃ (Mitra et al. 2004).

One predicted PI kinase was classified as a class I PI3K based on its catalytic domain and the presence of a PI3Ka domain; however, it lacks the ras domain (Fig. 3, group B). Two genes (Fig. 3, group C) encode PI3Ks partly resembling class I PI3Ks; they have an accessory domain (PI3Ka) together with a catalytic domain and a ras domain. The ras domain, however, is preceded by a PH domain (Pleckstrin homology; IPR011993) (Lemmon 2003) and followed by a PI3K_C2 domain. In addition, these two *Phytophthora* PI3Ks possess a second PH domain that is located between the PI3K-C2 domain and the accessory domain. Such a domain organization has not been found in any other PI kinase, suggesting that it is a novel class. One other predicted protein is related to the class I-III PI3Ks. It contains a PI3Ka domain, the catalytic domain, but also a FYVE domain (IPR000306) located at the N-terminus (Fig. 3, group D).

The *P. sojae* EST database contains ESTs for only one PI3K group; however, the *P. infestans* EST database contains at least one highly homologous EST for each individual PI3K described. For most PI3Ks, a *P. brassicae* EST also is found.

The more distantly related class IV PI3Ks are much larger than the true PI3Ks. They are thought to be functionally distinct from true PI3Ks because they have been shown to possess protein kinase activity rather than phospholipid kinase activity (Bosotti et al. 2000; Vanhaesebroeck et al. 2001). Homology with true PI3Ks is restricted to the catalytic domain. In addition to the catalytic domain, these proteins share two other domains: a so-called FAT domain (IPR003151) located N-terminal of the PI kinase domain and a FATC domain (IPR003152) located at the extreme C-terminal end. The similarity in the catalytic domain with the true PI3Ks is high; therefore, these PIK-related proteins were included in the genomewide search. The genus *Phytophthora* appears to have several class IV PI3Ks that range in size from 2,000 to over 4,500 aa (Fig. 3, group L). They have high homology to protein kinases such as TOR (target of rapamycin) and DNA-dependent protein kinases of various organisms (Bosotti et al. 2000; Vanhaesebroeck et al. 2001). ESTs were found for nearly all class IV PI3Ks.

PI4Ks.

PI4Ks are divided in type II and III based on their biochemical properties (Whitman et al. 1988). Type II PI4Ks are membrane-localized proteins that have hardly any sequence similarity with other protein or lipid kinases (Barylko et al. 2001), and the homology between mammalian, plant, and yeast type II PI4Ks is low (Minogue et al. 2001). Type II PI4Ks in plants are significantly larger than those in other species due to an N-terminal extension resembling one or two copies of an ubiquitin domain (IPR000626) (Mueller-Roeber and Pical 2002). The catalytic domains of type II PI4Ks have no close similarity to those of PI3Ks and the type III PI4Ks but contain conserved residues found in protein kinases, PIKs, and PIPKs (Barylko et al. 2001; Minogue et al. 2001).

In *Phytophthora* spp., four proteins were identified that, based on phylogenetic analysis, are highly related to the type II PI4Ks and align readily with PI4Ks from rice. Three of these are relatively small proteins (460 to 630 aa) that have a C-terminal PIKc domain (IPR000403) but lack any other defined domain (Fig. 3, group E). The fourth one is larger (710 aa) and has an ubiquitin domain at the N-terminus and a relatively large C-terminal tail downstream of the PIKc domain (Fig. 3, group F).

Type III PI4Ks mostly are larger than the canonical type II PI4Ks, and their catalytic domain is more related to those of the PI3Ks. Eight proteins were predicted that are categorized as type III PI4K. Three of them have a novel domain structure with a PH domain and a FYVE domain combined with a PIKc

Fig. 2. Alignment and phylogram of phosphatidylinositol synthase amino acid sequences. **A**, Multiple alignment of the CDP-alcohol phosphotransferase signature (shown in the first line) present in the deduced amino acid sequence of phosphatidylinositol synthase genes of four *Phytophthora* spp. (*P. sojae*, *P. ramorum*, *P. infestans*, and *P. brassicae*), yeast (ScPIS: AAA34876), human (HsPIS: AAB94860), *Arabidopsis thaliana* (AtPIS: CAA04172.1), rice (OsPIS: XP_463937), and *Brassica napus* (BnPIS: AF521301). Symbols below the alignment indicate identical residues (*), conserved substitutions (:), or semiconserved substitutions (.). **B**, Phylogenetic tree based on the full-length amino acid sequence of phosphatidylinositol synthase (PIS) from the various organisms listed in A. *P. infestans* and *P. brassicae* PIS were not included.

catalytic domain (Fig. 3, group G), whereas two others lack the FYVE domain but contain the PH domain (Fig. 3, group H). These five are phylogenetically closely related, suggesting that, during evolution, the FYVE domain either was lost in group H or gained in group G. In one predicted type III PI4K categorized as group I (Fig. 3), an N-terminal FYVE domain occurs in combination with the C-terminal catalytic domain. This protein has the highest homology with a PIK in *Anopheles gambiae*, the malaria mosquito; however, this homology is restricted to the catalytic domain. Apart from a FYVE domain, the protein has no significant homology to any other protein.

