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Computational Prediction of the Secretome of *Ganoderma lucidum*

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ABSTRACT: Almost all phytopathogens are known to secrete proteins that hinder the functioning of a host organism. These molecules work by either rendering the defence of the host cell weak or altering the physiology of the host cell to obtain nutrients in effect promoting infection. All the gene products secreted by a cell is known by the term secretome. *Ganoderma lucidum* is a fungus that works by degrading lignin in palms to causing stem rot eventually killing the palm tree. Understanding the secretome of *G.lucidum* would be beneficial in understanding the mode of propagation of the infection and help in the development of anti-fungal mechanisms which would help counter the spread of this deadly pathogen.

For identifying the secreted proteins, various bioinformatics tools like SignalP, TargetP, TMHMM and Protcomp were utilized to identify signal peptide sequences in the N-terminus while removing possible transmembrane sequences. After subcellular localization by using Protcomp, it was predicted that approximately 21 unique proteins make up part of the secretome of *G. lucidum*.

KEYWORDS: SignalP, TargetP, TMHMM, *Ganoderma lucidum*

I. INTRODUCTION

Ganoderma lucidum is a polyphore mushroom belonging to the genus *Ganoderma*, which was first described by the Finnish mycologist Petter Adolf Karsten in 1881 [1]. Identification of different species of *Ganoderma* is difficult due to various morphological similarities between different species. A Norwegian mycologist, Leif Randulff Ryvarden, studied the genus and stated that the shape and size varies across collections, the colour changes with age of the pileus and stipe and the pore size, even though constant, changes colour with age [2]. Whole genome sequencing of the *G. lucidum* genome has been completed along with its mitochondrial genome [3, 4]. Currently, genetic studies are done to identify members of the *Ganoderma* genus and there are more than 300 identified *Ganoderma* species across the world making it the largest genus in the order aphyllophorales [5]. *G. lucidum* causes extensive losses to coconut production in India by causing basal stem rot disease (also known as Thanjavur wilt, bole rot and Anabe) [6] .

Wood decaying fungi is known to produce a cocktail of enzymes including cellulases, hemicellulase and ligninase to aid it in the degradation of cellulose, hemicelluloses and lignin. The most abundant enzyme produced by *G. lucidum* in different studies is laccase [7, 8]. In liquid culture, tests have revealed that more than three isoenzymes can be produced by *G. lucidum* [9]. However, the quantity differs according to the various external factors like the temperature, which was demonstrated in a study conducted in 2011 at the Michigan State University [10]. For e.g., under laboratory settings, higher levels of laccase were found to be produced in a nitrogen medium with glucose serving as the source of carbon. Some other enymes that are found to be secreted by *G. lucidum* are manganese dependant peroxidase (MnP) and lignin peroxidase (LiP) [9]. This is sufficient evidence to state that the secretome of an organism varies according to different factors.

There have been many studies detailing the medicinal properties of *Ganoderma* [11], however, relatively fewer studies have been done to study the pathological effects that the fungus has on wood. Most secretory proteins have certain common characteristics between them like the presence of signal peptides [12]. Signal peptides are short sequences of length between 5–30 amino acids present in the N – terminus (start of a polypeptide). However, this cannot be termed as a surefire method to identify the whole secretome of an organism *per se* due to the existence of non-classical secretory proteins which lack

signal peptides [13]. In this study, we aim to identify the secretory protein sequences present in *G. lucidum* using a variety of bioinformatics tools that predicts the presence of signal peptides in protein sequences.

II. MATERIALS AND METHODS

The protein sequences for *G. lucidum,* already available on the fungal genomics database (MycoCosm) developed by the Joint Genome Institute [14], was obtained for the identification of secretory proteins. For identifying the presence of signal peptides in a sequence, the standalone package of a machine learning tool named SignalP (v4.1) (http://www.cbs.dtu.dk/services/SignalP/performance.php) [15] was utilized which has been trained with sequences containing transmembrane segments and no transmembrane segments so as to help it distinguish between the two. The input sequences were analyzed with the options set for eukaryotes with the default cutoff (D-cutoff) values set at 0.34 on both the Tm and non-Tm network parameters so as to increase sensitivity. The PRINSEQ server (v0.20.4) (http://edwards.sdsu.edu/cgi-bin/prinseq/prinseq.cgi) [16] was used to refine results by removing duplicates from the results before using as an input in the next tool.

The results obtained from SignalP analysis was further refined by identifying those signal peptides that could be part of a secretory pathway by utilizing a tool named TargetP (v1.1) (http://www.cbs.dtu.dk/services/TargetP/) [17]. Default parameters were set for the tool and the non-plant option was set for the organism group. Only those sequences that gave a prediction of S (secretory signal peptides) instead of M (mitochondrial targeting peptides) or unknown were selected for further analysis. A custom perl program was developed to match each id (TargetP result) and extract its respective sequence from the preliminary sequence file.

