



Genomic analyses and expression evaluation of thaumatin-like gene family in the cacao fungal pathogen *Moniliophthora perniciosa*



Sulamita de Freitas Franco^a, Renata Moro Baroni^{a,b}, Marcelo Falsarella Carazzolle^a, Paulo José Pereira Lima Teixeira^a, Osvaldo Reis^a, Gonçalo Amarante Guimarães Pereira^{a,*}, Jorge Maurício Costa Mondego^b

^a Laboratório de Genômica e Expressão, Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil

^b Centro de Pesquisa e Desenvolvimento de Recursos Genéticos Vegetais, Instituto Agronômico, Campinas, SP, Brazil

ARTICLE INFO

Article history:

Received 26 August 2015

Accepted 9 September 2015

Available online 11 September 2015

Keywords:

Thaumatin

Moniliophthora perniciosa

Cacao

Witches' broom disease

Gene expansion

Gene expression

ABSTRACT

Thaumatococin-like proteins (TLPs) are found in diverse eukaryotes. Plant TLPs, known as Pathogenicity Related Protein (PR-5), are considered fungal inhibitors. However, genes encoding TLPs are frequently found in fungal genomes. In this work, we have identified that *Moniliophthora perniciosa*, a basidiomycete pathogen that causes the Witches' Broom Disease (WBD) of cacao, presents thirteen putative TLPs from which four are expressed during WBD progression. One of them is similar to small TLPs, which are present in phytopathogenic basidiomycete, such as wheat stem rust fungus *Puccinia graminis*. Fungi genomes annotation and phylogenetic data revealed a larger number of TLPs in basidiomycetes when comparing with ascomycetes, suggesting that these proteins could be involved in specific traits of mushroom-forming species. Based on the present data, we discuss the contribution of TLPs in the combat against fungal competitors and hypothesize a role of these proteins in *M. perniciosa* pathogenicity.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Thaumatococin-like proteins (TLPs) are proteins with molecular weight of approximately 23 kDa found in plants, nematodes, arthropods and fungi [1]. TLPs share sequence similarity with thaumatin, a monomeric sweet-taste protein isolated from the fruit of *Thaumatococcus daniellii* [2,3]. These proteins have conserved domains that form an acidic cleft, which is related to the degradation of β -1,3-glucans, glucose polymers found in fungal cell walls [4,5]. Plant TLPs are highly expressed during the attack of fungal pathogens [6] being classified as Pathogenicity Related proteins (PR proteins) type 5 (PR-5).

Leal et al. [7], and Da Hora Jr. et al. [8], have reported the expression of plant TLPs during the interaction between the chocolate tree *Theobroma cacao* and *Moniliophthora perniciosa* (Stahel) Aime & Phillips–Mora [9], a basidiomycete hemibiotrophic pathogen that causes Witches' Broom Disease (WBD) of cacao. WBD is one of the most devastating plant diseases in the Americas, causing serious economical problems in cacao producing regions with yield

losses reaching from 50% to 90% [10,11]. The disease begins when basidiospores come in contact with meristematic tissues in stems, flower cushions or developing fruits [12]. Basidiospore germination produces monokaryotic biotrophic mycelia that slowly grow in the intercellular space of cacao. Stems become hypertrophic and hyperplastic turning into an anomalous structure called 'green broom' [13]. After approximately 60 days, the fungus becomes dikaryotic with mycelia containing clamp connections. These necrotrophic hyphae penetrate the plant cells promoting the necrosis responsible for the 'dry broom' phenotype [14,15]. After alternate dry and wet periods, the fungus produces basidiomata that release basidiospores, reinitiating the cycle.

Most of TLPs studied are from plants [6]. However, the researches about fungal TLPs have been increasing in the late years. For instance, Sakamoto et al. [16] found that *Lentinula edodes* thaumatococin-like protein TLG1 degrades lentinan, a β -1,3-glucan present in its own cell wall, likely being involved in cell wall degradation and remodeling. In addition, the wheat stem rust pathogen, *Puccinia graminis*, has an expansion of TLPs, most specially the small TLPs (sTLPs), which have molecular mass of 16–17 kDa resulted from a C-terminal peptide deletion that abolishes the formation of TLPs typical acidic cleft [1]. Interestingly, OPEL, a protein expressed by the oomycete *Phytophthora parasitica*, is

* Corresponding author.

E-mail address: goncalo@unicamp.br (G.A.G. Pereira).

an elicitor of plant basal immunity that contains a thaumatin-like domain [17].

Due to the occurrence of TLPs in plant pathogens, we decided to investigate the presence of TLPs in *M. perniciosa* genome, asking whether these proteins could be relevant for pathogenicity and development of this phytopathogen of cacao. In this manuscript, we report the identification of a TLP gene family in the *M. perniciosa* genome (*MpTLPs*). In addition, *MpTLPs* expression profiles in *planta* during WBD and in basidiomata and basidiospores of *M. perniciosa* were assessed. Furthermore, fungal genomes annotation revealed that TLPs presence seems to be pervasive and expanded in basidiomycetes. The results provide insights of possible contributions of TLPs in the prevention against fungal competitors and in fungal pathogenesis.

2. Materials and methods

2.1. Identification of TLPs in *M. perniciosa* genome

One TLP gene (*MpTLP1*) was identified during the annotation of *M. perniciosa* EST libraries [18]. Using the *MpTLP1* sequence as reference, tBLASTN searches were performed in a draft assembly of the *M. perniciosa* genome obtained by Sanger methodology [19]. This search led to the identification of three additional TLP genes with high similarity to *MpTLP1*. With the emergence of new DNA sequence technologies, the *M. perniciosa* genome assembly was improved with a dataset from 454-pyrosequencing and Illumina sequencing, and additional BLAST searches revealed 9 new TLP sequences. The complete open reading frames (ORFs) of these genes were predicted by Augustus software [20] and intron-exon boundaries were confirmed by alignment with RNAseq libraries from WBD transcriptome atlas (www.lge.ibi.unicamp.br/wbdAtlas) [21]. These sequences were deposited in GenBank under the accession numbers KT351092 to KT351104 (Table 1, Doc. S1).