One predicted type III PI4K has three or four putative transmembrane domains preceding a PI3Ka domain and the catalytic domain (Fig. 3, group J). Based on phylogenetic analysis, it is classified as a type III PI4K; however, the presence of a PI3Ka domain and transmembrane domains is rather peculiar. Related to Group J but lacking both the PI3Ka domain and the transmembrane domains is one predicted type III PI4K (Fig. 3, Group K). ESTs were found for all PI4K genes, suggesting that they are active genes.

Proteins with a PIPK domain.

PIPK (EC 2.7.1.68) phosphorylates PIP isomers at their inositol ring, yielding PIP₂ (Fig. 1). Three types of PIPKs are known that are present throughout the eukaryotic kingdom. Type I (PI4P 5-kinase; PI4P5K) and type II (PI5P 4-kinase;

PI5P4K) generate PI(4,5)P₂ from PI4P and PI5P, respectively (Rameh et al. 1997), whereas type III (PI3P 5-kinases; PI3P5K) generates PI(3,5)P₂ from PI3P (Cooke et al. 1998; Gary et al. 1998). Sequence identity between type I and II PIPKs is high in the catalytic domain; however, they have different enzymatic properties, different catalytic activities, and are functionally nonredundant (Anderson et al. 1999; Kunz et al. 2000). Type I PIPKs are located at the plasma membrane, in the nucleus, and on the Golgi (Doughman et al. 2003); type II PIPKs reside in the nucleus and cytosol, and are associated with the endoplasmic reticulum. Type III PIPKs are distinct from type I and II. They localize at internal membranes and are thought to be involved in vesicle membrane integrity, subcellular targeting, and vacuole homeostasis (Meijer and Munnik 2003; Michell et al. 2006). None of the known PIPKs is incorporated in the membrane because they lack transmembrane domains (Anderson et al. 1999).

The genus *Phytophthora* has 15 genes that code for proteins with homology to PIPKs and they are conserved between *P. sojae* and *P. ramorum*. Four distinct PIPK groups can be distinguished (Fig. 4), three of which contain only 1 member (A, B, and C), whereas group D contains 12 members. Phylogenetic analysis of the catalytic domain revealed that group A and B cluster with type III PIPK and group C with the type I/II PIPKs representatives (data not shown) (Bakthavatsalam et al. 2006). Group D clusters with a PIPK from *Dictyostelium* dis-

Fig. 3. Domain organization and composition of *Phytophthora* phosphatidylinositol kinases (PIKs). Domains were identified by various prediction programs (see Materials and Methods) and, based on domain organization, the PIKs were classified in groups (A to L). Members per group are indicated in brackets. Groups with a putative PI 3-kinase (PI3K) catalytic activity are marked with *, those with a putative PI4K catalytic activity with #. The sizes can be deduced from the bar of 200 amino acids (AA) for group A to K and the bar of 400 AA for group L. For groups possessing two or more members, the indicated size is an average.

coideum. Because the PIPKs in group D are unique and different from any PIPK described thus far, we have classified them as type IV PIPKs or G-protein-coupled receptor-PIPKs (GPCR-PIPK) (Bakthavatsalam et al. 2006). Below, the various PIPK groups are described in more detail.

PIPks.

The single group A PIPK in genus *Phytophthora* has the highest homology to type III PIPKs (Fig. 4). The prototype for type III is Fab1p from yeast (Cooke et al. 1998) and all model eukaryotes have Fab1p homologues. It contains a characteristic kinase domain at the C-terminus and a chaperonin-like domain in the center. At the N-terminus, there is a FYVE domain that is involved in protein targeting. Recently, a “PIPKIII-unique” domain was identified between the chaperonin domain and the catalytic domain that is present only in type III PIPKs (Michell et al. 2006).

The *Phytophthora* group A protein has a similar domain organization but, in addition, has an N-terminal extension of 200 aa that composes a PDZ domain (also known as DHR or GLGF; Interpro IPR001478). PDZ is a protein–protein interaction domain that is found in many different proteins in a variety of organisms (Ponting 1997). The genus *Phytophthora* encodes at least 70 proteins containing a PDZ domain; however, this seems to be the only one involved in phospholipid metabolism (data not shown). PDZ domains interact with sites located at the C-terminus of target proteins (Ponting 1997; van Ham and Hendriks 2003). Zimmermann and associates (2002) demonstrated that the PDZ domain in human syntenin binds PIP in vitro and in vivo, thus suggesting that PDZ domains play a role in phospholipid signaling.

