



# Molecular factors associated with pathogenicity of *Phytophthora cinnamomi*

## Fatores moleculares associados com a patogenicidade de *Phytophthora cinnamomi*

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### ABSTRACT

*Phytophthora cinnamomi* is soil pathogen that has a wide range of hosts in several countries and different climates. This fungus is responsible for the chestnut ink disease (*Castanea sativa* Miller) and death of the tree. Portugal stands out in the production of the European chestnut tree. However, between 2002 and 2004, there was a decrease of 27.3% in the distribution area of this tree due to *P. cinnamomi*. The aim of this study was to identify molecular factors possibly associated with the fungal pathogenicity through genomic sequences deposited at NCBI using bioinformatics tools. The first contig was used and the ORFs present in the sequences were identified. SmartBlast was used for homologous proteins. The prediction of cellular localization prediction of proteins was performed using four different tools: SignalP 4.1, Cello v.2.5, LOCTree3, Euk-mPLOC 2.0. Protein domains characterization was accomplished using PROSITE and the structure prediction was performed using Phyre2 server. We found 13 proteins probably associated with the pathogenicity of *P. cinnamomi* and its properties related to the infection were analyzed *in silico*. These results are important since they are a first step in the search for pathogenic factors.

**Keywords:** Chestnut ink disease, Oomycetes, Infection, Bioinformatics.

### RESUMO

*Phytophthora cinnamomi* é um patógeno do solo que possui uma ampla gama de hospedeiros em diversos países de diferentes climas. Esse fungo é responsável pela doença da tinta do castanheiro europeu (*Castanea sativa* Miller) e conduz à morte da árvore. Portugal se destaca na produção do castanheiro europeu. Entretanto, entre 2002 e 2004, houve uma redução de 27.3% na área de distribuição dessa área devido ao *P. cinnamomi*. O objetivo deste trabalho foi identificar fatores moleculares possivelmente associados à patogenicidade do fungo através de sequências genômicas depositadas no NCBI utilizando ferramentas de bioinformática. O primeiro *contig* foi utilizado e as ORFs presentes nas sequências foram identificadas. Foi utilizado o smartBlast em busca de proteínas homólogas. A predição da localização celular das proteínas foi feita através de quatro ferramentas: SignalP 4.1, Cello v.2.5, LOCTree3, Euk-mPLOC 2.0. A caracterização dos domínios dos produtos foi realizada utilizando o PROSITE e a predição das estruturas foi realizada utilizando o servidor Phyre2. Foram encontradas 13 proteínas provavelmente associadas com a patogenicidade de *P. cinnamomi* e as suas propriedades relacionadas com a infecção foram analisadas *in silico*. Esses resultados são importantes uma vez que são uma primeira etapa na busca por fatores patogênicos.

**Palavras-chave:** Tinta do castanheiro, Oomycetes, Infecção, Bioinformática.

## INTRODUCTION

The Oomycete fungus *Phytophthora cinnamomi* is a soil pathogen extremely aggressive and it has a wide range of hosts, that includes almost 500 plants species in more than 70 countries (Jung *et al.*, 2013). It was described firstly in Sumatra (Indonesia) by Rands (1922) as being the causative agent of cinnamon (*Cinnamomum burmannii*) stripe cancer.

Due to its potential pathogenicity, *P. cinnamomi* generates substantial economic losses in agriculture, afforestation, and horticulture. This pathogen is associated with the oak decline in Mediterranean Europe and with the oak disease in California (Brasier *et al.*, 1993; Robin *et al.*, 1998; Vettraino *et al.*, 2002; Garbelotto and Hüberli, 2006). Further, it causes root rot in fynbos in South Africa, leading to its death (von Broembsen & Kruger, 1985; Nagel *et al.*, 2013). In addition, its introduction in nature leads to severe consequences to the natural ecosystem and biodiversity (Hardham and Blackman, 2018).

