Phytopathology Research

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The La-related protein PsLARP4_5 is crucial for zoospore production and pathogenicity in *Phytophthora sojae*

Can Zhang[1†], Shanshan Chen[1†], Fan Zhang[1], Yuxin Zheng[1], Yuke Wang[1] and Xili Liu[1,2*]

Abstract

A large number of La-related proteins (LARPs), most of which share a La-motif and one or more adjacent RNA-recognition (RRM) domains, are known to play a function in diverse processes. Among the LAPRs, LARP4 and LARP5 have been mainly reported to act as positive translation factors. In *Phytophthora sojae*, only one protein with typical LARP protein features was identified, and it was named LARP4_5 due to the presence of an RRM_LARP4_5-like domain. The *PsLARP4_5* gene was significantly upregulated in zoospores and during the infection stage. By comparing the biological characteristics of a wild-type strain with three *PsLARP4_5* knockout transformants, it was found that *PsLARP4_5* was involved in mycelial growth, sporangium and zoospore production, and pathogenicity of *P. sojae*. Further analysis of the transcriptome indicated that many differentially expressed genes could participate in several essential biological processes in the *PsLARP4_5* transformant, including translation, as structural constituents of ribosomes or cytosolic large ribosomal subunits and others. Notably, 76 genes with a role in the ribosome pathway were downregulated in the *PsLARP4_5* transformant, suggesting that *PsLARP4_5* might affect translation. Overall, these findings indicate that PsLARP4_5 plays an essential role in the development and pathogenicity of *P. sojae*.

Keywords *Phytophthora*, La-related proteins, Pathogenicity, Ribosome pathway

Background

The La-motif (LaM) is an RNA-binding domain defining a superfamily of RNA-binding proteins conserved across eukaryotes (Bousquet-Antonelli and Deragon 2009). Most organisms generally possess a true La protein ortholog with an LaM and one or more adjacent RNA-recognition (RRM) domains. The functions of the La module have been best studied in genuine La proteins (Wolin and Cedervall 2002). The genuine La protein binds to 30-oligo (uridylic acid) stretches (UUU-3′OH) found in newly synthesized RNA polymerase III (Pol III) transcripts, such as tRNA and 5S rRNA precursors. The La module is thought to protect RNA trailers from 30-exonuclease digestion and to assist their folding via RNA chaperone activity (Koso et al. 2016).

There is a significant large number of La-related proteins (LARPs), most of which share the conserved adjacent LaM and RRM domains, but these proteins function in diverse processes (Yang et al. 2011; Aoki et al. 2013). Six different LARPs have been identified in humans (LARP1, 1B, 4, 5/4B, 6, and 7) based on their homology with genuine La proteins (Stavraka and Blagden 2015). LARP7 family members bind the 3′ end of Pol III transcripts. However, they have developed specificity for a subset of Pol III RNA targets (Prathapam et al. 2005;

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Muniz et al. 2013; Eichhorn et al. 2018) and facilitate specific functions (Aigner et al. 2003; Stone et al. 2007; Markert et al. 2008; Hasler et al. 2020; Wang et al. 2020). In contrast, other LARP members, including 1, 4, 5/4B, and 6, are mostly cytoplasmic and interact with mRNA rather than Pol III transcripts (Kuspert et al. 2015; Zhang et al. 2016; Mattijssen et al. 2017; Fonseca et al. 2018).

LARP4 and its paralog LARP5/4B were found to act as positive translation factors and interact with the poly(A)-binding protein (PABP) and receptor for activated C kinase (RACK1) via similar protein-protein interactions (Angenstein et al. 2002; Bayfield et al. 2010; Burrows et al. 2010; Schäffler et al. 2010; Yang et al. 2011). The *LARP4* genes are present in all animals tested and some protists, but not in plants and yeasts (Merret et al. 2013). The overexpression of human *LARP4* resulted in increased mRNA stability, indicating that LARP4 promotes mRNA stability (Yang et al. 2011). LARP4 could regulate cell morphology by binding to and translational regulation of mRNAs encoding cytoskeletal regulators. Recent research suggests that the observed increase in translational output upon the overexpression of *LARP5/4B* can be attributed, at least in part, to an increase in the poly(A) tail length and a stabilization of the transcripts (Mattijssen et al. 2017). The mammalian LARP4 has an affinity for poly(A) RNA, suggesting it could bind to mRNAs' poly(A) tail, while LARP4B binds to AU-rich regions in the 3′ untranslated regions of mRNAs. This observation implies that LARP4 and LARP4B may have distinct functions (Kuspert et al. 2015).

Oomycetes are filamentous microorganisms that are evolutionarily distant from fungi, but phylogenetically closer to brown algae and diatoms (Thines and Kamoun 2010; Kroon et al. 2012). *Phytophthora sojae* is an economically important plant pathogen that causes root and stem rot in soybean, leading to substantial economic losses to soybean production yearly (Tyler 2007; Zhang et al. 2020). With the completion and publication of its genome sequence, *P. sojae* has gradually become a model species for studying oomycete plant pathogens (Tyler 2007). No LARP4 and LARP5 proteins were ever described in *P. sojae*, but in this study, we identified a protein containing the RRM LARP 4_5-like domain (named PsLARP4_5). This study aims to investigate the functions of the *PsLARP4_5* gene in *P. sojae*.