Solely based on the catalytic domain of the single group B PIPK, this enzyme should be classified as a type III PIPK (Fig. 4) (Bakthavatsalam et al. 2006). The catalytic domain has the highest homology with PI3P5Ks from *Arabidopsis* and rice; however, apart from the catalytic domain, there is no homology to any other type III PIPK. Instead of the characteristic FYVE domain and a chaperonin domain, the group B PIPK contains an N-terminal-located PH domain.

The genus *Phytophthora* has only one PIPK that contains significant homology with the catalytic domains in type I or II PIPKs (Fig. 4, group C) and, thus, could function as a PI4P5K (Bakthavatsalam et al. 2006). Also, in yeast, there is just a single PI4P5K gene named *Mss4*. The yeast PI4P5K has dual activity because it phosphorylates PI4P to PI(4,5)P₂ and PI3P to PI(3,4)P₂, although the latter to a much lesser extent (Desrivieres et al. 1998). *Mss4p* contains a nuclear localization

signal and was shown to shuttle between the nucleus and the cytoplasm (Audhya and Emr 2003). Interestingly, the group C *Phytophthora* PIPK also has a putative nuclear localization signal, suggesting overlapping functions.

For group A and B as well as group C PIPKs, ESTs are found, suggesting that the putative PI3P5Ks and PI4P5K in *Phytophthora* spp. are active. Group A and C PIPKs have homologs throughout the animal and plant kingdoms; therefore, we anticipate that their signaling functions are ubiquitous and well conserved. The domain organization in the group B PIPK has not been described for any other PIPK thus far, which may point to a unique function for this PIPK in *Phytophthora* spp. Also, other organisms seem to have their own unique PIPKs. *Arabidopsis*, for example, has four type III PIPKs, two of which lack the FYVE domain (Meijer and Munnik 2003; Mueller-Roeber and Pical 2002). Another typical feature of some plant PIPKs is the presence of membrane occupation and recognition nexus (MORN) motifs at the N-terminus (Ma et al. 2004; Mikami et al. 1998) which can interact with the plasma membrane via phospholipids (Takeshima et al. 2000). In the *Phytophthora* PIPKs, no MORN motifs were found.

G-protein-coupled receptor-PIPks.

Twelve genes in *Phytophthora* encode a novel class of PIPKs. They have a catalytic domain that is phylogenetically distinct from that in type I or II or type III PIPKs and is preceded by putative transmembrane domains located at the N-terminus (Fig. 4, group D). In public databases, there is only one protein that shows significant homology with the group D PIPKs, not only with the catalytic domain but also with the transmembrane domains. This homolog is DdRpkA from *Dictyostelium discoideum* which belongs to the superfamily of GPCR receptors and shares homology with Frizzled/Smoothed receptors (Eichinger et al. 2005). Because DdRpkA and the 12 group-D proteins in *Phytophthora* spp. share both the GPCR signature and the novel PIPK domain, we recently proposed to classify them as type IV PIPKs or GPCR-PIPKs (Bakthavatsalam et al. 2006). DdRpkA knockout mutants of *D. discoideum* showed defects in cell density sensing in a G-protein-dependent and -independent manner (Bakthavatsalam et al. 2006). The GPCR signature in the type IV PIPKs suggests that these proteins can perceive and transmit extracellular signals, resulting directly in the activation of PIPK. It also is possible that the GPCR signature activates G proteins or that the PIPK domain has protein kinase activity. Bypassing G-protein signaling by direct activation of a PIPK might explain why the genus *Phytophthora* has only one G-protein α and one G-pro-

Fig. 4. Domain organization and composition of *Phytophthora* phosphatidylinositol phosphate kinases (PIPks). The domain organization was determined by various domain prediction programs (see Materials and Methods) and, based on the domain organization, the PIPks were classified in groups (A to D). Members per group are indicated in brackets. A, Nearly typical type III PIPK (“PIPKIII-unique” domain, present between the chaperonin-like region and the catalytic domain, is not shown). B, Nontypical type III PIPK; C, type I or II PIPK, and D are the GPCR-PIPks. The approximate sizes can be deduced from the bar of 200 amino acids. For group D GPCR-PIPks with 12 members, the average size is shown.

tein β subunit (Tyler et al. 2006). The high number of these unique GPCR-PIPKs in the genus *Phytophthora* is remarkable. Of the 24 GPCRs annotated in *Phytophthora* spp., 12 have the GPCR signature combined with a PIPK catalytic domain (Tyler et al. 2006) and, for 5 of these, ESTs were found in *P. infestans*, *P. sojae*, or both. Some of the *P. infestans* ESTs span the region between the transmembrane domains and the PIPK domain. The presence of these unique GPCR-PIPKs suggests that they have an important physiological role in genus *Phytophthora*.

PLC.

