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REVIEW

In silico characterization of molecular factors involved in metabolism and pathogenicity of *Phytophthora cinnamomi*

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Abstract

Phytophthora cinnamomi is classified as one of the most devastating plant pathogens in the world. It has a destructive effect on more than 5000 horticultural and forestry species in the world, and especially on *Castanea sativa*. The genus *Phytophthora* belongs to the Class Oomycetes, a group of fungus like organisms which provoke plant diseases via motile zoospores. Control of this organism is considered very challenging because of the limited range of effective chemical inhibitors. The development of sustainable control measures for the future management of *P. cinnamomi* requires in-depth knowledge of the cellular and molecular bases of development and metabolism. The aim of this review was to identify molecular factors associated with the metabolism of *P. cinnamomi* by studying the genes implicated in fundamental metabolism using tools of bioinformatics. Also, some genes involved in pathogenicity will be cited and characterized, such as genes coding for transglycosylases. Genomic sequences of *P. cinnamomi* were analyzed using an open reading frame (ORF) finder. The identified ORFs products (proteins) were compared to sequences already described and with known functions present in databases such as NCBI and fungi database. In this way, homologous proteins were found, with the respective specific domains, to proteins involved in the metabolism and pathogenicity of *Phytophthora* ssp.

Keywords *Phytophthora cinnamomi* · *Oomycetes* · Zoospores · *Castanea sativa* · Open reading frame

Introduction

Castanea Sativa, known commonly as the chestnut tree, was originally located on the sprinkled reliefs of the north of the Mediterranean, in particular south of the Balkans (especially in Greece), in Italy, in Corsica, in the south-east of France, but also in the Iberian Peninsula (Trás-os-montes region in Portugal and northern Spain), south of the Black Sea in Turkey, then east into the Caucasus, Georgia, Armenia, and Azerbaijan. Its distribution area has been greatly extended by humans into Southern Europe and especially into Western Europe, north to Scotland, and also locally into North Africa [1].

It occupies almost 2 million hectares with a very significant economic contribution in European agriculture [1]. Since the twentieth century, the production of chestnuts has decreased considerably, with losses of around 300 million euros, and chestnuts continue to be seriously threatened by pests such as the disparate woodworm that attacks the wood, and by the codling moth which damages the fruit. However, the real threat comes from pseudo-fungi belonging to the genus *Phytophthora*. *P. cinnamomi* (the most virulent species) and *P. cambivora*, both causing ink disease, in addition to the fungus *Cryphonectria parasitica*, responsible for chestnut cancer [2, 3].

Ink disease is one of the biggest threats to *C. sativa* affecting both the development and economic yield of this important culture. It causes root and collar rot of both adult trees and of seedlings in plantations, nurseries, and forests. In addition, symptoms of the disease include chlorotic leaves which are reduced in size, immature husks remaining on the tree after the leaves have fallen, and thinning of the crown [4].

Moreover, flame shaped dark necrosis is evident on the collar of the tree after debarking. During the spring and the period corresponding to leaves falling, large roots are mainly infected, they start producing a black exudate that stains the surrounding soil. On young trees with

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smooth bark, the necrosis is visible without debarking as depressed, slightly cracked areas at the base of the stem. There is extensive necrosis of the taproot that extends until the lateral roots and up the stem for some centimeters, causing stem cankers [4].

Symptoms of ink disease in the European chestnut (*C. sativa* Miller) were first observed in Spain in 1726. In 1859, the symptoms of the disease were found in the chestnuts in many European countries, including northern Italy and France, as well as in the United States [5, 6]. In Portugal, the first symptoms of ink disease were described in 1838 and more precisely on the banks of the Lima River [7].

This is the taxonomy of *Phytophthora cinnamomi* Rands; Kingdom Chromista; Phylum *Oomycota* or Pseudofungi; Class *Oomycetes*; Order *Peronosporales*; Family *Peronosporaceae*; genus *Phytophthora* [8]. Since the identification of *P. cinnamomi*, it was believed to belong to the fungi kingdom due to similarities with "real fungi" in terms of growth allowed by the polarization of the hyphae, vegetative spores adapted to dispersion by wind or water, and infection strategies employed. Unlike true fungi, this soil-borne pathogen passes most of its life cycle as a diploid, the cell walls are composed of cellulose and β-glucans instead of chitin, it produces biflagellated zoospores and it does not synthesize sterols, they are resistant to poly- genic antibiotics, such as pimaricin, but requires sterols to sporulate [9].