Results

Sequence and phylogenetic analyses of putative LAPRs in *P. sojae*

We conducted a search for "La motif" in the *P. sojae* database (http://www.jgi.doe.gov) and identified six related proteins. Upon domain analysis of these proteins, we found that only one displayed the typical feature of a LARP protein (protein ID: 554114). The corrected *PsLARP4_5* gene model is 1692 bp in length, with an 87 bp intron, and encodes a protein of 534 amino acids. The corresponding protein contains a LAM superfamily domain, RRM_LARP4_5-like domain, and PRK12323 superfamily domain; thus, it was named LARP4_5 (Fig. 1a).

The phylogenetic analysis of LARP4_5 proteins from different organisms indicated that they are relatively conserved within oomycetes (Fig. 1b). Interestingly, LARP4_5 proteins in *Phytophthora* form a distinct cluster, while LARP4 or LARP5 of other species can also be clustered separately. However, LARP4_5 of *Phytophthora* was not clustered with either LARP4 or LARP5, indicating that it may have a unique function in *Phytophthora*.

Expression analysis of *PsLARP4_5*

The *PsLARP4_5* gene was expressed in all tested developmental stages and during infection stages. The expression level of *PsLARP4_5* in zoospores was significantly upregulated, more than tenfold compared to mycelia. In the sporangium and cyst stage, the expression of *PsLARP4_5* was upregulated and downregulated, respectively, about twofold compared to mycelia (Fig. 2). During infection, the expression of *PsLARP4_5* was upregulated about 20-fold compared to the mycelium stage. This result suggests that *PsLARP4_5* may play an essential role in the development of zoospores and during the infection stage.

The phenotypes of *PsLARP4_5* deletion transformants in *P. sojae*

We obtained six *PsLARP4_5* homozygous deletion transformants, three of which (KT1, KT18, and KT40) are independent transgenic lines displayed in Fig. 3. The biological characteristics of the transformants were compared to those of WT (the parental wild-type isolate), EV (the control isolate of *P. sojae* transformed with an empty vector), and LC (the *PsLARP4_5* complemented isolate). The *PsLARP4_5* deletion transformants displayed reduced mycelial growth compared with the wild-type strain P6497 (Fig. 3a). Moreover, the sporangium number, sporangium release rate, and zoospore production were significantly decreased in the *PsLARP4_5* mutants (Fig. 3b–d). The cyst germination and oospore production of *PsLARP4_5* knockout transformants were similar to P6497 (Additional file 1: Figure S1). In addition, the mycelial growth rates of the *PsLARP4_5* transformants on sorbitol or KCl media, remained unchanged (Additional file 1: Figure S2). Therefore, *PsLARP4_5* is involved in the mycelial growth, sporangial, and zoospore production of *P. sojae*.