Two important intracellular messengers (DAG and IP_3) are derived from $PI(4,5)P_2$ via a phosphoinositide-specific PLC (EC 3.1.4.11) (Rhee and Bae 1997). All known PLCs contain distinct conserved domains that include the characteristic catalytic X- (IPR000909) and Y-domains (IPR001711) and a C2 domain. Variation is found in the occurrence of a PH domain, calcium-binding EF-hands (IPR002048), and other domains, and PLCs have been classified into subtypes based on the presence of various domains (Meijer and Munnik 2003; Mueller-Roeber and Pical 2002; Rhee and Bae 1997). In nearly all studied eukaryotes, PLC genes are present as a multigene family; however, in the two sequenced *Phytophthora* spp., we were unable to identify any PLC gene. Automatic annotation did not reveal a gene model for a PLC gene. Two genes were predicted to encode a protein containing an X-domain; however, because there was no further homology, they could not be classified as PLC genes. Also, thorough blasting with PLC sequences from plants, mammals, yeast, and bacteria did not result in the identification of any clear putative PLC gene. Because oomycetes are closely related to diatoms, we retrieved all PLC sequences from the *Thalassiosira pseudonana* genome database and used these to screen the *Phytophthora* sequences, again without success. Because the sequences cover the two *Phytophthora* genomes several-fold, the chance that a particular gene is absent in the assembled genome sequences is very low. Nevertheless, we screened the TRACE sequence files (available via the National Center for Biotechnology Information [NCBI] website), but no PLC-like sequences were found. Also, the *Phytophthora* EST databases did not contain anything that resembled a PLC sequence. Among the PLC sequences deposited in the NCBI database, there is one accession (CB174626) that is defined as a PLC cDNA clone from the obligate oomycete pathogen *Plasmopara halstedii*. It concerns an SSH clone from infected sunflower; however, detailed analysis revealed that this is a cDNA sequence from the host plant and not from the pathogen.

In addition to the canonical PLCs, several organisms have a phosphatidylcholine-hydrolyzing PLC. Genes encoding this enzyme have been cloned from a wide variety of bacteria and from *Arabidopsis thaliana* (Nakamura et al. 2005; Preuss et al. 2001). They are highly conserved but lack the characteristic phosphoinositide-specific PLC domains such as the X- and Y-domains or EF-hand motifs. Putative homologous were detected but not considered to encode PLCs due to the limited homology and inconsistencies between *Phytophthora sojae* and *P. ramorum*. In bacteria, various PLCs exist with only a PLC X-domain and often containing a signal peptide (Titball 1998); however, these also were not detected in the *Phytophthora* genomes.

The apparent absence of PLC genes questions whether $PI(4,5)P_2$ hydrolysis occurs in *Phytophthora* spp. PIK and PIPK genes are present and expressed; therefore, it is likely that $PI(4,5)P_2$ is synthesized, and phospholipid analysis indeed revealed that $PI(4,5)P_2$ is present in *P. infestans*, albeit in very low amounts (*unpublished results*). It is very well possible that

Phytophthora spp. contain other enzymes with $PI(4,5)P_2$ hydrolyzing activities that lack sequence homology with known PLCs; however, thus far there is no biochemical evidence for PLC activity in *Phytophthora* spp. Latijnhouwers and associates (2002) demonstrated that in *P. infestans* PLD, and not PLC, is responsible for the production of PA and, with their labeling procedures, they could not detect PLC activity. In contrast, Tani and associates (2004) reported that PLC inhibitory compounds influenced gene expression levels in *P. infestans*, suggesting that PLC activity does exist. However, in that study, the effect of the inhibitors on phospholipid turnover or IP_3 production was not analyzed and, therefore, it cannot be excluded that the inhibitors affect other enzymes or signaling pathways. Moreover, the lack of sequence homology suggests that regular PLC inhibitors are not functional in inhibiting a putative PLC activity in *Phytophthora* spp. To find indications for the existence of IP_3 receptors, we scanned the *P. sojae* and *P. ramorum* genome sequences for genes encoding a putative IP_3 -binding site that was identified in various IP_3 receptors (Ponting 2000), but no such genes were found.

DGK.

DGK (EC 2.7.1.107) phosphorylates DAG, yielding PA. DGKs are widespread in eukaryotes, and most plants and animals contain multiple DGKs. In yeast, however, no DGK gene has yet been identified. All mammalian DGK isoforms contain a conserved catalytic domain that is composed of a DGK accessory domain (DGKa [IPR000756]) and a DGK catalytic (DGKc [IPR001206]) domain (van Blitterswijk and Houssa 2000), and two cysteine-rich (C1) domains which are essential for DAG or phorbol ester (PE) binding. *Arabidopsis* has two DGKs with a similar architecture (AtDGK1 and AtDGK2) and five that lack the C1 domains (AtDGK3 to AtDGK7) (Gomez-Merino et al. 2004; Meijer and Munnik 2003). Both types are able to phosphorylate DAG (Gomez-Merino et al. 2004, 2005).