One of the major characteristics of Chromista is the production of motile asexual spores possessing a flagellum adorned by tubular hairs that are responsible for forward movement [10, 11]. For many species of *Phytophthora*, the motile zoospores are the main infective agent initiating plant disease. *Phytophthora cinnamomi* has been ranked in the top 10 *Oomycete* plant pathogens due to their scientific importance and economic impact [12]. In California, it has been estimated that losses in avocado crops caused by *P. cinnamomi* exceed US$40 million annually [13].

Phytophthora cinnamomi is a soil-borne pathogen with sexual and asexual phases in its lifecycle [14]. It can grow on dead organic matter in the saprophytic phase or directly on susceptible hosts during the parasitic phase. Characteristically, *P. cinnamomi* infects fine, feeder roots, but it can also invade woody stems, especially by exploiting wounds or natural breaks in the peridermal layer [15].

The pathogen's growth within the root system causes root necrosis, which influences negatively water uptake and the transport of essential nutrients to the plant, causing wilting and chlorosis of the foliage. Infected plants can remain asymptomatic for many years, or they can die quickly. The ability of *P. cinnamomi* to grow saprophytically in the soil or symptomlessly in infected plants is a major contributing factor to the long-term survival of the pathogen. It is believed that sexual chlamydospores, sexual oospores, and intracellular hyphal aggregates permit the pathogen to survive for long periods under unfavorable conditions and therefore make disease management extremely difficult [16].

Although we know very well the economic, ecological and environmental problems caused by oomycete *P. cinnamomi* and their mechanisms of infection, increased in recent years by climate change, there is still a lack of knowledge about the metabolism specific and pathogenicity factors of this oomycete.

Some works developed allowed us to characterize some factors (genes and proteins) as necrosis-inducing protein, lyases, transglutaminases, glucanases associated with pathogenicity, however, the metabolism factors specific to this oomycete are little known [3, 8, 17–21]. However, in oomycetes such as *Phytophthora infestans* these factors have been studied and deepened for a long time [12]. With the genomic sequences deposited in the biological databases (NCBI, EMBL, DDJB, and FungiDB) and using the extraordinary capabilities of modern bioinformatic tools, starting from homology criteria with factors known in oomycetes models like *P. infestans*, we could advance further in the characterization the pathogenicity factors of *P. cinnamomi* and deduce some specific factors of the metabolism of this phytopathogen. These are the factors that allow us to understand how the organism grows, multiplies, and responds to the changing environment.

Methodologies

Sequence editing and comparison

For the detailed editing of the sequences was used the BioEdit program, whose version is available at http://www.mbio.ncsu.edu/bioedit/page2.html. We used the Sequencher program from Gene Codes Corporation for sequence comparison and annealing, whose demo copy of version 5.2.4 is available at www.genecodes.com.

Biological information databases

For the research of genes, we used the sequences of the genome and the transcriptome of *P. cinnamomi*, deposited in the databases:

NCBI (National Center for Biotechnology Information) at https://www.ncbi.nlm.nih.gov/;

EMBL (*European Molecular Biology Laboratory) at* www.ebi.ac.uk/ena

DDJB (DNA Data Bank of Japan) at www.ddbj.nig.ac.jp/

*FungiDB (*The Fungal and *Oomycete* Genomics Resource) at fungidb.org.

The genomic sequence used in this work is deposited in NCBI under the reference: *P. cinnamomi* isolate MP94-48(5,831 rc linear DNA).

Open reading frame search

For the search of open reading frames, in the sequences of the genome and the transcriptome of *P. cinnamomi*, the following programs were used:

- *ORF finder NCBI at* https://www.ncbi.nlm.nih.gov/orffinder/;
- GGENEinfinity at http://www.geneinfinity.org/sms/sms_orffinder.html;
- GGenScript at https://www.genscript.com/sms2/orf_find.html.

Gene predictions

For gene prediction from the Contig 1 sequence of *P. cinnamomi* isolate MP94-48 assembly, we use the Maker program. MAKER identifies repeats, aligns ESTs and proteins to a genome, produces ab initio gene predictions, and automatically synthesizes these data into gene annotations having evidence-based quality indices [22].

Homology of proteins encoded by open reading frames

Detection of protein homology and sequence alignment underlies the prediction of protein structure, function, and evolution. Protein homologies were determined in the Blast (smartBlast), Clustal Omega, and Fasta programs independently or through NCBI (National Center for Biotechnology Information). Homology is established with the Protein Databases sequences: UniProt Universal Resource (EBI), Protein Information Resource [PIR, Georgetown University Medical Center (GUMC)], Swiss-Prot Protein Knowledgebase (Swiss Institute of Bioinformatics), and PROSITE (Database of Protein Famines and Domains).