The Oomycetes class, which includes genus *Phytophthora*, belongs to Pseudofungi phylum within the Chromista kingdom. The characteristics that define the Chromista are the asexual reproduction of mobile spores endowed with a flagellum adorned by tubular hairs, which are responsible for motility. These spores start the plant infection in many species of *Phytophthora*, including *P. cinnamomi* (Beakes *et al.*, 2012; Hardham and Blackman, 2018). *P. cinnamomi* also is responsible for the European chestnut ink disease (*Castanea sativa* Miller) and, worsening this problem, *C. sativa* is very susceptible to infections by this pathogen (de Sampaio e Paiva Camilo-Alves *et al.*, 2013). The symptoms of this disease include yellowing and premature fall off the leaves and the appearance of wet rot in the roots, which will lead to the death of the tree (Fernandes, 1953; Shattock, 2001).

The European chestnut tree has economic interests due to the fruit and wood production, as well as other *Castanea* species. Portugal is the third-largest European chestnut tree producer, with 27.337 tons in 35.426 hectares (INE, 2016) and the Cold Land Chestnut Production Zone is responsible for the production of 80% of the national chestnut (INIAV, 2016). However, the *C. sativa* production has

been declining over the years due to the epidemic proportions of *P. cinnamomi*, only between 2002 and 2004, the area of distribution decreased 27.3 % (Martins *et al.*, 2007; Pereira, 2017).

Despite several species are affected by *P. cinnamomi*, European chestnut trees receive special highlights in Portugal due to the economic importance for the country (INIAV, 2016). Additionally, *P. cinnamomi* is ranked as one of the most aggressive species of *Phytophthora* genus to the *C. sativa* (Vettraino *et al.*, 2001). However, the ink disease still the main threat once *P. cinnamomi* is resistant to the available fungicides and there are no effective options of pest control (Santos *et al.*, 2016). Still, the molecular mechanisms involved in the pathogenicity of *P. cinnamomi* remains unclear.

The plant infection by *P. cinnamomi* is initiated by binding the zoospores to the plant root elongation region (Hardham, 2005). This binding is provided by some proteins, like adhesine Vsv1 (Hardham and Gubler, 1990; Robold and Hardham, 2005). Then, zoospores enter the stage of encystment with the formation of biofilm from secreted proteins. Between 20-30 minutes after the zoospores encystment, the cyst germinates and the hyphae are formed (Hua *et al.*, 2013; Hardham and Blackman, 2018). These hyphae are responsible for the production of some enzymes that will degrade the plant cell wall, such as the ones that belong to the Cell Wall-Degrading Enzymes (CWDEs) family (Götesson *et al.*, 2002; Ospina-Giraldo *et al.*, 2010; Larroque *et al.*, 2012; Blackman *et al.*, 2014). The intracellular and intercellular growth of hyphae in the radicular cortex towards the cortical and vascular tissues leads to water stress and necrosis (Hosseini, 2010; Hardham and Blackman, 2018).

Bioinformatic provide promising tools for analysis in silico and to support in vivo assays. In the context of this work, ORFFinder and smartBLAST are two tools from NCBI that enable an easy and trustworthy search for open read frames in genomic sequences and provides determination of homology of these sequences (Rawat *et al.*, 2018). Concerning the protein structures, the SignalP 4.1 is a reliable tool for the prediction of the signal peptide (Petersen *et al.*, 2011; Ayalew *et al.*, 2017). Considering that secreted proteins are highly important to the pathogenicity of pathogens, the prediction of subcellular

localization is indispensable. Among the available online bioinformatics tools LocTree3 (Nair and Rost, 2005; Sanasam and Kumar, 2019), Cello (Yu *et al.*, 2004; Thakare *et al.*, 2016) and Euk-mPLoc (Chou, 2019) stand out. In line with this, the identification of domains and motifs of proteins are very useful to determine the proteins functions. PROSITE is an online tool for searching for significant sites in amino acid sequences (Gellért *et al.*, 2006; Sigrist *et al.*, 2009). Finally, determine the protein structure through modeling is essential to analyze its conformation. For this use, PHYRE2 is a very used web-based tool for modeling amino acid sequences (Zhang *et al.*, 2011; Singh and Gupta, 2016).