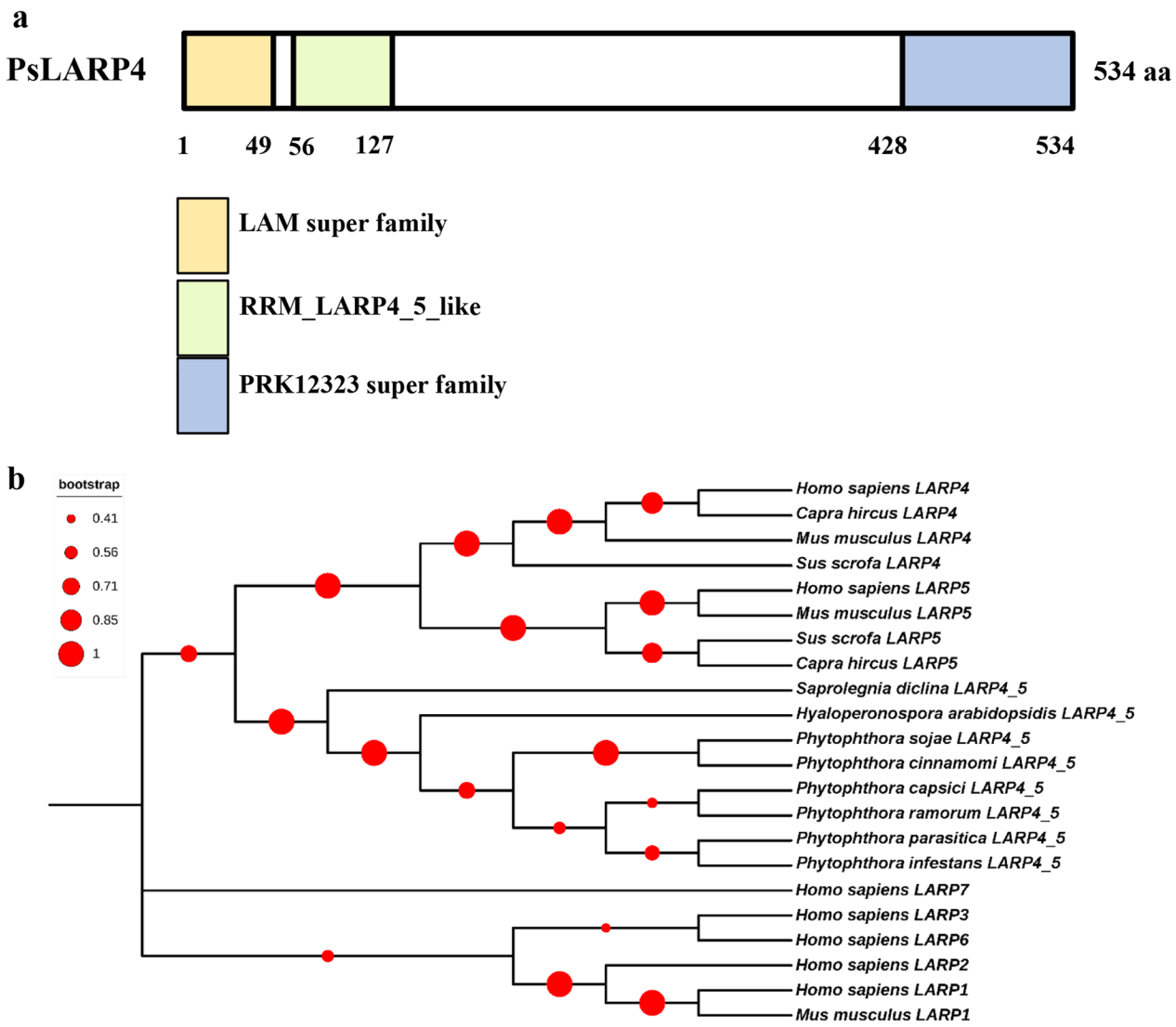

Fig. 1 Sequence characteristics of PsLARP4_5. **a** Domain structures of the PsLARP4_5 protein predicted by the NCBI CD search program. **b** Phylogeny analysis of PsLARP4_5 with other LARP proteins reported in different species

PsLARP4_5 is required for the pathogenicity of *P. sojae*

The interaction between *Phytophthora* spp. and plants is complex and involves various factors and pathways (Naveed et al. 2020). To explore whether *PsLARP4_5* mediates a role in this interaction, the pathogenicity of *PsLARP4_5* transformants was compared with that of the wild-type strain. The etiolated soybean seedlings inoculated with WT, EV, and LC zoospores or mycelial plug displayed typical and severe symptoms on entire plants at two days postinoculation (dpi). However, the *PsLARP4_5* transformants produced significantly smaller lesions beyond the inoculation site ($P < 0.05$) (Fig. 4a, b). The biomass was significantly reduced in soybean seedlings inoculated with the *PsLARP4_5* transformants as shown in Fig. 4c, d. Moreover, the *PsLARP4_5* transformants lost its ability to penetrate cellophanehas (Fig. 4e). These results indicate that *PsLARP4_5* is associated with *Phytophthora* pathogenicity.

P. sojae may remodel its transcriptome to compensate for *PsLARP4_5* deficiency

To further address whether the loss of *PsLARP4_5* has an effect on *P. sojae*, RNA-Seq was performed in mycelia during sporangium production. The transcriptome comparison was conducted between the *PsLARP4_5* transformant KT1 and the wild-type strain P6497 (Additional file 2: Table S1). Among approximately 22,000 genes expressed at this stage, 1168 were significantly

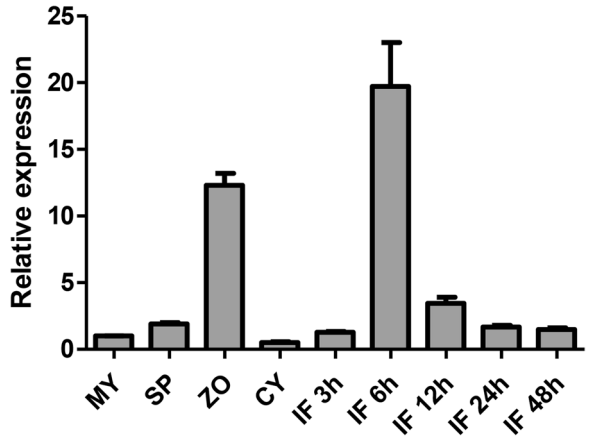

Fig. 2 *PsLARP4_5* transcript levels. The transcript levels of *PsLARP4_5* were determined by qRT-PCR. RNA was extracted from vegetative mycelia (MY), sporulating hyphae (SP), zoospores (ZO), cysts (CY), and during the early stages of infection (IF, 3, 6, 12, 24, and 48 h post-inoculation) on soybean leaves. The expression values of *PsLARP4_5* were normalized using two housekeeping genes and are shown relative to MY, which was used as a reference with a value of 1.0. Error bars represent standard errors calculated using three biological replicates for each sample

upregulated, and 1338 were downregulated in the knockout transformant compared to the wild-type strain.

Gene ontology (GO) analysis indicated that many differentially expressed genes (DEGs) participate in several essential biological processes, such as translation, and as structural constituents of ribosomes, cytosolic large ribosomal subunits, cytosolic small ribosomal subunits, ribosomal small subunit assembly, and others (Fig. 5a). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment indicated that the DEGs are involved in different pathways (Fig. 5b). Notably, 76 genes with a role in the ribosome pathway were downregulated in the *PsLARP4_5* transformant. Genes displaying more than threefold differential expression levels are listed in Table 1. The expression levels of five differentially expressed genes of interest were verified by qRT-PCR using the mycelium samples with sporangia (Fig. 6). The consistency between the results of the two methods verified the reliability of this quantitative transcriptomics technique.