Subcellular location of identified proteins of *P. cinnamomi*

The prediction of the subcellular location of proteins predicts the fate of a protein in the cell, using computational methods with the protein sequence.

There are several publicly available softwares, using different methods to predict protein localization (amino acid composition, signal peptide composition, physicochemical composition, among others), which is a very important part of the prediction based on protein function bioinformatics and genome annotation.

The packages used for the prediction of protein localization were accessed at the following URL:

SignalP 3.0: http://www.cbs.dtu.dk/services/SignalP-3.0/;

Cello: http://cello.life.nctu.edu.tw/;

LOCtree: https://rostlab.org/services/loctree2/;

EuK-mPLoc 2.0: http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/;

Esl pred: http://www.imtech.res.in/raghava/eslpred/index.html

Bioinformatics characterization of molecular factors

After decoding the open reading frame of a gene, a number of bioinformatics tools can be used to characterize the deduced sequence of the protein. A search on the Expasy Proteomics Server website (http://expasy.org/tools) and a nucleotide sequence allows us to identify and characterize proteins, identify motifs, patterns, and profiles, infer their stability, cell location or function, predictions of secondary and tertiary structures, look for similar sequences deposited in databases and compare them, and establish phylogenetic relationships.

The detection of the physical–chemical characteristics of the proteins was carried out in PROSITE (http://prosite.expasy.org/scanprosite/), in the neural network system of the Pôle BioInformatique Lionnais/Network Protein Sequence Analysis or in the application DiANNA 1.1 (http://clavius.bc.edu/~clotelab/DiANNA/). Different sites were predicted for post-translational modifications on the Center of Biological Sequence Analysis website (http://www.cbs.dtu.dk/services).

Gene prediction from the Contig 1 sequence of *P. cinnamomi* isolate MP94-48

We used the Maker program, as it is a robust analysis program, to gene prediction from the Contig 1 sequence of *P. cinnamomi* isolate MP94-48, in order to obtain broader functional genomics analysis and prediction of metabolic pathways. Table 1 shows the results of this analysis with the NCBI reference number of the homologous gene, the organism, the designation of the protein product, and the region of the contig where the sequence is found. Genes with the most significant homology (greater than 85%) and with participation in significant aspects of cellular metabolism were selected for the table. As we can see, these genes have homology with genes from species of the genus *Phytophthora* as expected by the phylogenetic proximity.

As expected, these genes encode products that participate in different aspects of the cellular metabolism of *P. cinnamomi*, from enzymes that participate in the synthesis of Aminoacyl-tRNA synthetases, essential in protein synthesis, genes that encode products of biosynthesis and metabolism of various biomolecules such as glycolipids, to genes that encode products with a significant role in cell phase changes and in the synthesis of environmental adaptation and genes that encode pathogenicity products [23–26].

Table 1 Molecular factors of *Phytophthora cinnamomi* metabolism, deduced with the Maker tool from contig 1 of strain MP94-48