Considering the economic losses caused by *P. cinnamomi* in European chestnut trees it is extremely important that molecular factors responsible for the pathogenicity of this pathogen are identified. Thus, the aim of this study was to identify genes and its products related to the pathogenicity of *P. cinnamomi*, as well as the characterization of the results obtained through bioinformatics tools.

## MATERIAL AND METHODS

### Database of biological information

For the search of genes, the genomic sequences from *Phytophthora cinnamomi* used are deposited in the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>). To continue the research from the group used the sequence (REF: LGSK01000001.1) deposited by Studholme *et al.* (2016) which belongs to the strain MP94-48 (BioSample: SAMN03921829; BioProject: PRJNA290836; Assembly: GCA\_001314365.1).

### Open Read Frames (ORFs) search and homology

In order to find de ORFs present in genomic sequences we used the ORFFinder tool (<https://www.ncbi.nlm.nih.gov/orffinder/>), available by NCBI, with the following parameters: minimal ORF length: 300 nucleotides; genetic code: Standard; ORF start codon to use: "ATG" only.

The homology of proteins encoded by the ORFs founded was determined using the Basic Local

Alignment Search Tool (Blast), specifically the smartBlast version, also available by NCBI. This tool establishes the homology between the sequences deposited in several protein databases available.

### Prediction of cellular localization of proteins

The software SignalP 4.1 (<http://www.cbs.dtu.dk/services/SignalP/>) was used to analyze the presence of signal-peptide. The following softwares were used to predict the sub-cellular location of proteins: Cello v.2.5 (<http://cello.life.nctu.edu.tw/>); LOCTree3 (<https://roslab.org/services/loctree3/>); and Euk-mPLoc 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/>). Each one analyzes proteins in different ways and for all tools, the selected parameters were: "Eukaryotes" for organism or domain and "Protein" for the type of sequence.

### Gene and proteins characterization

Genes and their products were characterized through bioinformatics tools that allow the identification of domains, patterns, and motifs, as well as active center and physical-chemical characteristics. Therefore, we used the PROSITE (<https://prosite.expasy.org/>) tool, in "Quick Scan" mode, excluding motifs with a high probability of occurrence.

The structural prediction of the proteins found was performed using the Phyre2 (<http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>) server, using the normal modeling mode. For visualization and representation of the structures, we used the Jmol program.

## RESULTS AND DISCUSSION

1980 ORFs, larger than 300 nucleotides, established by the ORFFinder program, were analyzed using the smartBlast in search of homology. Of these ORF's, we found 13 ORFs that encoded for proteins homologous related to the pathogenicity in different organisms mostly of the genus *Phytophthora*, as shown in Table 1. The selection method was based on a link between the protein sequence and the pathogenicity in other organisms. This link was

**Table 1** - Selected genes possibly related to the pathogenicity of *P. cinnamomi* and their products according to the data found in the study

Gene	Accession	Protein	Uniprot accession	Function	Organism of origin homology
PHALS_03704	NCBI: 36398759	Engulfment and cell motility elm family protein	A0A0P1AZ20	Phagocytosis and cellular motility	<i>Plasmodium halstedii</i>
AM587_10014656	EMBL: KUG02203.1	Nephrocystin-3	A0A0W8E0S2	Development of cilia, motility.	<i>Phytophthora nicotianae</i>
PHPALM_11761	EMBL: POM71642.1	Cysteine protease family C26	A0A2P4Y1E2	Peptidase activity	<i>Phytophthora palmivora</i> var. <i>palmivora</i>
PHMEG_000772	EMBL: OWZ24236.1	Carbohydrate esterase	A0A225X2T7	Esterase activity	<i>Phytophthora megakarya</i>
PITG_03453	NCBI: 9467416	Phosphatidylinositol kinase (PIK-G1)	D0N0A9	Kinase activity	<i>Phytophthora infestans</i> T30-4
PHMEG_0003727	EMBL: OWZ21687.1	Transmembrane protein	A0A225WX86	Integral membrane component	<i>Phytophthora megakarya</i>
AM587_10007505	EMBL: KUF93685.1	E3 ubiquitin-protein ligase HERC4	A0A0W8DBN8	Ligase activity	<i>Phytophthora nicotianae</i>
AM587_10008051	EMBL: KUF76294.1	E3 ubiquitin-protein ligase HERC2	A0A0W8BX49	Ligase and transferase activity	<i>Phytophthora nicotianae</i>
PITG_03425	NCBI: 9467389	Glycoside hydrolase, putative	D0N082	Hydrolase and transferase activity	<i>Phytophthora infestans</i> T30-4
PHMEG_000217	EMBL: OWZ24694.1	Serine protease	A0A225X3M6	Serine carboxypeptidase activity	<i>Phytophthora megakarya</i>
PHMEG_000637	EMBL: OWZ24320.1	Cysteine protease	A0A225X505	Cysteine peptidase activity	<i>Phytophthora megakarya</i>
PHYSODRAFT_551981	NCBI: 20662776	Papain-like cysteine protease C1	G4YMD2	Cysteine peptidase activity	<i>Phytophthora sojae</i>
PITG_03400	NCBI: 9464554	Crinkler (CRN) family protein, putative	D0N058	Integral membrane component	<i>Phytophthora infestans</i> T30-4

