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Analysis of β-tubulin protein in *Phytophthora infestans* using computational tools

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ABSTRACT: Fungal β-tubulins, a major constituent of microtubules, are an important part of studies on phytopathogens as they play a vital role in the infection process. The knowledge about molecular mechanisms responsible for pathogenicity is a prerequisite to evolve suitable management strategies against the pathogen. In the present study, computational analyses of β -tubulin protein of *Phytophthora infestans*, the causal organism of late blight of potato was carried out. The network analysis of *P. infestans* β-tubulin protein revealed its interacting partners. Five motif regions could be predicted. Conserved domain search showed the presence of nucleotide binding site, Taxol binding site, α/β domain interface and α domain interface.

KEYWORDS: β-tubulin, *P. infestans*, network analysis.

I. INTRODUCTION

Phytophthora is an oomycete which belongs to the Kingdom Stramenopila. It is a diploid organism, whose life cycle includes both sexual and asexual reproduction. The genus name *Phytophthora* meaning 'plant destroyer' was given by Anton de Bary, when he described the potato late blight pathogen *P. infestans*. *Phytophthora* spp. attack a wide range of plants, and are responsible for some of the world's most destructive plant diseases. Almost all species within the genus *Phytophthora* are plant pathogens.

In eukaryote, tubulins constitute the major components of the microtubules. Microtubules are involved in a plethora of cellular processes, including cell division, motility of cilia or flagella and intracellular transport [1]. The-, β-, and γ-tubulins are ubiquitous and can be found in all eukaryotes. Assembly of α- and β-tubulins occurs as heterodimers in a head-to-tail fashion and results in the formation of the basic building block of the microtubule [2]. The γ-tubulins are chiefly found in the microtubule organizing center and it has been implicated in the initiation of microtubule assembly [2].

Fungal β-tubulins assume importance in control of oomycetes as they are targets of zoxamide fungicides. This fungicide disrupts microtubule cytoskeleton and inhibits nuclear division in oomycetes as the result of a highly specific covalent binding to the β subunit of tubulin [3].

The microtubular cytoskeleton of *Phytophthora* hyphae and spores is of fundamental importance for their growth and development. In hyphae, microtubules form meiotic and mitotic spindles and longitudinal cytoplasmic arrays [4]. In zoospores, microtubules form flagellar axonemes, basal bodies, flagellar rootlets and cytoplasmic arrays that are likely to shape and position the nucleus [5]. B-tubulin gene sequences has been found to be useful in reconstructing the phylogenetic relationships among various *Phytophthora* spp.

In view of the extremely high diversity, adaptability and pathogenic nature of the *P. infestans*, it is imperative to dissect the multiple roles of the β-tubulin and is functional interplay in pathogenicity. In the present study, detailed analysis of β-tubulins protein of *P. infestans* was undertaken using computational tools.

II. METHODOLOGY

The sequence of β-tubulin protein of *P. infestans was* retrieved from Universal Protein Resource (http://www.uniprot.org/) [6]. Similarity search of β-tubulin protein was carried out BLASTp (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [7]. The protein interaction network of β-tubulin protein was constructed using STRING 10 (http://string-db.org/) [8]. The motif prediction was carried out by MOTIF search tool

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(http://www.genome.jp/tools/motif/). Detailed analysis of motifs was carried out by MEME (http://meme-suite.org/tools/meme). The conserved domain was predicted by CDD (http://www.ncbi.nlm.nih.gov/cdd).

III. RESULTS

Protein sequence retrieval: The β-tubulin protein of *P. infestans* (Accession no. PITG_00156) was found to be 446 amino acids in length. This protein was found to be closely related to *P. capsici* β-tubulin protein with 99% identity with 100% query coverage.

Interaction diagram: The interaction diagram of *P. infestans* β-tubulin protein, generated by STRING 10, is given in Figure 1.

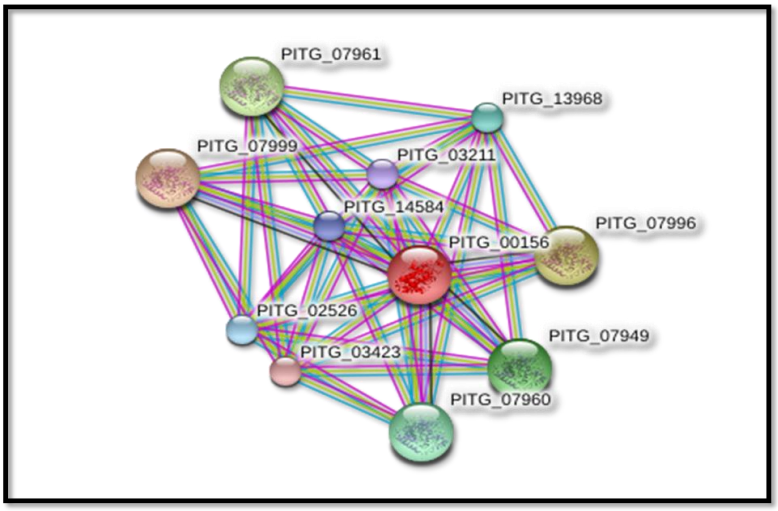

Figure 1: Network diagram of predicted functional associations of β-tubulin protein.