NCBI ref	Contig region	Product definition	Species
POM73604.2	NCKW01005070.1:1..7903 (+strand)	GPI ethanolamine phosphate transferase	*Phytophthora palmivora var. palmivora*
KAF1795552.1	NCKW01005070.1:5353..13255 (+strand)	Rof/RNase P-like	*Phytophthora cactorum*
POM70932.1	NCKW01005070.1:5662..13564 (+strand)	Pleckstrin-like protein	*Phytophthora palmivora var. palmivora*
KAF1795547.1	NCKW01005070.1:5662..13564 (+strand)	Serine-tRNA synthetase, type1, N-terminal	*Phytophthora cactorum*
OWZ24232.1	NCKW01005070.1:5662..13564 (+strand)	COP9 signalosome complex subunit	*Phytophthora megakarya*
POM71256.1	LNFO01000046.1:33571..47822 (+strand)	Pol protein	*Phytophthora palmivora var. palmivora*
KAE8878987.1	LNFO01000046.1:33571..47822 (+strand)	Transposon Tf2-6 polyprotein	*Phytophthora fragariae*
KAF1795549.1	LNFO01000046.1:33571..47822 (+strand)	Sterile alpha motif/pointed domain	*Phytophthora cactorum*
ABG66531.1	LNFO01000046.1:32716..46967 (+strand)	Reverse transcriptase, partial	*Phytophthora sojae*
OWZ00916.1	LNFO01000046.1:32716..46967 (+strand)	Pol Polyprotein	*Phytophthora megakarya*
OWZ00482.1	LNFO01000046.1:33005..47256 (+strand)	Polyprotein	*Phytophthora megakarya*
KAE9028524.1	LNFO01000046.1:1..14252	Callose synthase 1	*Phytophthora fragariae*
KAF1795554.1	LNFO01000046.1:1..14252	1,3-beta-glucan synthase subunit FKS1-like, domain-1	*Phytophthora cactorum*
KAE9233925.1	LNFO01000046.1:1..14252	Callose synthase 9	*Phytophthora fragariae*
OWZ24222.1	LNFO01000046.1:14399..28650 (+strand)	Endoplasmic oxidoreductin	*Phytophthora megakarya*
KAF1795547.1	LNFO01000046.1:14399..28650 (+strand)	Serine-tRNA synthetase, type1, N-terminal	*Phytophthora cactorum*
OWZ24233.1	LNFO01000046.1:14399..28650 (+strand)	Seryl-tRNA synthetase	*Phytophthora megakarya*
KAE8911029.1	LNFO01000046.1:14399..28650 (+strand)	Serine–tRNA ligase, cytoplasmic	*Phytophthora fragariae*
OWZ24236.1	LNFO01000046.1:14399..28650 (+strand)	Carbohydrate esterase	*Phytophthora megakarya*
KAF1795539.1	LNFO01000046.1:14399..28650 (+strand)	NAD(P)-binding domain	*Phytophthora cactorum*
KAF1795555.1	LNFO01000046.1:14399..28650 (+strand)	Class I glutamine amidotransferase-like	*Phytophthora cactorum*
KAF1795557.1	LNFO01000046.1:14399..28650 (+strand)	Aldehyde dehydrogenase, glutamic acid active site	*Phytophthora cactorum*
POM71642.1	LNFO01000046.1:14399..28650 (+strand)	Cysteine protease family C26	*Phytophthora palmivora var. palmivora*
POM71643.1	CKW01006442.1:5255..17840 (+strand)	Endoplasmic oxidoreductin	*Phytophthora palmivora var. palmivora*
KAE9012269.1	NBNE01003014.1:1..7752	Eukaryotic translation initiation factor 3 subunit C	*Phytophthora rubi*
KAE9305923.1	NBNE01003014.1:1..7753	Eukaryotic translation initiation factor 3 subunit C	*Phytophthora fragariae*
POM80617.1	NBNE01003014.1:1..7754	Eukaryotic translation initiation factor 3 subunit C	*Phytophthora palmivora var. palmivora*
POM59598.1	NBNE01003014.1:1..7755	MFS transporter, LAT3 family, solute carrier family 43, member 3	*Phytophthora palmivora var. palmivora*
OWZ08811.1	NBNE01003014.1:1..7756	Aspartyl protease	*Phytophthora megakarya*
KAF1794292.1	NBNE01003014.1:1..7757	DNA/RNA-binding protein Alba-like	*Phytophthora cactorum*

Molecular factors

In this work, we used the genomic sequence deposited in NCBI under the following reference: *P. cinnamomi* isolate MP94-48(5,831 rc linear DNA). Firstly, Contigs from this assembly were analysed via the ORF finder tool in order to find out the ORFs from these random sequences (contigs). Secondly, we performed a smart blast with the identified ORFs to find homologs of molecular factors already described in other pseudo-fungi, such as *Phytophthora sojae, P. infestans,* with known pathways and functions. These molecular factors are the divided into two classes: implicated in fundamental metabolism and others involved in pathogenicity (Tables 2 and 3). This classification was based on bibliography, conserved domains, and subcellular location of molecular factors of interest using SignalP and Cello tools. Furthermore, the subcellular location of the proteins may be carried out in another way through the design of cassettes containing GFP sequences, followed by fluorescence microscopy, which is more precise, but also more time and money consuming. Proteins which have extracellular locations are considered to be involved in pathogenicity, those which do not have extracellular locations (nuclear, cytoplasmic, and mitochondrial) are considered to be involved in fundamental metabolism. This classification was in agreement with what has been shown in the literature.

In Tables 2 and 3, each cited protein is codified by a specific ORF, which was identified as described in the previous part with its contig number and the name of the homolog organism. Proteins are described in terms of function and subcellular location as mentioned above. Each protein is defined by its NCBI gene bank accession.