Discussion

A large number of LARPs have been reported to function in diverse processes in mammals, and six different LARPs were identified in humans (LARP1, 1B, 4, 5/4B, 6, and 7) (Bousquet-Antonelli and Deragon 2009; Bayfield et al. 2010; Stavraka and Blagden 2015). However, only one LARP4_5 protein with the typical feature of a LARP protein was identified in *P. sojae* and was found to be conserved in *Phytophthora*. In particular, LARPs are absent in plants and yeasts (Merret et al. 2013). The lack of LARPs in plants and the limited number of LARPs in pathogens require further investigation. In addition, the LARP4_5 protein of *Phytophthora*

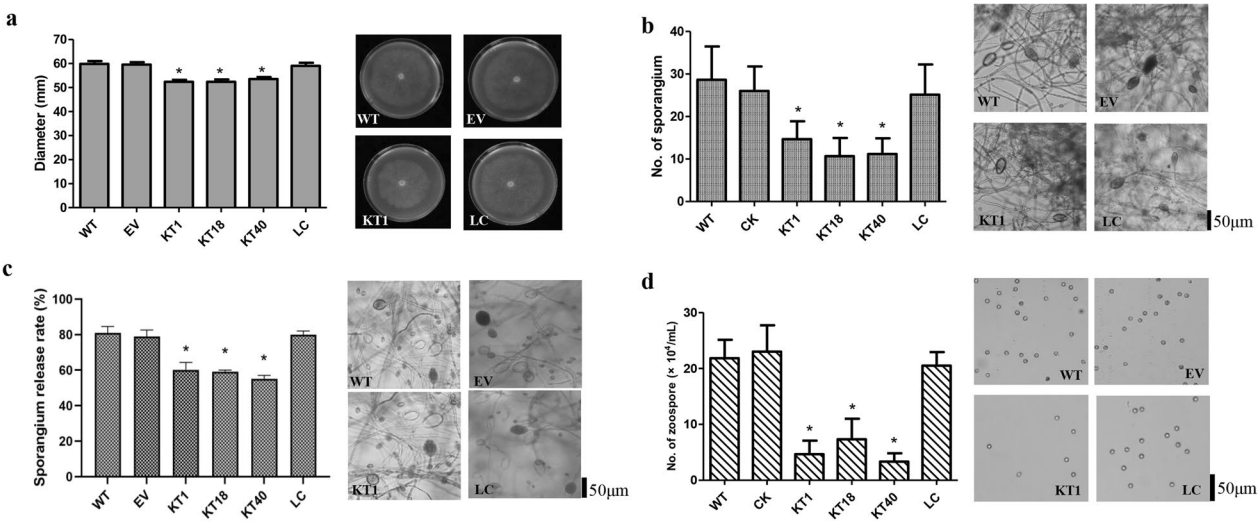

Fig. 3 The characteristics of *PsLARP4_5* deletion transformants compared to the wild-type controls in *P. sojae*. The parental wild-type isolate (WT) is P6497; EV is the control isolate of *P. sojae* transformed with an empty vector; LC is the *PsLARP4_5* complemented isolate; KT1, KT18, and KT40 are the *PsLARP4_5* gene deletion transformants. **a** Mycelial growth, **b** number of sporangia, **c** release rate of sporangia, and **d** zoospore production in the mutant and WT or EV or LC isolates. Bars represent the mean ± standard error (SD) from three biological replicates, with each having three technical replicates. All statistical analyses were conducted using DPS software 7.05. Differences between the means were determined by the least significant difference (LSD) multiple range test at $p = 0.05$