Results from TargetP were used as an input for TMHMM (v2.0) (http://www.cbs.dtu.dk/services/TMHMM/) which utilizes a machine learning approach to find transmembrane helices [18] so as to separate them from signal peptides. Only those sequences predicted with zero to one transmembrane helix present was considered to be part of the secretory pathway. SignalP, TargetP and TMHMM are all tools developed by the Center for Biological Sequence Analysis (CBS) in the Technical University of Denmark (DTU).

Further filtering of the results of TMHMM was done through Protcomp (v9.0) (http://linux1.softberry.com/berry.phtml) which uses a neural network based prediction methodology to calculate the subcellular localization of both animal and fungal proteins [19]. This helps to exclude those proteins that have a high probability of not being located in the extracellular space.

A BlastP search was done with the final set of proteins to identify the possible functions that these proteins could play in aiding the fungus or to disrupt the host cellular mechanisms.

III. RESULTS AND DISCUSSION

The methodology followed to predict the secretome of *G. lucidum* is given in Fig.1. A total of 2, 89, 550 proteins were present in the protein file available in the *G. lucidum* database. Running these sequences through the standalone version of SignalP with the set sensitive parameters, provided us with a total of 31, 216 proteins with potential signal peptides present in their N-terminal region. Removing exact duplicates from these sequences helped to keep the results unique and decrease the number of sequences to be tested to a manageable level. Thus, a total of 6, 054 proteins were obtained and used as an input in TMHMM. Signal peptide sequences generally have a N-region that is positively charged and a hydrophobic helical H-region which makes it very similar to transmembrane sequence which also display hydrophobicity [20]. All sequences that were predicted with more than two transmembranes were removed from the set of probable sequences comprising the secretome.

This gave us a set of 205 sequences which were eventually used as an input for the Protcomp tool which predicted a set of 21 proteins that would be present in the secretome of *G. lucidum* (Table 1). One probable reason as to the low number of sequences predicted would be due to the stringent parameters that were set during each analysis so as to remove any probable doubt as to if a protein was part of the secretome.

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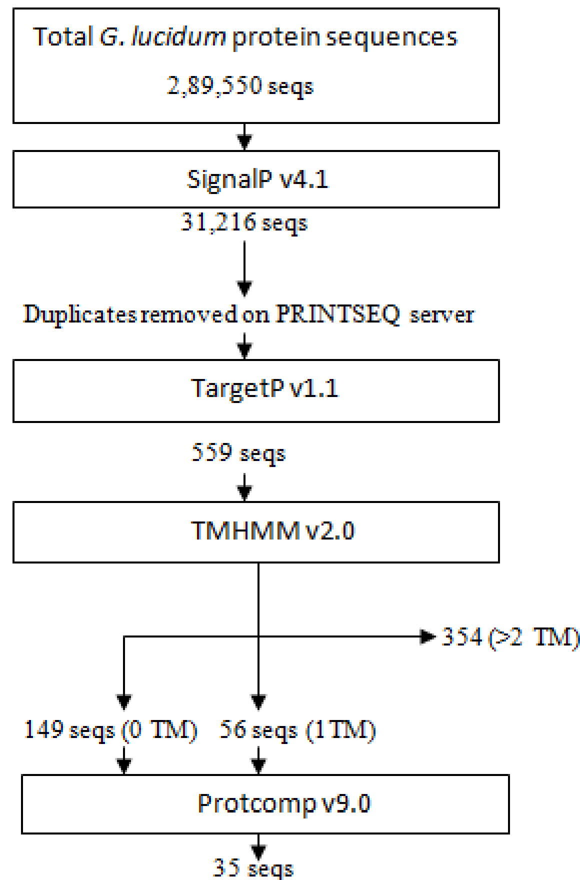

Fig.1: The methodology followed to predict the secretome of *G. lucidum*

Interactions between the host and pathogen proteins and metabolites defines the outcome of pathogenesis in most cases. A functional prediction of the protein sequences obtained revealed that a major set of the proteins had hydrolase activity that helps the fungus degrade polysaccharides and cause rot [21]. Previous transcriptome studies of *G. lucidum* have also identified hydrolases (28.86%) as the most dominant set of genes present [22]. Histidine protein kinases were present that help the fungi sense their external environment and adapt accordingly [23]. Cytochrome P450 enzymes were found to be present in the secretome that works to detoxify pollutants in the surroundings covert hydrophobic components in the secondary and primary metabolic pathways [24]. Analysis of the *Trichoderma* secretome has also identified the presence of Cytochrome P450 enzymes in small quantities [25].

A few proteins were singled out to be part of the cell wall anchor family that form part of the cell wall and represent the insoluble part of the secreted proteins [26]. Meprin and TRAF (TNF receptor associated factors) homology (MATH) domains have been detected through homology searches in a variety of eukaryotes including fungi where they function in protein processing [27]. RCC2 like protein have been identified to be expressed by the genome of the basidomycete fungi *Termitomyces* [28] though the exact role that these proteins play in the secretome has yet to be determined.