Despite the large amount of research and analysis carried out on the *Phytophthora* genome, until now there is not sufficient information to carry out a treatment to eradicate or at least mitigate the impact of ink disease. In previous reviews, the major focus was given to genes directly implicated in pathogenicity, such as the NPP1 gene, which has been shown to trigger root necrosis in host cells. The GIP gene responsible for the suppression of the host defense response. The GLUCANASES gene family responsible for adhesion, penetration, and colonization in host tissues have also been well studied [17, 27] (see Table 4).

We made a comparison of the proteins deduced from the obtained open reading phases, in order to find homologous proteins characterized in other living organisms and deposited in the Uniprot and Swiss-Prot protein databases. In both sources, most of the open reading phases encode proteins homologous to metabolic and pathogenic proteins

Table 2 *Phytophthora cinnamomi,* molecular factors involved in fundamental metabolism

Contig	Protein length	Homolog organism	Name of protein	Localization	Function	Accession
1	137aa	*Phytophthora megakarya*	Polycomb protein	Nuclear	Direct Assembly of transcriptionaly repressed chromatin [50]	GenBank: OWZ24764.1
	132aa	*Phytophthora cactorum*				GenBank: KAF1784310.1
	113aa	*Phytophthora cactorum*	Nucleotide-binding alpha–beta plait domain	Nuclear	RNA binding [51]	GenBank: KAF1773404.1
2	166aa	*Phytophthora megakarya*	P21-activated protein kinase-interacting protein	Nuclear and mainly cytoplasmic	Regulate cell shape and polarity [52]	GenBank: OWZ18359.1
	121aa	*Phytophthora megakarya*	Glucose repression regulatory protein TUP1	Nuclear	Greatly implicated in glucose repression and affect mating type [28]	GenBank: OWZ07049.1
	241aa	*Phytophthora cactorum*	Frag1/DRAM/Sfk1	Plasma membrane	Induces macroautophagy as an effector of p53-mediated death [53]	GenBank: KAF1793710.1
3	177aa	*Phytophthora infestans*	Rap1 Myb domain		critical for maintaining normal telomere length and structure [54]	GenBank: KAF4146400.1
102	791aa	*Phytophthora megakarya*	Clavaminate synthase	Cytoplasmic	conversion of proclavaminic acid to clavaminic acid [40]	GenBank: OWZ22087.1

Table 3 *Phytophthora cinnamomi*, molecular factors involved in pathogenicity

Contig	ORF's length	Homolog organism	Name of protein	Localization	Function	Accession
102	406	*Phytophthora megakarya*	Murein transglycosylase	Extracellular	Involved in the biogenesis of the cell wall, required for growth, invading ecological niches, and counteracting the host immune response [40]	GenBank: OWZ01054.1
	135aa	*Phytophthora sojae*	Elicitin	Extracellular	Elicitin proteins produce metabolic or structural changes in host cells that aid pathogen growth and favour the development of the disease [8]	GenBank: XP_009524940.1
81		*Phytophthora ramorum*	Elicitin-like protein RAL11D	Extracellular		GenBank: ABB55948.1
	135aa					
	478aa	*Phytophthora megakarya*	Transglutaminase elicitor, partial	Extracellular	Involved in the establishment of *Phytophthora* disease during early phases [8]	GenBank: OWY99932.1
101	478aa	*Phytophthora palmivora var. palmivora*	Transglutaminase elicitor-like protein	Extracellular		GenBank: POM62262.1
95	118aa	*Phytophthora palmivora var. palmivora*	Polysaccharide lyase, partial	Extracellular	Enzyme involved in plant polysaccharide and pectin degradations [55]	GenBank: POM79686.1
108	115aa	*Phytophthora palmivora var. palmivora*	Zinc ion binding protein	Extracellular	Sequester zinc from host cells and tissues [56]	GenBank: POM72001.1

Table 4 Genes linked to pathogenicity in *P. cinnamomi* [18]