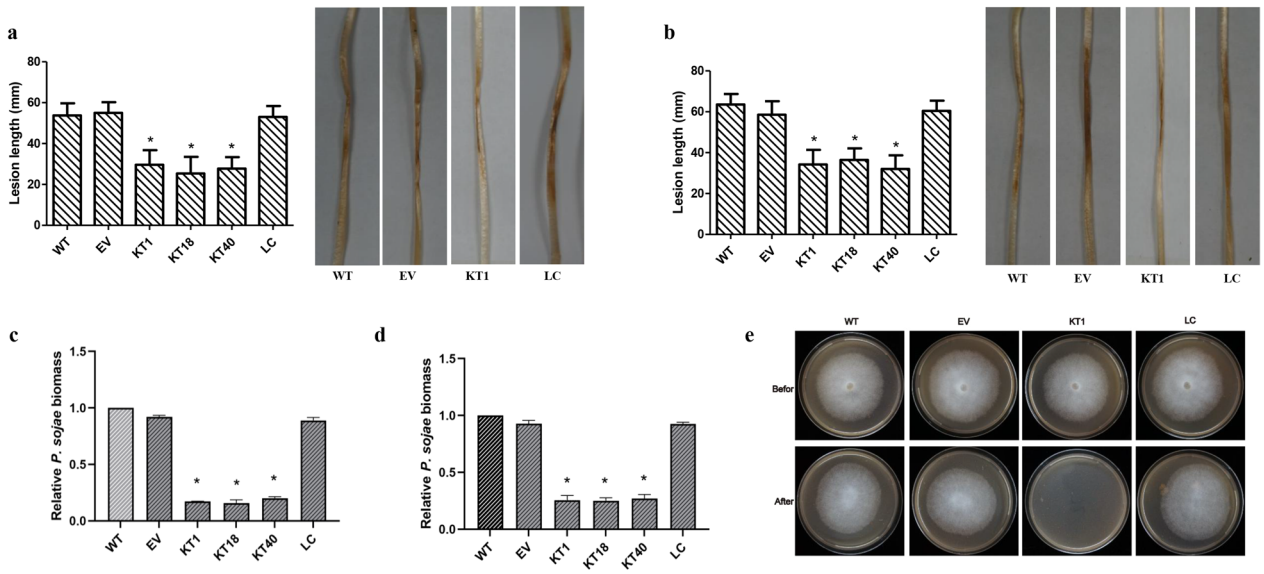

Fig. 4 Pathogenicity assays on soybean leaves and seedlings. The parental wild-type isolate (WT) is P6497; EV is the control isolate of *P. sojae* transformed with an empty vector; LC is the *PsLARP4_5* complemented isolate; KT1, KT18, and KT40 are *PsLARP4_5* deletion transformants. **a** Soybean seedlings were inoculated with 10 µL of zoospore suspension (1×10^4 zoospores/mL) for 3 days. **b** Soybean seedlings were inoculated with a 5-mm mycelial plug for 3 days. **c** The *P. sojae* biomass in soybean seedlings inoculated with 10 µL of zoospore suspension (1×10^4 zoospores/mL). **d** The *P. sojae* biomass in soybean seedlings inoculated with a 5 mm mycelial plug. **e** The mycelial growth of strains on V8 media for 5 days after removal of the two-day cellophane culture. Bars represent the mean ± standard error from at least six seedlings. All statistical analyses were conducted using DPS software 7.05. Differences between the means were determined by the least significant difference (LSD) multiple range test at $p = 0.05$

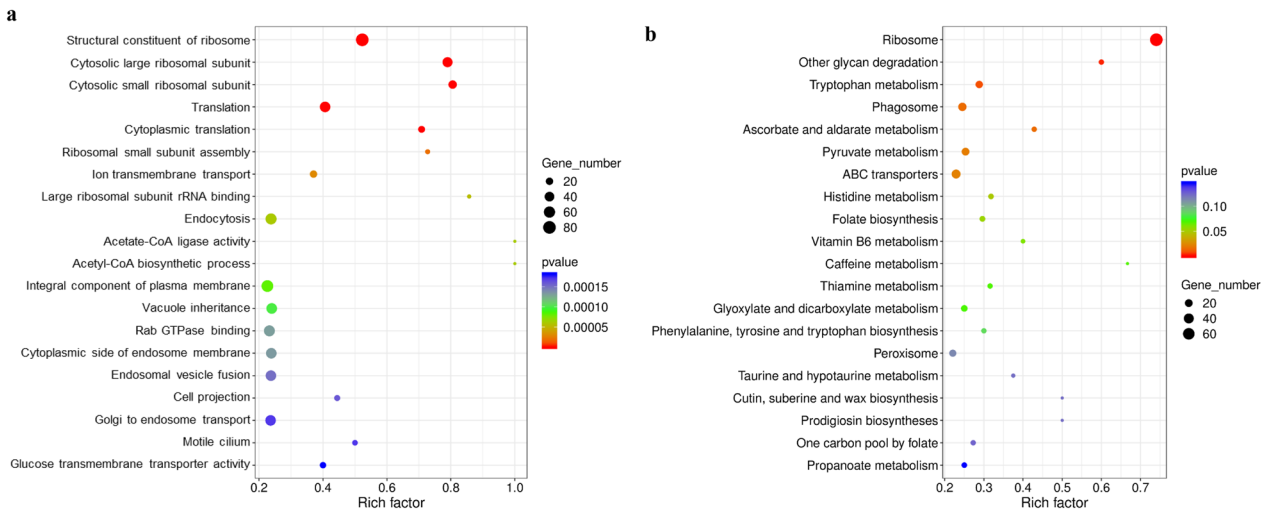

Fig. 5 Comparison of the wild-type and *PsLARP4_5* deletion mutant transcriptomes. Clusters of enriched GO terms **a** and KEGG pathways **b** on differentially expressed genes in the knockout transformant compared to the wild-type strain

was neither clustered with LARP4 and LARP5 nor other LAPRs of mammals, indicating the specificity of *PsLARP4_5* in *P. sojae*. We found that a domain belonging to the PRK12323 superfamily, which is absent in mammal LARP4 and LARP5 proteins, was predicted in the LARP4_5 protein. The PRK12323 superfamily is related to the DNA polymerase III subunit gamma, and previous studies showed that LARP7 could bind the 3′ end of Pol III transcripts (Aigner et al. 2003; Markert et al. 2008; Hasler et al. 2020; Wang et al. 2020). However, it is unknown whether LARP4_5 combines the functions of different LARP proteins. Overall, the

Table 1 List of the differentially expressed genes enriched in the ribosome KEGG pathway

Accession No.[a]	Description	Fold change[b]
PHYSODRAFT_285181	40S ribosomal protein S20	0.25
PHYSODRAFT_354758	40S ribosomal protein S14	0.25
PHYSODRAFT_285573	Ribosomal protein S2	0.26
PHYSODRAFT_289114	60S ribosomal protein L44-like protein	0.26
PHYSODRAFT_558348	Putative ribosomal protein	0.27
PHYSODRAFT_354074	60S ribosomal protein L27A	0.28
PHYSODRAFT_494630	Ribosomal protein S5	0.28
PHYSODRAFT_285079	40S ribosomal protein S24	0.28
PHYSODRAFT_353469	40S ribosomal protein S18	0.28
PHYSODRAFT_310652	Ribosomal protein L34e	0.28
PHYSODRAFT_284181	60S ribosomal protein L35a	0.28
PHYSODRAFT_401211	60S ribosomal protein L38	0.28
PHYSODRAFT_287105	40S ribosomal protein S3	0.28
PHYSODRAFT_285581	Ribosomal protein L14 domain-containing protein	0.29
PHYSODRAFT_353704	Ribosomal_L23eN domain-containing protein	0.29
PHYSODRAFT_285013	40S ribosomal protein S1	0.29
PHYSODRAFT_286426	S10_plectin domain-containing protein	0.29