The genomes of *P. sojae* and *P. ramorum* each contain one putative DGK gene. The *Phytophthora* DGKs have the same domain organization as the mammalian DGKs, with the catalytic domain consisting of DGKa and DGKc and two C1 domains (Fig. 5A). The genes have an intron at a conserved position and encode proteins that share 88% identity. Screening of the *P. infestans* trace files at the NCBI website revealed a highly homologous DGK gene and, from this species, DGK ESTs also were found, demonstrating that the gene is expressed. An alignment of the conserved amino acid segment resembling part of the ATP-binding site and containing the consensus sequence G-x-G-x(2)-G is shown in Figure 5B. *Phytophthora* DGKs are most homologous to one DGK derived from *T. pseudonana* (diatom; which encodes at least two DGKs) and the C1-DGKs from *Arabidopsis*. The smaller *Arabidopsis* DGKs fall into a distinct phylogenetic cluster (Fig. 5C) (Gomez-Merino et al. 2004). *Phytophthora* spp. have other putative proteins that have a DGKc domain but lack a DGKa domain, and these are thought to be sphingosine kinases (e.g., model Ps_144369). The fact the genus *Phytophthora* has only a single-copy DGK gene may be related to the absence of PLC. Although DAG can be produced via other pathways (e.g., dephosphorylation of PA or the addition of a fatty acyl chain to monoacylglycerol) (Lee et al. 2001), the low amount of substrate might diminish the need for multiple isoforms.

Proteins with a PLD catalytic domain.

A typical feature of PLD (EC 3.1.4.4.) is the catalytic domain (PLDc) that contains two motifs with the sequence HxKxxxxD, the so-called HKD motif (IPR001736). In eukaryotes, there are two major classes of PLDs, the PXP-PLDs and C2-PLDs (Eliš et al. 2002). In PXP-PLDs, the catalytic domain is pre-

ceded by two phospholipid-binding domains: PX (or PHOX; IPR001683) and PH. They both serve as recruitment domains that target the protein to distinctive membranes. C2-PLDs are smaller and, thus far, are found only in plants. They lack a PX or PH domain but, instead, have a C2 domain that interacts with phospholipids in a Ca^{2+} -dependent manner (Testerink and Munnik 2005; Wang 2005). The genus *Phytophthora* has 18 genes that code for proteins having a PLDc with two HKD motifs or motifs closely resembling HDK (PLD-like) and are

Fig. 5. Diacylglycerol kinase (DGK). **A**, The domain organization of DGK identified in *Phytophthora* spp. C1: conserved cysteine-rich domain; DGKc, catalytic domain; DGKa, accessory domain. **B**, Sequence alignment of a conserved amino acid segment in the catalytic domain representing the ATP-binding site. The consensus sequence is shown in the first line. Symbols below the alignment indicate identical residues (*), conserved substitutions (:), or semiconserved substitutions (.). **C**, Phylogenetic tree with characteristic DGKs of human (Hs), *Arabidopsis thaliana* (At), and the diatom *Thalassiosira pseudonana* (Tp). Accession numbers: HsDGK α : P23743; HsDGK β XP_044625.1; HsDGK γ : P49619; AtDGK1: NP_196409.1; AtDGK2: AAR28755; AtDGK3: AAL57635; AtDGK4: BAB09587; AtDGK5: AAK26010; AtDGK6: NP_194542; AtDGK7: AAU04880; PsDGK1: PsDGK1_130457; PrDGK1: PrDGK_81672; TpDGK1: Tp_105913.

conserved between *P. sojae* and *P. ramorum*. Two subfamilies that each contain one member have perfect HDK motifs (Fig. 6, PXPH-PLD and PXTM-PLD). PXPH-PLD is the universal PLD, whereas the PXTM-PLD contains a number of transmembrane domains. None of the *Phytophthora* PLDs has a C2 domain, demonstrating that the genus *Phytophthora* also lacks C2-PLDs.

The *P. sojae* and *P. ramorum* PXPH-PLD gene both have one intron at a conserved position and the encoded proteins are 1,121 and 1,113 aa, respectively. This size is comparable with PXPH-PLDs in plants, and the overall similarity to the *Arabidopsis* PXPH-PLD PLD ζ 1 is 39%. The most conserved region spans the catalytic domain (>50% identity with AtPLD ζ 1). In between the HKD motifs there are other regulatory domains, including a PIP_2 -binding domain, the IYIENQFF motif, and a modified DRY-motif (Fig. 6, not shown) (Meijer et al. 2005). The *Phytophthora* PXPH-PLD is expressed as shown by EST data.

Membrane-associated PLDs.