2.2. Search for TLPs in public genomic databases and sequence analyses

Forty six fungal genome sequences available on the Joint Genome Institute (JGI, <http://www.jgi.doe.gov>), in the Broad Institute (<http://www.broadinstitute.org>) and in the NCBI protein websites were mined using *MpTLPs* and a maize thaumatin [22] sequences as references in BLAST searches. Similar sequences to *MpTLPs* were retrieved from filtered models databases from JGI and Broad Institute (Cut-off E-value was $<1 E^{-10}$). TLP sequences from plants, arthropods and nematodes present in NCBI database were individually examined and amino acid sequences that presented a complete thaumatin family domain were retained for further analyses. For amino acid sequence alignments, the procedure described by Petre et al. [1] was applied, limiting the TLP domain as N-x-C-x(3)-V/I-W and Y-x-I/V-x-F-C-x in the N- and C-terminal ends, respectively. Only proteins covering almost 95% of the mature TLP domain were considered.

2.3. Biological material

The basidiomata of *M. perniciosa* were produced in the laboratory according to procedures previously described [23]. Briefly, cacao stems were sterilized inside glass flasks and then inoculated with agar plugs containing single cultures of *M. perniciosa* necrotrophic hyphae. After 7 weeks, *M. perniciosa* basidiomata developed on the stems. After the expansion of the pileus, mature basidiomata released basidiospores, which were harvested in a solution containing 16% glycerol, 0.01 M MES and 0.01% Tween, pH 6.1 [24]. Mature basidiomata were then collected and frozen in liquid nitrogen.

M. perniciosa isolate BP10 and *Theobroma cacao* cv. “Comum” (a WBD susceptible Forastero genotype) were used to perform the infection experiments. Three month old plantlets were inoculated

Table 1
Characteristics of the thirteen *MpTLP* genes identified in the *M. perniciosa* genome.

Gene	<i>M. perniciosa</i> database ID	Genbank ID (Nucleotide/Protein)	Gene size (bp)	CDS size (bp)	Number of introns	Protein size (aa)	Cysteine number	BlastP (NCBI) ^a	PI/MW (kDa) ^b
<i>MpTLP1</i>	MP04497	KT351092/ALA44963	1403	771	11	257	16	EKV44020.1- hypothetical protein (3e-130) <i>Agaricus bisporus</i>	5.55/27.2
<i>MpTLP2</i>	MP04510	KT351093/ALA44964	1284	771	10	257	16	ELU36916.1- thaumatin-like protein (6e-134) <i>Rhizoctonia solani</i>	4.56/27.1
<i>MpTLP3</i>	MP08014	KT351094/ALA44965	1563	750	8	250	18	EJD01931.1- thaumatin-like protein (5e-94) <i>Fomitiporia mediterranea</i>	4.80/26.4
<i>MpTLP4</i>	MP08020	KT351095/ALA44966	1228	750	8	250	18	EIM87365.1- Osmotin thaumatin-like protein (5e-92) <i>Stereum hirsutum</i>	4.90/26.3
<i>MpTLP5</i>	MP07273	KT351096/ALA44967	1254	732	7	244	13	EKV44094.1- hypothetical protein (1e-92) <i>Agaricus bisporus</i>	4.59/25.4
<i>MpTLP6</i>	MP01417	KT351097/ALA44968	1279	747	7	249	16	EJD04853.1- thaumatin-like protein (5e-121) <i>Fomitiporia mediterranea</i>	3.99/26.0
<i>MpTLP7</i>	MP07692	KT351098/ALA44969	1252	726	8	241	14	EJD04867- 1thaumatin-like protein (7e-80) <i>Fomitiporia mediterranea</i>	3.90/25.4
<i>MpTLP8</i>	MP04575	KT351099/ALA44970	1193	759	7	253	16	EJD04867.1- thaumatin-like protein (2e-90) <i>Fomitiporia mediterranea</i>	4.52/26.3
<i>MpTLP9</i>	MP07276	KT351100/ALA44971	1239	741	8	247	16	EKV44094.1- hypothetical protein (9e-112) <i>Agaricus bisporus</i>	4.00/25.7
<i>MpTLP10</i>	MP08739	KT351101/ALA44972	1485	783	7	261	20	EIW57348.1- thaumatin-like protein (1e-71) <i>Trametes versicolor</i>	4.22/27.3
<i>MpTLP11</i>	MP09043	KT351102/ALA44973	887	558	6	186	11	XP_003034800.1- hypothetical protein (8e-68) <i>Schizophyllum commune</i>	4.69/19.2
<i>MpTLP12</i>	MP09053	KT351103/ALA44974	891	498	7	164	12	EKV41735.1- hypothetical protein (8e-68) <i>Agaricus bisporus</i>	7.98/18.5
<i>MpTLP13</i>	MP12084	KT351104/ALA44975	1098	759	8	253	16	EMD36872.1-hypothetical protein (2e-93) <i>Ceriporiopsis subvermispora</i>	4.49/26.5

^a E-values are shown in parentheses.

^b Theoretical values from ExPaSy tool (http://web.expasy.org/compute_pi/).

Download English Version:

<https://daneshyari.com/en/article/10749407>

Download Persian Version:

<https://daneshyari.com/article/10749407>

[Daneshyari.com](https://daneshyari.com)