The interaction analysis revealed that β-tubulin protein of *P. infestans* was networked with different proteins in *P. infestans* (α-tubulin, putative (PITG_07999), α-tubulin (PITG_07996), cleavage induced tubulin α chain (PITG_07961), putative α-tubulin (PITG_07949 and PITG_07960), dynein heavy chain (PITG_13968 and PITG_02526), microtubule-associated protein EB1 (PITG_14584), putative uncharacterized protein (PITG_03211) and dynein heavy chain (PITG_03423). The functionally linked β-tubulin protein of *P. infestans* is shown in Figure 1. The 10 predicted network partners of *P. infestans* β-tubulin protein are shown in Figure 2.

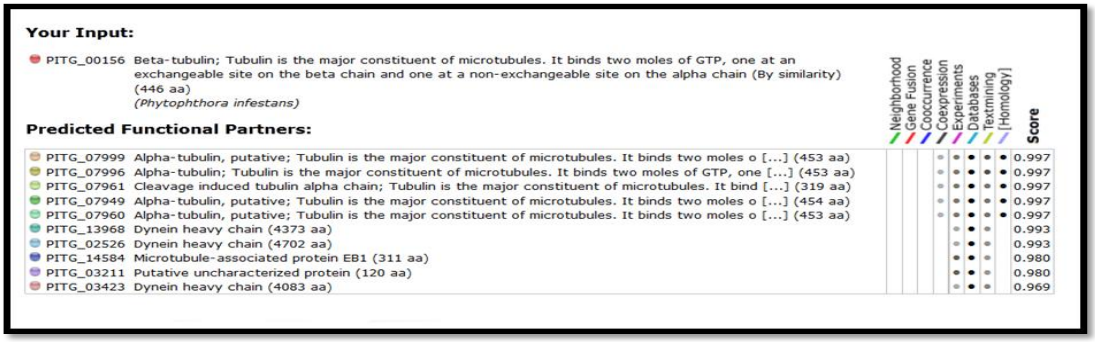

Figure 2: Predicted functional partner's tubulin protein of *P. infestans*.

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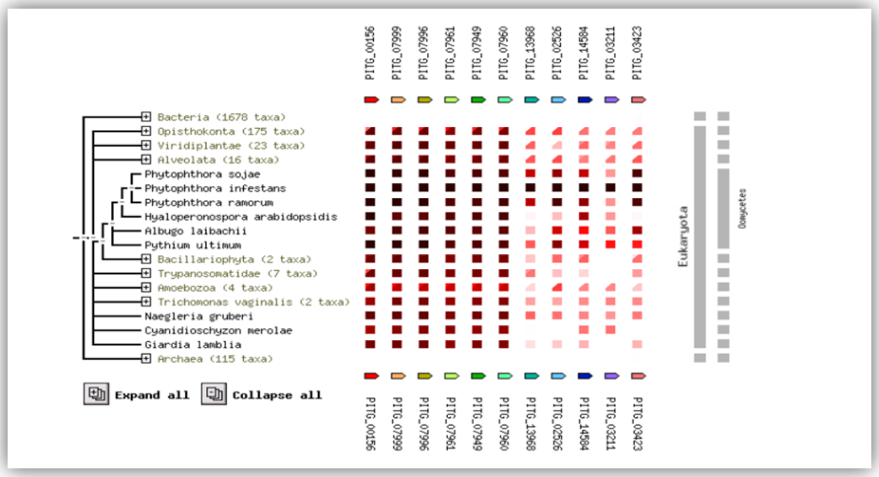

Figure 3: Occurrence of β-tubulin proteins in different organism's

The β-tubulin protein also occurs in other organisms (Figure 3), which include other *Phytophthora* spp., which includes *P. sojae* and *P. ramorum* which is based on the similar occurrence of patterns. The result reveal that β-tubulin proteins are conserved in oomycetes.

Motif search: The structural motif of the β-tubulin protein was predicted by MOTIF search (Figure 4). The motif search predicted five motifs namely Tubulin: Tubulin/FtsZ family, GTPase domain (3-211), Tubulin_C: Tubulin C-terminal domain (261-381), Misat_Tub_SegII: Misato Segment II tubulin-like domain (2-71), Tubulin_3: Tubulin domain (120-194) and Tubulin_2: Tubulin like (113-176).