established with references in the scientific literature available. The data about the genes chosen and their related products are presented in Table 1. All information about these genes and their products including scientific references can be obtained from the databases referred to in Table 1 through the accession number of each gene in the respective database.

The prediction of cellular localization of proteins informs their destination after leaving the endoplasmic reticulum. This destination often depends on the signaling of a signal peptide. The results of the prediction of cellular localization of the proteins encoded by the ORFs selected are in Table 2. To obtain these results we used: SignalP to determine the presence or absence of signal peptide in the protein; Cello, LocTree3, and Euk-mPloc, which from different algorithms, predict the cellular localization of proteins.

The results obtained with the three prediction tools diverge sometimes due to the different algorithms that they use. In addition, some of these proteins are not determined molecularly by experiments in vitro, being determined only in silico by bioinformatics methods, a factor that hinders the prediction by the tools. According to Dönnies and Höglund (2004), the several web-based methods for the prediction that are available online have different localization coverage and different means to assess their accuracy, making it impossible to compare all methods against each other. Some examples of this divergence can be seen in Table 2 where it can also be observed that most proteins with a role in pathogenicity have extracellular or membrane fate as expected as it is outside the parasite cell and in interaction with host cells that they will exercise their action.

**Table 2** - Results of the sub-cellular localization of proteins predicted by SignalP, Cello, LOCTree3 and Euk-mPloc

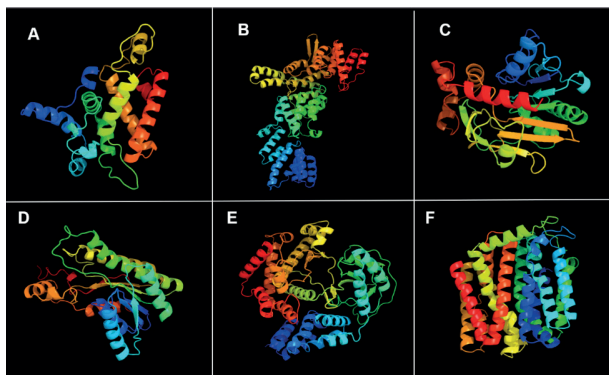
Protein	Signal peptide (Signal P)	Localization (reliable index) (Cello)	Localization (expected accuracy) (LOCTree3)	Localization (Euk-mPloc)
Engulfment and cell motility elm family protein	No	Plasma membrane (0,716)	Cytoplasm (87%)	Cell membrane; cytoplasm. endoplasmic reticulum; nucleus
Nephrocystin-3	No	Cytoplasm (0,598)	Cytoplasm (84%)	Cytoplasm; nucleus
Cysteine protease family C26	No	Mitochondria (0,578)	Mitochondria (82%)	Cytoplasm
Carbohydrate esterase	No	Chloroplast (0,618)	Mitochondria (84%)	Chloroplast; cytoplasm
Phosphatidylinositol kinase (PIK-G1)	No	Nucleus (0,886)	Cytoplasm (86%)	Cytoplasm
Transmembrane protein	No	Plasma membrane (0,997)	Plasma membrane (89%)	Endoplasmic reticulum
E3 ubiquitin-protein ligase HERC4	No	Extracellular (0,448)	Cytoplasm (80%)	Extracellular; nucleus
E3 ubiquitin-protein ligase HERC2	No	Nucleus (0,368)	Cytoplasm (81%)	*
Glycoside hydrolase, putative	Yes	Extracellular (0,650)	Secreted (87%)	Plasma membrane; extracellular
Serine protease	No	Mitochondria (0,285)	Secreted (83%)	Extracellular
Cysteine protease	Yes	Extracellular (0,943)	Secreted (83%)	Extracellular
Papain-like cysteine protease C1	Yes	Extracellular (0,786)	Secreted (82%)	Extracellular
Crinkler (CRN) family protein, putative	No	Mitochondria (0,273)	Cytoplasm (86%)	Nucleus