[a] Accession number from *P. sojae* JGI database

[b] The down regulation level of gene expression in the *PsLARP4_5* transformant compared with the wild-type parental strain

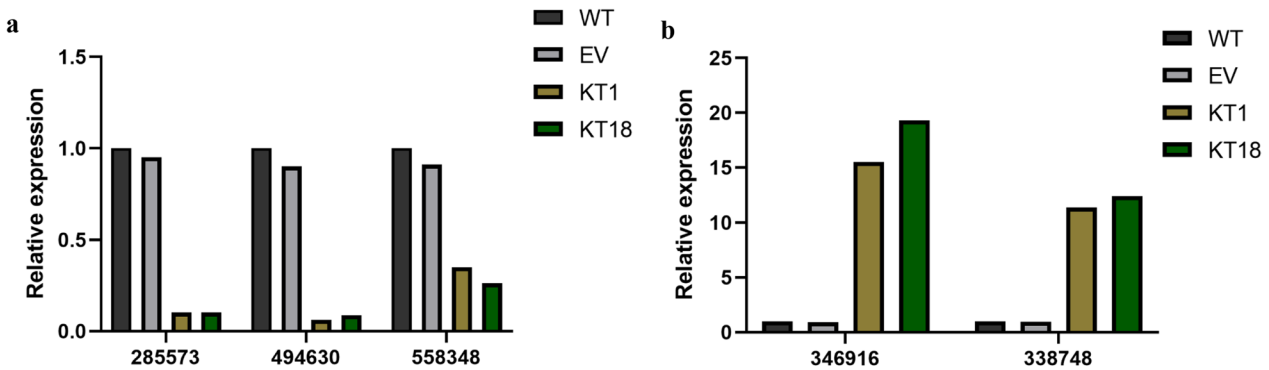

Fig. 6 Assessment of gene expression level of five genes selected from quantitative transcriptomics results by qRT-PCR. The parental wild-type isolate (WT) is P6497; EV is the control isolate of *P. sojae* transformed with an empty vector; KT1, KT18, and KT40 are *PsLARP4_5* deletion transformants. The expression values of each sample at the sporangium production stage are shown, **a** relative to the 285573 gene of the WT strain for the down-regulated genes, and **b** relative to the 346916 gene of the WT strain for the up-regulated genes. Two housekeeping genes were used to normalize the expression of target genes

results indicate that LARP4_5 might have a unique function.

Until now, few studies have reported the function of LARPs in plant pathogens. In order to investigate the function of *P. sojae* LARP4_5, the biological characteristics of different growth and development stages in the *LARP4_5* mutants were determined. *PsLARP4_5* was involved in the zoospore production of *P. sojae*, which was consistent with the expression level of the gene. Coherently, previous studies reported that the overexpression of the human *LARP4* could modulate cell morphology through the translational regulation of mRNAs encoding cytoskeletal regulators (Yang et al. 2011). However, no significant differential expression of genes encoding cytoskeletal regulators was found in the *PsLARP4_5* mutants. Further research is required to unravel how PsLARP4_5 affects zoospore production.

Many factors can affect the pathogenicity of pathogens in plants, including parameters like the mycelial growth rate, spore germination, and formation of infection structure (Xie et al. 2016; Liu et al. 2017; Zhang et al. 2021). In this study, the mycelial growth of the *PsLARP4_5*

knockout transformants was inhibited, which may explain why these transformants' pathogenicity was affected. However, the reduction in virulence was greater than the rate of mycelial growth, and we also observed a decrease in virulence when the plants were challenged with zoospores. The loss of cellophane penetration suggests that knockout transformants' ability to penetrate the host epidermis may also be reduced. The pathogens could secrete distinct pathogenic effectors to fight plants' immune responses during infection (Hogenhout et al. 2009; Xu and Mandgen 1997; Tyler 2009). The decreased virulence of the *PsLARP4_5* knockout mutants may be caused by different mechanisms.

Interestingly, over 2500 genes were differentially expressed in the *LARP4_5* knockout mutants compared to the WT. The single-gene knockout mutants of *Phytophthora* species displayed fewer differentially expressed genes than the *LARP4_5* knockout mutants (Wang et al. 2022a, b). In this study, the ribosome pathway was significantly enriched after deleting the *PsLARP4_5* gene. Ribosomes are RNA-protein complexes that constitute the protein factory and are present in all living organisms (Wang et al. 2011). They form a translational machinery catalyzing protein synthesis (Khatter et al. 2015). The observation of enrichment in the ribosome pathway in the knockout mutant is consistent with LARP4 and LARP5 acting as positive translation factors in humans (Angenstein et al. 2002; Schäffler et al. 2010; Yang et al. 2011). This result is also consistent with the increased sensitivity of the *PsLARP4_5*-disruption transformants to protein synthesis inhibitor cycloheximide. Moreover, LARP4 and LARP5 could interact with PABP and RACK1 proteins via similar protein-protein interactions (Schäffler et al. 2010; Yang et al. 2011). However, only a PABP protein but no direct RACK1 was identified in *P. sojae*, and the expression level of *PABP* was unchanged in the mutants. These results indicate that the PsLARP4_5 might be involved in translation in a different way, such as interacting with proteins that are not PABP and RACK1, which needs follow-up research.