Secretome analysis is a field generating much interest in recent times and there have been studies done on the genome on other fungi like *Fusarium graminearum* [29], *Trichoderma harzianum* [30] etc. Multiple databases are also currently available like the Fungal Secretome Database [31] and FunSecKB [27]. Studies of the secretomes of basidomycota has generally revealed a significant number of H_2O_2 producing enzymes and peroxidases [31]. *Phanerochaete chrysosporium* cultures were the first to reveal the presence of LiP and MnP [32]. Considering that conditions change the secretome of *G.*

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lucidum and that there are more than 30,000 species [33] in the Basidomycota division, it would be more interesting to see discover new naturally produced, novel compounds that can be put to use in our pharmacological or other vital sectors.

Table 1: Predicted sequences present in the secretome of *G. lucidum*

SI. No:	Protein id	Functional prediction	Transmembrane helices identified	Protein location prediction	Score in Protcomp
1	jgi|Gansp1|125621|fgenesh1_pm	HAD hydrolase, family IA, variant 1	PredHel=0	Extracellular (Secreted)	5.4
2	jgi|Gansp1|127542|fgenesh1_pm	no result	PredHel=0	Extracellular (Secreted)	4.6
3	jgi|Gansp1|127551|fgenesh1_pm	no result	PredHel=0	Extracellular (Secreted)	9.7
4	jgi|Gansp1|128913|fgenesh1_pm	RCC2 like protein	PredHel=0	Extracellular (Secreted)	8
5	jgi|Gansp1|129212|fgenesh1_pm	cell wall surface anchor family protein	PredHel=0	Extracellular (Secreted)	9.6
6	jgi|Gansp1|132418|fgenesh1_pm	hypothetical protein DICSQDRAFT_133460	PredHel=0	Extracellular (Secreted)	8.9
7	jgi|Gansp1|107765|fgenesh1_pg	endoglucanase	PredHel=0	Membrane bound Extracellular (Secreted)	9.6
8	jgi|Gansp1|108353|fgenesh1_pg	glycoside hydrolase family 5 protein	PredHel=0	Membrane bound Extracellular (Secreted)	9.9
9	jgi|Gansp1|108375|fgenesh1_pg	glycoside hydrolase family 5 protein	PredHel=0	Membrane bound Extracellular (Secreted)	2.5
10	jgi|Gansp1|108668|fgenesh1_pg	putative threonine-rich GPI-anchored glycoprotein	PredHel=0	Extracellular (Secreted)	9.7
11	jgi|Gansp1|111561|fgenesh1_pg	two-component system sensor histidine kinase	PredHel=0	Membrane bound Extracellular (Secreted)	6.5
12	jgi|Gansp1|112370|fgenesh1_pg	Glycoside Hydrolase Family 3 protein	PredHel=0	Membrane bound Extracellular (Secreted)	9.6
13	jgi|Gansp1|121512|fgenesh1_kg	Cytochrome P450	PredHel=0	Membrane bound Extracellular (Secreted)	4
14	jgi|Gansp1|134216|estExt_fgenesh1_pm	Unknown	PredHel=0	Extracellular (Secreted)	7.1
15	jgi|Gansp1|139166|estExt_fgenesh1_pm	hypothetical protein STEHIDRAFT_112496	PredHel=0	Membrane bound Extracellular (Secreted)	9.4
16	jgi|Gansp1|13864|gw1.4.113.1	No result	PredHel=0	Extracellular (Secreted)	3.9
17	jgi|Gansp1|145018|estExt_fgenesh1_pg	Cytochrome P450	PredHel=1	Membrane bound Extracellular (Secreted)	7.5
18	jgi|Gansp1|146360|estExt_fgenesh1_pg	hypothetical protein TRAVEDRAFT_109170	PredHel=1	Membrane bound Extracellular (Secreted)	8
19	jgi|Gansp1|147630|gm1	zf-TRAF domain protein	PredHel=1	Extracellular (Secreted)	5.8
20	jgi|Gansp1|150489|gm1.3188_g	no result	PredHel=1	Membrane bound Extracellular (Secreted)	3.8
21	jgi|Gansp1|154618|gm1.7317_g	putative transmembrane protein	PredHel=1	Extracellular (Secreted)	6.8

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IV. CONCLUSION

These results provide a base in identifying some of the proteins in the secretome of *G. lucidum* but lab studies need to be conducted to confirm these results. Currently, 2-D gel electrophoresis is considered to be the best method to understand fungal secretome [29]. An intuitive step forward on this research subject would be to compare the secretomes of different *Ganoderma* spp. to identify the common proteins and understand the mode of infection followed by members of the genus. A lot more studies need to be conducted so as to completely elucidate the role played by fungal extracellular biocatalysts and develop counter-measures for crop protection.

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