\* very long amino acid chain.

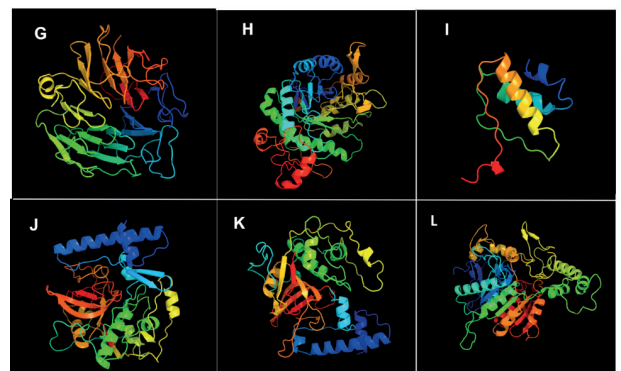
The characterization of genes and its products was performed using Phyre2 server for modeling and structure prediction. The domains and active sites were identified using PROSITE tool. The structures predicted by modeling are in Figure 1 and 2 and in Table 3 is possible to analyze the domains and active sites detected. However, the structure

of E3 ubiquitin-protein ligase HERC2 could not be predicted since it has a very long amino acid chain.

The most promising protein found was the crinkler (CRN) family. This protein is widely related to the damage caused by the *Phytophthora* genus in its hosts (Lamour *et al.*, 2007; Fawke *et al.*, 2015).



**Figure 1** - Structural prediction of the following proteins. (A) engulfment and cell motility elm family protein, (B) nephrocystin-3, (C) cysteine protease family C26, (D) carbohydrate esterase, (E) phosphatidylinositol kinase (PIK-G1), (F) Transmembrane protein. The confidence is represented using a rainbow spectrum (red to blue) whereupon red indicates high confidence and blue indicates low confidence.



**Figure 2** - Structural prediction of the following proteins. (G) E3 ubiquitin-protein ligase HERC4, (H) glycoside hydrolase, putative, (I) serine protease, (J) cysteine protease, (K) papain-like cysteine protease C1, (L) crinkler (CRN). The confidence is represented using a rainbow spectrum (red to blue) whereupon red indicates high confidence and blue indicates low confidence.

**Table 3** - Results from structural prediction of proteins using Phyre2 and characterization of proteins using PROSITE

Protein	Phyre2		PROSITE	
	Confidence	Coverage	Domain	Active sites
Engulfment and cell motility elm family protein	100%	21%	PH, ELMO	-
Nephrocystin-3	100%	27%	SAM, TPR, TPR_REGION	-
Cysteine protease family C26	100%	67%	GATASE TYPE 1	GATASE TYPE 1
Carbohydrate esterase	100%	92%	-	-
Phosphatidylinositol kinase (PIK-G1)	100 %	41%	PH, PI3_4_KINASE_3	-
Transmembrane protein	99.6%	64%	-	-
E3 ubiquitin-protein ligase HERC4	100%	14%	B30.2_SPRY, RCC1_3, FHA	-
E3 ubiquitin-protein ligase HERC2	Very long chain		RCC1_3, FHA, B302_SPRY, UBA, HECT	HECT
Glycoside hydrolase, putative	100%	68%	EGF_3	-
Serine protease	100%	75%	CARBOXYPEPT_SER_HIS, CARBOXYPEPT_SER_SER	CARBOXYPEPT_SER_HIS
Cysteine protease	100%	59%	THIOL_PROTEASE_CYS, THIOL_PROTEASE_HIS, PFKB_KINASES_2	THIOL_PROTEASE_HIS
Papain-like cysteine protease C1	100%	59%	THIOL_PROTEASE_HIS, PFKB_KINASES_2	THIOL_PROTEASE_HIS
Crinkler (CRN) family protein, putative	96.9%	11%	-	-