Conclusions

The La-related proteins LARP4 and LARP5 are present in animals and some protists, but not in plants or yeasts. These proteins were found to act as positive translation factors. In this study, only the LARP protein PsLARP4_5 containing an RRM LARP 4_5-like domain was identified in *P. sojae*. This gene is involved in the mycelial growth, sporangial, zoospore production, and pathogenicity of *P. sojae*. Several important biological processes, primarily related to the ribosome pathway, were significantly affected in the *PsLARP4_5* transformant. Future research is needed to determine whether the *PsLARP4_5* gene, maybe a paralog of human *LARP4* and *LARP5*, could affect the translation of *Phytophthora*.

Methods

P. sojae strains, plant cultivars, and culture conditions

The *P. sojae* isolate P6497 was used as the wild-type strain in this study. P6497 and all transgenic strains were grown in 10% V8 juice agar at 25 °C in the dark. Soybean (cv. 'Japan Blue') plants were grown in a glasshouse at 25 °C for 5–7 days in the dark, and etiolated soybean seedlings were harvested.

Sequence and phylogenetic analyses of LARPs

The *PsLARP4_5* gene was blasted against the JGI (Joint Genome Institute) genome database (https://genome.jgi.doe.gov/portal/). The *LARP* sequences from *Phytophthora* were retrieved from the Fungi DB database (http://Fungidb.org), and the *LARP* sequences from other species were collected from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). The valid accession numbers of protein sequences downloaded from different databases are listed in Additional file 2: Table S2. The putative domains of PsLARP4_5 were predicted by the Conserved Domain Database on NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The Mega 6.0 software was used to construct the phylogenetic tree (Tamura et al. 2013).

PsLARP4_5 gene deletion and complementation in *P. sojae*

The CRISPR/Cas9 system was used to knock out the *PsLARP4_5* gene. The sgRNA was designed using *EuPaGDT* (http://grna.ctegd.uga.edu/) and cloned into pYF515 plasmids following the previously described protocol (Fang et al. 2017). The sequences 1 kb upstream and 1 kb downstream of the target gene were amplified and cloned into a pBluescript II KS^+ donor vector. The replacement gene *NPTII* was then inserted between them for deletion, and the entire *PsLARP4_5* gene was inserted for complementation. The primers used in this study are listed in Additional file 2: Table S3.

Using previously described methods, we transformed the wild-type *P. sojae* strain P6497 through PEG-mediated protoplast transformation (Fang and Tyler. 2016). Putative transformants were transferred to the 10% V8 agar medium containing 50 μg/mL G418 and incubated for 3 days at 25 °C. The gene insertion into *P. sojae* was confirmed by PCR using the genomic DNA of transformants. The used primers are listed in Additional file 2: Table S3. At least three independent transformants were detected in each sample.

Phenotypic analysis of transformants in *P. sojae*

Mycelial growth

The diameter of the colony was measured by placing a mycelial plug (5 mm in diameter) on a 10% V8 agar medium and incubating it in the dark for 5 days.

Sporangium production

Ten 5 mm diameter mycelial plugs were placed on a Petri dish containing 20 mL of 10% V8 broth. The plates were incubated at 25 °C in the dark for 3 days, and the hyphae were repeatedly washed with 20 mL of sterile distilled water five times, followed by an incubation in the dark at 25 °C for 4–8 h, until sporangia developed. Sporangium production was determined by microscopy at 10× magnification.

Zoospore production

The production of zoospores was induced as described above. After washing the hyphae five times, 10 mL of sterile water was added to the dishes, and the samples were incubated in darkness at 25 °C for 8–10 h. The numbers of released sporangium and zoospore production were quantified with a hemacytometer.

Cyst germination

The zoospore suspensions were shaken using a vortex for 1 min to complete cystospore encystment. Cytospore germination was determined by microscopy after incubation at 25 °C for about 6 h in the dark (Sheng et al. 2021).

Oospore production

The strains were cultured on 10% V8 agar at 25 °C for 7 days in darkness. Oospore production was determined by microscopy at 10× magnification.

Infection assays

Pathogenicity on etiolated soybean seedlings was determined by infection with a 10 μL zoospore suspension (1×10^4 zoospores/mL) or a 5 mm mycelial plug. The seedlings were then incubated at 25 °C and 80% relative humidity in the dark for 3 days, and the length of the resulting lesions was measured. Each strain was tested using at least six seedlings.

qRT-PCR was used to measure relative pathogen biomass (the ratios of pathogen DNA to host DNA in the infected tissues). Infected plant tissues were harvested, and total genomic DNA was extracted using a DNeasy plant mini-kit (QIAGEN) following the recommended protocol. The *P. sojae Actin* gene was used to target the pathogen, and the soybean housekeeping gene *GmCYP2* served as the host target genes. The primer pairs (Additional file 2: Table S3) were designed to anneal specifically to target genes. Each experiment was repeated three times, each time with five replicates.

For the penetration assays, a sterilized cellophane membrane was overlaid onto V8 medium. After 2 days, half of the membranes on the media were removed. The cultures were incubated with or without the cellophane membrane for another 4 days. The hyphae were observed on the cellophane surface and in the underlying medium to determine if there were any breaches in the cellophane.