Name of Gene	source	Name of the protein	Function
Endo1	NCBI Accession: AM259651	Glucan 1,3-beta-D-glucosidase	Cell morphogenetic processes, especially in the infection process
Gip	NCBI Accession: AM259384	Glucanase inhibitor protein	Responsible for suppressing the host's defense response
Npp1	NCBI Accession: AM403130	Protein 1	It causes necrosis in the leaves and roots of the plant
Transglu	NCBI Accession: AM403129	Transglutaminase elicitor precursor	It induces defense responses and symptoms similar to that of the disease
Endoglucanase	[60]	Endo-1,3-beta-glucanase	Responsible for infection mechanisms
Exoglucanase	[60]	exo-glucanase	Responsible for adhesion, penetration and colonization in the host's tissues
ADP-ribosylation factor	[61]	ADP-ribosylation factor	Responsible for infection mechanisms
Ric1 protein mrna	[61]	Ric1 protein mRNA	Responsible for infection mechanisms
Polygalacturonase 4 1, 2, 3 e 4, 5, 6	[62]	Polygalacturonase 4	Breakdown in the cell wall composition of plant cells
Highly acidic elicitin	[63]	Highly acidic elicitin	Responsible for infection mechanisms

of different species of the genus *Phytophthora*, as expected, especially *P. infestans*, *P. sojae*, *Phytophthora nicotinea,* and *Phytophthora parasitica*. There were also a large number of open reading frames that coded for hypothetical proteins and/or with unknown functions.

Characterization of genes involved in metabolism

A bioinformatic analysis of the *Phytophthora* genomic sequences allowed us to identify several genes involved in metabolism, the most important of which code for proteins belonging to several families, namely kinases, ligases, polymerases, transferases, and methylases. Metabolism is the set of thousands of chemical reactions of anabolism and catabolism by proteins which are in turn coded for by specific genes. The good functioning of the mechanisms will allow survival and development and consequently enhance the virulent power of the pathogen.

Characterization of TUP 1 gene in *P. cinnamomi*

In contig 2 (LGSJ01000002.1 sequence in NCBI), an ORF with a length of 121aa (366nt) has been identified as a homolog of TUP 1 (glucose repression regulatory protein). It has a high percentage of homology equal to 83.19% with TUP1 of *Phytophthora megakarya* according to smart blast; also this isolated ORF has a very significant coverage up to 93% with the same gene of *P. megakarya*. TUP 1 is a gene involved in the repression of glucose for a multitude of genes and also influences the type of mating, and an induced mutation of this gene has reduced the degree of repression of glucose considerably, changed mating type, and disrupted some other pathways [28]. TUP 1 affecting the reproduction of the species, can be considered as one of the ways to inhibit the pathogen. The analysis of the sequence revealed 2 conserved domains, WD40 (accession: cl29593) and DUF2205 (accession: cl10911).

Characterization of Rap1 gene in *P. cinnamomi*

Analysis of the sequence LGSJ01000003.1 from *P. cinnamomi* taken from NCBI, with the ORF research platform, as described in the Methodologies section, revealed an ORF of length equal to 366 nucleotides/121aa, which has a significant coverage and homology of 98% and 65.28%, respectively, with Myb Rap 1 domain of *P. infestans*. Rap1 (repressor activator protein 1) was first discovered in budding yeast, *Saccharomyces cerevisiae,* as a positive transcriptional regulator of multiple growth-related genes, such as ribosomal protein genes [29]. Other studies have identified Rap1 as the main repeat binding protein for double-stranded telomeres in *S. cerevisiae* and necessary for maintaining the length and structural integrity of telomeres [30, 31]. Indeed, telomeres are specialized nucleoprotein structures that preserve the integrity of eukaryotic chromosomal terminations by protecting them from fusion and recombination and by initiating their replication (for reviews, see references 32–34). In most organisms, telomeric DNA is made up of short repetitive sequences characterized by an abundant presence of G residues on the strand containing the 3 'end. These repeat sequences are maintained by a ribonucleoprotein (RNP) known as telomerase, acting as an unusual reverse transcriptase [35, 36]. Telomeric binding proteins and telomerase are both essential for preserving the integrity of telomeres through multiple cell divisions, which in turn are essential for supporting genome stability and extend the lifespan of cell. The Myb domain binding to the DNA of *C. albicans* Rap1 is sufficient to suppress most of the aberrations of the telomeres observed in the null mutant. In addition, in a recent analysis, Rap1 was recognized as a key component of the coupling-type silencer and has been shown to be essential for transcriptional silence [37–39].

Characterization of gene coding for clavaminate synthase in *P. cinnamomi*

Clavaminate synthase is an enzyme involved in metabolism. The gene coding for this protein has been identified in the following reference sequence LGSJ01000102.1 of the genome of *P. cinnamomi* deposited in the NCBI platform using the ORF finder tool. The identified ORF has a length of 2376 nucleotides which corresponded to 792 amino acids.