Two PLD genes in genus *Phytophthora* encode proteins that have transmembrane domains. Each of these is classified to a different PLD subfamily named PXTM-PLD and TM-PLD (Fig. 6). Similar to the PXPH-PLD, PXTM-PLD has a PX domain at the N-terminus and two perfect HKD motifs in the catalytic domain. The PH domain, however, is replaced by five putative transmembrane domains favoring a model with an extracellular PX domain and an intracellular PLD catalytic domain. Recently, we described the cloning of the single-copy PXTM-PLD gene from *P. infestans* (Meijer et al. 2005). The PXTM-PLD genes in *P. infestans*, *P. sojae*, and *P. ramorum* are highly homologous and, in between the HKD motifs, PXTM-PLD has the same regulatory domains as described above for PXPH-PLD.

The TM-PLD lacks any known domain in the N-terminus but is packed with 9 or 10 transmembrane domains. Moreover, the first HKD motif is as expected; however, in the second motif, the lysine (K) is replaced by asparagine (N), resulting in HxNxxxxD. This is peculiar because HKD motifs are extremely conserved within the PLD superfamily, which includes not only PLDs but also phosphatidyltransferases, endonucleases, and some viral envelope proteins (Koonin 1996; Liscovitch et al. 2000). Further analysis and database mining showed that TM-PLD has some homology to voltage-gated ion channels (ProtFun2.1), suggesting a function as a transporter. Moreover, the transmembrane domains have homology with the DUF803 domain (IPR008521) present in the protein encoded by the *ichthyin* gene. This is a multiple transmembrane domain pro-

Fig. 6. Domain organization and composition of *Phytophthora* phospholipase Ds (PLDs). The domain organization of the five PLD subfamilies was determined by various domain prediction programs (see Materials and Methods). The duplicated HKD motifs represent the catalytic domain. Members per subfamily are indicated in brackets. The approximate size can be deduced from the bar of 200 amino acids (AA). For sPLD-like and PLD-like genes, the indicated size is an average.

tein belonging to a family of approximately 50 plasma membrane proteins with homology to both transporters and G-protein-coupled receptors (Lefevre et al. 2004). None of these have PLD catalytic domains. TM-PLDs are well conserved between *Phytophthora* spp. (92% homology), but the homology with other PLDs is low.

EST data show that both PXTM-PLD and TM-PLD are expressed. The combination of a PLD catalytic domain together with the transmembrane-spanning regions provides unrecorded possibilities for activation and function. Although the function of PXTM-PLD currently is not known, its topology suggests the ability to sense external signals via binding of PPI α to the PX domain and subsequent activation of internal signaling via the catalytic domain. With respect to TM-PLD, the function is more obscure. The fact that one of the two HDK motifs is aberrant questions its PLD activity, and further studies are needed to determine whether TM-PLD has enzymatic activity at all.

PLD-likes.

Besides the single-copy PLD genes described above, genus *Phytophthora* has two PLD-like gene families. Like TM-PLD, these PLDs have no perfect HDK motifs. In the first HDK motif, the aspartate (D) is changed into an alanine (A), arginine (R), or valine (V). The second HKD motif is intact and highly similar in all family members. Because of the aberrant HKD motif, they are named PLD-like. The largest family consisting of 12 members encodes PLD-like proteins that contain a putative N-terminal secretion signal varying in length from 21 to 31 aa (Fig. 6, sPLD-like). In all cases, SignalP predicted the secretion signals with a probability higher than 99%. The PLD-likes belonging to the small family of three have no secretion signal (Fig. 6, PLD-like). In between the HDK motifs, all the sPLD likes and PLD likes contain the PIP $_2$ -binding region and the IYIENQFF motif, but a DRY motif cannot be recognized (not shown).

More than half of the sPLD-like and PLD-like genes are clustered on the genome. In *P. sojae*, seven sPLD-like genes are located within a 150-kb sequence contig on scaffold 66 and the *P. ramorum* homologs are located within a 75-kb sequence contig on scaffold 44. In *Phytophthora* spp., many of the genes encoding secreted proteins belong to gene families and they often are found in clusters on the genome (Jiang 2006, Jiang et al. 2006). Most of the PLD-like genes have a single exon. The ones that do contain introns are most likely pseudogenes because the predicted proteins are truncated or contain a frameshift. The EST data suggests that only one or a few members of each family are expressed. Remarkably, one of the three PLD-like genes showed an extremely high expression level, with nearly 50 ESTs in *P. infestans* and even 10 in the relatively small set of ESTs from *P. brassicae*.

The sPLD-like and PLD-like genes have the highest homology to predicted PLDs from *Janibacter* spp. and *Kineococcus radiotolerans*. These bacterial PLDs have an arginine (R) instead of an aspartate (D) in the first HKD motif (Fig. 7). Moreover, the predicted *K. radiotolerans* PLD has a putative signal peptide as found for the sPLD likes. Significant homology of the *Phytophthora* sPLD-like and PLD-like genes also was found with C2-PLDs and PXP-PLDs, but hardly with other secreted PLDs that are characterized in bacteria (not shown).