It was first identified in *Phytophthora infestans* but is known to be present in almost all species of plant pathogenic oomycetes (Torto *et al.*, 2003). It is known that CRN family interacts with the host DNA, leading to leaf wrinkles and necrosis (Amaro *et al.*, 2017). The mature CRNs are able to induce cell death through translocation of cytoplasmic factors and also by action on the host nucleus (Schornack *et al.*, 2010; Stam *et al.*, 2013). This protein family probably is present at the base of the late stages of *Phytophthora cinnamomi* infection due to its functioning.

Carbohydrate esterase is an enzyme that catalyzes the reaction of hydrolysis in ester bonds in order to remove oxygen (O) or nitrogen (N) from carbohydrates (Cantarel *et al.*, 2009). Although the sequence found does not have any specific domain found, it is still possible to perceive its probable importance in *P. cinnamomi* infection, through the breakdown of complex carbohydrates and glycoconjugates of the host, such as cellulose from cells wall. Cysteine protease family C26 is a protein with peptidase activity and involved in the metabolic process of glutamine due to its GATASE\_TYPE\_1 (Glutamine amidotransferase type 1) domain. This domain is responsible for catalyzing the reaction of removal

of the ammonia group from glutamine and transfer to another substrate to form a new carbon-nitrogen group (Buchanan, 1973). Cysteine proteases are conserved among the animal and plant pathogenic microorganisms, besides proteases facilitate penetration of the plant host's physical barriers.

Additionally, both cysteine protease and papain-like cysteine protease C1 are enzymes with the proteolytic activity of the family eukaryotic thiol proteases, which have cysteine in their active sites (Dufour, 1988). In addition to the proteolytic action, both also have the domain of the family phosphofructokinase B-type of carbohydrate kinases, which has a function not yet well defined, however, it seems to be involved with the plastidial genome (Yokota *et al.*, 2009). Thus, due to the activity of these protein, it is possible to assume that they have an influence on the pathogenicity of *P. cinnamomi* since it degrades peptides through their proteolytic actions, facilitating the pathogen penetration into the host's tissues.

In line with this, serine protease has two carboxypeptidase active sites: serine/serine and serine/histidine, involving an aspartic acid residue in the catalysis system (Liao and Remington, 1990).

Carboxypeptidases have proteolytic action and cleave near the carboxyl end site (Beekman *et al.*, 2018). Several studies already associate the presence of carboxypeptidases with pathogenicity fungi, such as *Pseudogymnoascus destructans* (Beekman *et al.*, 2018), and the bacteria of the genus *Neisseria* sp. (Schaub & Dillard, 2019).

E3 ubiquitin-protein ligase HERC4 and E3 ubiquitin-protein ligase HERC2 have the B30.2\_SPRY domain, which is well-conserved and helps to regulate spore differentiation (Nuckolls *et al.*, 1996). Considering the importance of the spores in the infection of *C. sativa* by *P. cinnamomi* is possible that these enzymes are related to the pathogenicity of the present pathogen in the early stages. Glycoside hydrolase has hydrolase and transferase activity, i.e., has the ability to cleave glycosidic bonds as well as assist in cell wall elongation (Haas *et al.*, 2009). It has two distinct domains, with one active site in each. The action of this enzyme in the pathogenicity of *P. cinnamomi* is possibly originated in its lysing activity in glycosidic bonds, altering the permeability of the host.