This fragment has a very significant percentage of query cover and homology with the pseudo fungus of the same genus, *P. megakarya,* having percentages of 100% and 79.90%, respectively. It is an enzyme that has industrial utility and more specifically in the pharmaceutical field. It catalyzes the reaction of the transformation of proclavaminic acid into clavaminic acid [40]. This molecule has proven synergistic effects when combined with beta-lactam antibiotics, such as amoxicillin. This combination is used only in patients suspected of infections with beta-lactamase-producing bacteria.

Amoxicillin is a beta-lactam antibiotic that disrupts the synthesis of the bacterial cell wall by binding to penicillin-binding proteins present inside the bacterial cell wall, thereby preventing the synthesis of the peptidoglycan layer in the cell membrane [41]. This disruption of cell wall synthesis results in cell lysis and bacterial death. In some cases, the bacterial species produce the beta-lactamase enzyme, which can inactivate beta-lactam drugs by hydrolyzing the beta-lactam bond in the antibiotic compound, leading

to drug resistance. In this case, clavulanic acid at its beta-lactam bond binds to the active site of beta-lactamase and inactivates the enzyme, thereby improving the antibacterial effect of beta-lactam antibiotics.

Characterization of genes involved in mechanisms of pathogenicity of *P. cinnamomi*

In this section, we will shed light on the molecular factors thought to directly trigger an infection in a host cell. Thus, all the genes cited code for extracellular proteins according to the bioinformatic tools described in the methodologies. The function of each protein will be discussed by referring to the function of its counterpart already described in other living organisms.

Characterization of murein tranglycosylase gene in *P. cinnamomi*

Analysis of the sequence LGSJ01000102.1 from *P. cinnamomi* available in the NCBI, with the ORF finder tool revealed an ORF of length equal to 1221 nucleotides/406aa, which has a significant coverage and homology equal to 99% and 83.74%, respectively, with a gene coding for murein transglutaminase from *P. megakarya.*

The fungal cell wall is an essential structure that maintains cell form and protects fungi against environmental critical conditions. Glycosyltransferases, glycoside hydrolases, and transglycosylases are involved in the biogenesis of the cell wall, responsible for growth, invading ecological niches, and counteracting the host immune response. Murein transglycosylase is a lytic enzyme that belongs to the class of autolysin that cleaves the cell wall heteropolymer peptidoglycan (murein) to facilitate its biosynthesis and regeneration [42]. Moreover, the protein function described is in agreement with the extracellular localization deduced, since this enzyme is involved in the maintenance of cell structure and growth. In Aspergillus fumigatus, a family of five Crh transglycosylases was detected. Indeed, in vitro biochemical assays and localization studies demonstrated that detected enzymes are specifically transglycosylases for both chitin-glucan and chitin-chitin cell wall linkages forming a three-dimensional network mesh required to strengthen cell wall and ensure its integrity. Furthermore, crh genes aren't only dispensable for cell viability when ensuring cellular turnover but also renders cells sensitive to cell wall interfering compounds [43]. This makes this gene a target to be taken into consideration in order to inhibit or disrupt pathogen growth.

It was found that in the fungus *Botrytis cinerea* the protein Crh (BcCrh) is a cytoplasmic effector that is internalized in the plant cell for the induction of cell death and consequently with a great function in the pathogenicity of the fungus. During saprophytic growth, the BcCrh1 protein is localized in vacuoles and ER. Upon plant infection the protein accumulates to high levels in infection cushions, it is then secreted to the apoplast and translocated into plant cells, where it induces cell death and defense responses, as was well explained by the authors of the reference [44].

Characterization of polysaccharide lyase gene in *P. cinnamomi*

Polysaccharide lyase is an enzyme involved in plant polysaccharide and pectin degradation. The gene coding for this protein has been identified in the following reference sequence LGSJ01000095.1 of the genome of *P.cinnamomi* deposited in the NCBI platform using the ORF finder tool, and the identified ORF has a length of 357 nucleotides, which correspond to 118 amino acids.

This fragment has a very significant percentage of query cover and homology with the pseudo fungus of the same genus *P. palmivora var. palmivora,* having percentages of 100% and 91.53%, respectively. Fungal enzymes involved in plant polysaccharide degradation are assigned to at least 35 glycoside hydrolase (GH) families, three carbohydrate esterase (CE) families, and six polysaccharide lyase (PL) families [45, 46]. Thereby, the identified query coding for polysaccharide lyase has the power to degrade pectin backbones when combined with glycoside hydrolases [46, 47]. In addition to that, the analysis of this protein has shown the conservation of specific domain pectate lyase (accession: pfam03211) responsible for pectin degradation belonging to the pectate lyase superfamily according to sequence analysis of conserved domains carried out with NCBI [19–21].