In *Arabidopsis*, it was noted that the first HKD motif displays diversity in the PXP-PLD and C2-PLD subfamilies (Qin and Wang 2002). This diversity is observed in an amino acid stretch directly following the HKD motif. Also in *Phytophthora* PLDs, this region shows diversity (Fig. 7). The first HKD region in the *Phytophthora* PXP-PLD and PXTM-PLD is similar to that in other PXP-PLDs, indicating a close relationship. Similar

to the C2-PLDs, the PLD-like and sPLD-like genes have an insertion in that region which is different in the two subfamilies.

Concluding remarks.

In this study, we focused on the annotation of *Phytophthora* genes encoding enzymes involved in phospholipid metabolism and signaling. Comparative analysis of two species revealed that the annotated genes and the gene organization are well conserved within the genus *Phytophthora*. Apart from the sPLD-like and PLD-like genes, all genes are single copy and scattered over the genome. Some of the sPLD-like and PLD-like genes, however, occur in clusters. Based on the homology and gene organization, we conclude that the gene families already existed before the two species diverged, without further expansion of the families after speciation.

We found several enzymes with a novel, surprising domain organization; however, in all cases, the novelties found in one species also were observed in the other, thus validating their true existence. This was essential because comparison with their mammalian and plant counterparts showed that homology is based mainly on conserved catalytic and regulatory domains. In general, the number of genes for each class of enzymes are comparable with those observed in animals and plants, with some exceptions. Most striking was the absence of a traditional PLC gene. This could be due to a lack of homology; however, analyses in other species have shown that defined parts in PLC are well conserved and are easily recognized by homology and domain searches. For DGK, only one gene was found, whereas plants and animals have multiple DGK genes. Both PLC and DGK are involved in the same pathway leading to production of PA. Because PA also can be generated via PLD, PLC and DGK are not absolutely required.

The diversity in *Phytophthora* PLDs is remarkable. In the sPLD-like and PLD-like genes, the aberrant HKD motif and the surroundings regions have high homology to some bacterial PLDs but, at the same time, resemble the catalytic domain

HxKxxxxD	HKD motif	
YLWAHHEKLVIIIDQSV-----AFVGGIDLAYGR	HsPLD1	PXP-PLD
YLWAHHEKLVIIIDQSV-----AFVGGIDLAYGR	MmPLD1	
GLWSHHEKIVSVQQRV-----SFVGGIDLCFGR	PLD_134882	
YLWSHHEKLVIVDNQV-----CFIGGLDLCFGR	AtPLD ζ 1	PXTM-PLD
GFWSHHEKIVCIDSLS-----AFVGGIDLCFGR	PLD_163010	
TIYTHHQRNVIVDADA---GNNRRKIIAFVGGIDLCFGR	AtPLD β 1	
TIYTHHQRNVIVDADA---GNNRRKIIAFVGGIDLCFGR	AtPLD γ 1	C2-PLD
TMFTTHHQRNVIVDADA---GNNRRKIIAFVGGIDLCFGR	AtPLD α 1	
RGGAHHRQFVVIR-----HRDDATRDVAFVGGIDVHNR	<i>Janibacter</i>	
TLGSHHQRFLVIR-----HLHRPQDDVAFVGGIDLAHSR	<i>Kineococcus</i>	Bacterial
MTSSHHQKTLIFAANS---SSDKKDDQPVAVVGGIDFTKDR	PLD_138732	
ALSSHHQKTLVIAANS---SSDKKDDQPVAVVGGIDFTTGR	PLD_138733	
LIASHHQRKTLVIAADI---SSGKADQPVAVVGGIDLAHDR	PLD_138729	sPLD-like
LIASHHQRKTLVIAANI---SKDEDDQPVAVVGGIDLANDR	PLD_138762	
VVSSHQRKTLAIVANE---SSSETDQPVAVVGGIDLANDR	PLD_140963	
ALSSHHQRNMVIAVNS---SKDERAQPVAVVGGIDLANDR	PLD_138727	PLD-like
ALASHHQRNMVIAANS---STDPPDDQPVAVVGGIDLANDR	PLD_163069	
VASSHHQRKTLVIAANK---STGKDEHPVAVVGGIDLTNDR	PLD_138537	
GLSSHHQRKTLVIAANK---STGKDEH---YVGGIDLTNDR	PLD_138544	TM-PLD
GSGILHQRKIWFDSRH-----VYVGSANMDWKS	PLD_139486	
ATSSHHQRKSVIVR-----KGRDL---VAVVGGVDTNDR	PLD_127024	
PTSSHHQRKSVIVR-----KGRDL---VAVVGGVDTNDR	PLD_127026	
PTSSHHQRKSVIVR-----KGRDL---VAVVGGVDTNDR	PLD_129242	
RYAFHHQRKLCIDEET-----IMATGCBVNTER	PLD_128805	