As less promising proteins, yet very important, we found that the engulfment and cell motility elm family protein (Figure 1 A) belongs to the ELMO (Engulfment and Cell Motility) family of ELMO and PH domains. The ELMO domain is responsible for the regulatory activity of GTPases (Bowzard *et al.*, 2007), whereas the PH (Pleckstrin homology) domain occurs in several proteins that perform intracellular signaling or constitute the cytoskeleton (Haslam *et al.*, 1993; Mayer *et al.*, 1993; Musacchio *et al.*, 1993; Gibson *et al.*, 1994; Ingley and Hemmings, 1994; Pawson, 1995; Saraste and Hyvönen, 1995). Besides that, it is known that some pathogens, such as *Shigella flexneri*, promotes bacterial entry in cells through the ELMO-Dock180 machinery (Hachani *et al.*, 2008). Due to its importance in cell motility and the evidences involving the ELMO domain with pathogenicity, it is possible to infer that this protein participates in the infectious process by *P. cinnamomi* in the initial stages utilizing some homolog protein to Dock180.

The nephrocystin-3 is a protein involved in the ciliary development and function and thus, involved

in cell movement (Bergmann *et al.*, 2008). It has the SAM domain (Sterile  $\alpha$  Motif), which is related to protein-protein interaction (Thanos *et al.*, 1999), as well as the TPR (Tetratricopeptide Repeat) domain, which is responsible for several processes, such as cell cycle control, stress response, transcription repression, among others (Lamb *et al.*, 1995). TPR domain is present in some proteins that are directly involved in bacterial virulence-associated functions in animal cells and tissue. These functions include the translocation of virulence factors into host cells and adhesion to host cells (Edqvist *et al.*, 2006; Chakraborty *et al.*, 2008; Chao *et al.*, 2010). Due to the activities performed by the domains of nephrocystin-3, it is possible to infer that it has high importance in pathogenicity and cellular movements and development – both of these processes, being essential for the infection by *P. cinnamomi*.

Phosphatidylinositol kinase (PIK-G1) is an enzyme responsible for the molecular function of the kinase (transference of phosphate groups) and complexation of metal ions. Due to this, it is involved in the biological processes of phosphatidylinositol phosphorylation and signaling (Kaur *et al.*, 2010). It has PH domain, reinforcing its function in intracellular signaling and, the PI3\_4\_KINASE\_3 (Phosphatidylinositol 3- and 4-kinases) domain, involved in cell signaling of several metabolic pathways and cell cycle (Hiles *et al.*, 1992; Haslam *et al.*, 1993; Mayer *et al.*, 1993; Musacchio *et al.*, 1993; Ingley and Hemmings, 1994; Pawson, 1995; Saraste and Hyvönen, 1995). In line with this, PIK-G1 must play a key role in the stage of cell proliferation during the infectious process.

Although the transmembrane proteins do not integrate any metabolic pathway, they have already been reported in the pathogenicity of several microorganisms, since it is through them that effector molecules and toxins can be released to the host (Silveira *et al.*, 2016). Said that it is important to highlight the relevance of the transmembrane proteins in the process of infection by *P. cinnamomi* through the release of pathogenic effector molecules produced intracellularly.

## CONCLUSIONS

Bioinformatic tools are a reliable source to perform prediction ins proteins sequences available in databases while *in vitro* studies still not performed, and also as a previous step to better target these experimental studies.

New research using *P. cinnamomi* genomic sequence must be carried out in order to determine its pathogenicity mechanisms and molecules associated, and possibly develop methods of control of the pathogens and reduce the damage to European chestnut tree.

It is possible to deduce the role of many molecules in the metabolic pathways by their similitude and homology, however, there is the possibility that many molecules have roles that are still unknown today.

As demonstrated by (Pascoal *et al.*, 2018), we also believe that the silencing of these genes in phytopathogenic fungi and the subsequent infection of plants by these fungi may help to better understand the role of the enzymes expressed by these genes in the infection mechanism.. The research for molecular factors as well as the metabolic pathways involved in the infectious processes is an important tool for the development of preventive strategies and control of pathogens in plants.

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