Characterization of the gene coding for the zinc ion binding protein in *P. cinnamomi*

Analysis of the sequence LGSJ01000108.1 from *P. cinnamomi* available in the NCBI, with the ORF finder tool, revealed an ORF of length equal to 348 nucleotides/115aa, which has a significant coverage and homology respectively equal to 80% and 55.9%, with a gene coding for a zincophore-like protein from *P. palmivora var. palmivora.*

Zinc is fundamental for all domains of life, as it composes the catalytic and structural center of a large array of proteins. It is a ubiquitous metal in all life forms, as it is a structural component of almost 10% of eukaryotic proteins. In fact, zinc-depleting conditions are known to reduce fungal growth and evidence suggests that host cells employ sequestration of zinc to inhibit fungal development [48]. In order to overcome this defence system, pathogenic fungi activate the expression of several systems to enhance the uptake of zinc, through secretion of zincophores, which are proteins able to chelate zinc.

Characterization of a gene coding for elicitor protein in *P. cinnamomi*

Analysis of the sequence LGSJ01000081.1 from *P. cinnamomi* genome available in the NCBI, with the ORF finder tool, revealed an ORF of length equal to 408 nucleotides/135aa, which has significant homology with elicitin proteins from *P. sojae* and *P. rammourm,* equal to 75.56% and 68.22%, respectively.

During infection, *P. cinnamomi* secretes a diverse range of effector molecules in order to infect the plant. The intended function of these effectors is to facilitate the establishment of disease during the infection. From the plant's point of view, the goal is to recognize the effectors and induce a defense response that will inhibit or mitigate the development of the disease. When an intended effector is recognized by the plant and elicits a defense response, it is termed an avirulence factor or elicitor. Elicitins are classified as apoplastic effectors, meaning that they are secreted into the plant extracellular space. In fact, elicitin proteins produce metabolic or structural changes in host cells that aid pathogen growth and favour the development of the disease [8].

The analysis of conserved domains in this identified homolog with NCBI conserved domain option has revealed the presence of elicitin domain (Accession: pfam00964). This domain occupies a significant part of the protein in terms of length, from position 21 to position 113, and is believed to induce leaf necrosis in infected plants and elicit an incompatible hypersensitive-like reaction.

Characterization of a gene coding for transglutaminase elicitor-like protein in *P. cinnamomi*

As in other's cases described, analysis of the sequence LGSJ01000101.1 from *P. cinnamomi* genome available in the NCBI, with the ORF finder tool, revealed an ORF of length equal to 1437 nucleotides/478aa, which has significant homology with transglutaminase elicitin like-protein from *P. megakarya* and *Phytophthora palmivora var. palmivora* equal to 59.86% and 54.61%, respectively.

Transglutaminases are omnipresent in multiple *Phytophthora* species and catalyze an acyl transfer reaction that provides peptide bonds with more resistance to proteolytic degradation. They are expressed during early infection, suggesting that they may function during the establishment of *Phytophthora* disease [49].The analysis of conserved domains in this identified homolog with NCBI conserved domain option has revealed the presence of the TGase_elicitor domain (Accession: cl25039). This domain occupies a significant part of the protein in terms of length, from position 147 to position 475, and is believed to trigger infections in the early stages.

Conclusions and future trends

With the advances in sequencing capacity, with the emergence of the recent large-scale sequencing platforms, it was possible to advance the sequencing of the *P. cinnamomi* genome. However, this structural genomics is just a code whose meaning is necessary to deduce to understand that genes and proteins are involved in the growth, multiplication, and adaptation to the constantly changing environment as well as explaining the great capacity in a number of hosts and aggressiveness of this devastating oomycete. Thus, this work sought to increase the list of these factors (genes and proteins) using modern bioinformatics tools and *P. cinnamomi* sequences deposited in databases. In future research, it is necessary to continue to identify more key molecular factors of metabolism and pathogenicity to understand how it infects plants. The infection of small plants of *C. sativa* or another host with *P. cinnamomi* strains and the consequent sequencing of transcripts with the RNA-seq technique will be an interesting approach that will allow us to determine the main factors in a comprehensive way, as well as the interaction between them in the development of the infection.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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