Fig. 7. Sequence comparison of the first HKD motif of *Phytophthora* phospholipase Ds (PLDs). Alignment of the first HKD motif of *Phytophthora* PLDs and representatives of the PXP-PLD (HsPLD1, MmPLD1, AtPLD ζ 1) and C2-PLDs (AtPLD α 1, AtPLD β 1, and AtPLD γ 1). The consensus residues for the HKD motif are shown in the first line. Symbols below the alignment indicate identical residues (*), conserved substitutions (:), or semiconserved substitutions (.). *Phytophthora sojae* pseudogenes that lack the first HKD motif were omitted. Amino acids that are identical in at least 75% of the 24 homologues are shaded in gray. Accession numbers: HsPLD1: NP_002653.1; MmPLD1: NP_032901; AtPLD ζ 1: NP_188302; AtPLD α 1: NP_188194; AtPLD β 1: NP_565963; AtPLD γ 1: NP_192922.

in plant C2-PLDs and, to a lesser extent, in PXP-PLD and PXTM-PLD. In contrast, other organisms have secreted PLDs that are much more divergent from PXP-PLD. Moreover, the combination of a PLD catalytic domain and transmembrane regions in one protein is unprecedented and suggests that extracellular signals can directly initiate PLD activation. Also, in other enzymes, unexpected transmembrane regions were found. One is a GPCR signature combined with a PIPK domain which was found in more than 10 proteins. The only known homologue outside genus *Phytophthora* has a role in cell density signaling and we anticipate that, similar to PXTM-PLDs, GPCR-PIPKs monitor extracellular signals that initiate PIPK activity. Another example is a protein with a combination of transmembrane regions and a PIK catalytic domain; several other proteins with PIK and PIPK catalytic regions also appear to have domain compositions not observed before.

Based on the diversity and the many novel features that we observed in phospholipid-modifying enzymes in *Phytophthora* spp., we speculate that oomycetes have biochemical pathways that deviate from the traditional ones. This inventory is just a first step. Next is further characterization of the enzymes, their activities, and their substrates.

MATERIALS AND METHODS

Putative genes that encode phospholipid-signaling enzymes initially were identified based on the automatic annotation at the publicly accessible database at the Joint Genome Institute (JGI) of the United States Department of Energy. Additionally, searches were performed with the use of a range of sequences of phospholipid-signaling proteins and genes available from plants, animals, fungi, and oomycetes to probe the genome databases of both *P. sojae* and *P. ramorum* using the BLASTN, TBLASTN, and BLASTP algorithms as incorporated in the JGI accession page. In most cases, the parameters were set at the default values; however, occasionally, modifications were used. The sequences used included many representatives from each family of phospholipid-signaling enzymes that was investigated. The putative homologues that were detected were characterized based on conserved domains, identities, and E values. The presence of homologous between *P. sojae* and *P. ramorum* in the genome sequence was investigated and models were corrected when necessary. Each homologue found was used for blasting the NCBI database to check for similar genes in other organisms and for the identification of domain compositions.

P. sojae- and *P. ramorum*-derived sequences were used for BLAST analysis of EST databases available from *P. sojae* and *P. infestans* (Randall et al. 2005). The Syngenta *Phytophthora* Consortium database was used for *P. brassicae* EST mining.

Multiple alignments were constructed by Vector NTI align program (Informax) or with ClustalX (version 1.83) with default parameters. Distance-based trees were generated using the bootstrap option and Njplot. Alternatively, multiple alignments were performed by CLUSTALW at the EMBL-EBI search launcher.

Conserved domains were transformed from either the JGI derived sequences or multiple databases, including SMART, NCBI, and Interproscan, and results were merged and incorporated in the overview tables. Signal peptides were detected via SignalP using default parameters (Bendtsen et al. 2004). Predictions of putative transmembrane segments were performed and verified using the PredCon II program (Arai et al. 2004) or the protein predict program (Rost et al. 2003). For the TM-PLDs, the ProtFun2.1 server was used for specific analysis. Other genome sequences were accessed via the JGI website and several others described by Galperin (Galperin 2004).

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AUTHOR-RECOMMENDED INTERNET RESOURCES

- DOE Joint Genome Institute (JGI) *T. pseudonana* genome database: genome.jgi-psf.org/thaps1/thaps1.home.html
- DOE JGI *P. ramorum* genome database: genome.jgi-psf.org/ramorum1/ramorum1.home.html
- DOE Joint Genome Institute (JGI) *P. sojae* genome database: genome.jgi-psf.org/sojae1/sojae1.home.html
- The Phytophthora Functional Genomics database: www.pfgd.org
- United States Department of Energy: www.jgi.doe.gov
- The Virginia Bioinformatics Institute (VBI) microbial database: phytophthora.vbi.vt.edu

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