Mycelial growth in response to several stress inhibitors

The effect of the osmotic stress inhibitors sorbitol (0.5 M and 1 M) and KCl (0.25 M and 0.5 M) on the growth of the *PsLARP4_5* transformants and the wild-type strain P6497 was assessed. The experiment was carried out using the mycelial growth assay, where the strains were inoculated on V8 agar plates containing one of the inhibitors. The plates were then incubated at 25 °C in darkness for 4 days. Colony diameters were measured with a fine crosshair. Each treatment was represented by three replicates, and the entire experiment was conducted three times.

RNA-Seq and transcriptome analysis

The wild-type strain P6497 and the transformant KT1 were used for RNA-Seq analysis. Mycelium samples with sporangia were collected, frozen in liquid nitrogen, and stored at -80 °C for RNA extraction. Total RNA was extracted using the SV Total RNA Isolation kit (Promega, Beijing, China), and two biological replicates for each isolate were used for transcriptome. After quality control and mRNA purification, the cDNA libraries were constructed by LC-Bio Technology Co., Ltd. (Hangzhou, China), and the sequencing was performed on an Illumina NovaSeq™ 6000.

The preprocessed RNA-Seq reads were mapped to the reference genome of the *P. sojae* strain P6497 on the Fungi DB database. The DEGs were identified from the RNA-Seq data with a cut-off of the corrected p-value < 0.05, using a $\log_2$-fold change greater than or equal to 1 as a threshold. To evaluate repeatability between replicates, we calculated the Pearson correlation coefficient between two samples. For the functional classification of DEGs, we conducted GO enrichment analysis (http://geneontology.org/). KEGG analysis was performed using an online database (www.genome.jp/kegg) as a reference.

RNA extraction and qRT-PCR assays

The *PsLARP4_5* gene transcription levels were examined by quantitative qRT-PCR at different developmental stages and during infection. Samples included vegetative mycelia (MY), hyphae with sporangia (SP), zoospores

(ZO), cystospores (CY), and infection (IF 3, 6, 12, 24, and 48 h) stages. The SP samples of the wild-type strain P6497, a control isolate, and the *PsLARP4_5* transformants were also collected for the determination of the expression of five genes selected from quantitative transcriptomics results. Total RNA was extracted using an SV Total RNA Isolation kit (Promega, Beijing, China) following the manufacturer's instructions. RNA with sufficient quantity and quality was used as a template for first-strand cDNA synthesis using the PrimeScript™ Reverse Transcriptase (Takara, Beijing, China).

The qRT-PCR reactions were performed following the manufacturer's instructions (FastSYBR Mixture, CW Biotech, Beijing, China) using a qPCRsoft 3.4 system (qTower 2.2, Analytik Jena AG, Jena, Germany). The primers used for this experiment are listed in Additional file 2: Table S3. The *P. sojae* housekeeping genes *RPS* and *RPL13α* were used as endogenous controls. Data were analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). All treatments were represented by at least three replicates, and the entire experiment was conducted three times.

Statistical analysis

All statistical analyses were conducted using DPS software version 7.05. Mean differences were determined by the least significant difference (LSD) multiple range test at $p=0.05$.

Abbreviations

DEGs	Differentially expressed genes
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LaM	La motif
LARPs	La-related proteins
LSD	Least significance difference
PABP	Poly(A)-binding protein
PCR	Polymerase chain reaction
PsLARP4_5	A protein containing the RRM LARP4_5-like domain in *P. sojae*
qRT-PCR	Quantitative real-time PCR
RACK1	Receptor for activated C kinase
RRM	RNA-recognition

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00210-z.

Additional file 1: Figure S1. The cyst germination rate and oospore production of PsLARP4_5 deletion mutants compared to the wild-type controls in *P. sojae*. The parental wild-type isolate (WT) was P6497; EV was the control isolate of *P. sojae* transformed with an empty vector; KT1, KT18, and KT40 were PsLARP4_5 deletion mutants. The cyst germination **a** and oospore production **b** in mutants, WT, or EV control isolates. Bars represent the mean ± standard error (SD) from three biological replicates, and three repeated experiments. **Figure S2.** Mycelial growths of *PsLARP4_5* deletion transformants under osmotic stress conditions. The parental wild-type isolate (WT) is P6497; EV is the control isolate of *P. sojae* transformed with an empty vector; KT1, KT18, and KT40 are PsLARP4_5 deletion transformants. **a** The mycelial growth diameters on V8 media added with 0, 0.5, and 1 M sorbitol. **b** The mycelial growth diameters on V8 media added with 0, 0.25, and 0.5 M KCl. Bars represent the mean ± standard error (SD) from three biological replicates and three repeated experiments.

Additional file 2: Table S1. List of all genes identified in the parental wild-type isolate and a *PsLARP4_5* deletion mutant in *P. sojae*. **Table S2.** Accession numbers of the LARP sequences of different organisms. **Table S3.** Primers used in this study.

Acknowledgements

Not applicable.

Author contributions

XL and CZ designed the research. CZ, SC, FZ, and YZ performed the research. CZ and YW analyzed the data. CZ and XL wrote the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (32172447; 31801761).

Availability of data and materials

Not applicable.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 5 June 2023 Accepted: 23 October 2023
Published online: 30 November 2023

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