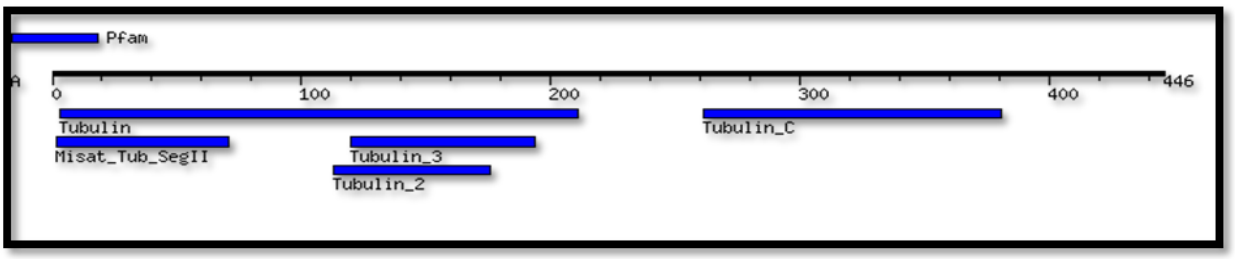

Figure 4: Motif's identified using Motif search

The motif analysis of β-tubulin protein of *P. infestans* was carried out by MEME. The details of five discovered motif is given in Table 2. The first motif , of ~13 amino acids length, was found in three locations of the sequence (204 to 216, 252 to 264 and 350 to 362). The second motif was of ~6 amino acid length (19 to 24 and 340 to 345). There was ~15 amino acids length in third motif, situated in three sites (85 to 99,133 to 147 and 389 to 403). The fourth motif was situated in two locations (165 to 174 and 315 to 324) and contained ~10 amino acids. In fifth motif, ~7 amino acids were present and two sites were predicted in this sequence (226 to 232 and 297-303)

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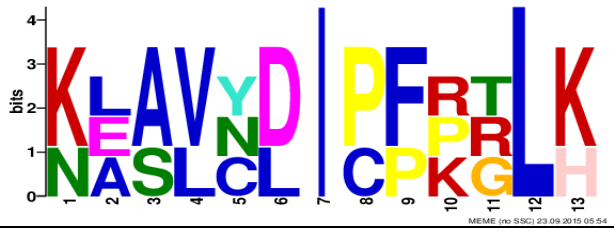
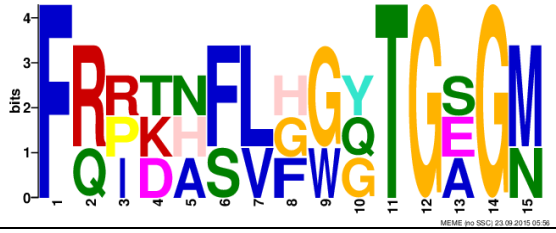
No.	Predicted Motif Logo	E-value	Sites	Width
1		5.2e+000	3	13
2		1.2e+001	2	6
3		2.9e+001	3	15
4		1.0e+002	2	10
5		51.4e+002	2	7

Table 2: Details of discovered motifs of β-tubulin protein of *P. infestans*

Conserved domains Search: The conserved domains were predicted by using Conserved Domains Database (CDD). The domains were found to possess nucleotide binding site, Taxol binding site, α/β domain interface and α domain interface (Figure 5). The sequence was classified under 'tubulin superfamily'. There were seven domain hits (The beta-tubulin family, Tubulin C-terminal domain, Tubulin/FtsZ family, GTPase domain, Tubulin beta chain, Tubulin beta chain, Tubulin [Cytoskeleton], Tubulin/FtsZ family and GTPase domain). Detailed information of the same is furnished in Table 3.

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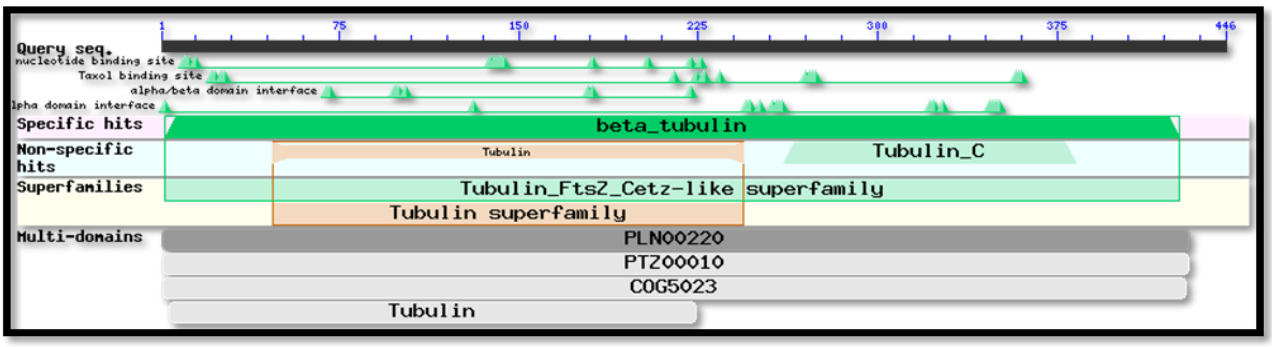

Figure 5: The identified conserved domain of β-tubulin protein of *P. infestans*

Sl. No.	Name	Accession	Description	Interval	E-value
1	beta_tubulin	cd02187	The beta-tubulin family;	2-426	0e+00
2	Tubulin_C	pfam03953	Tubulin C-terminal domain;	261-383	4.16e-70
3	Tubulin	smart00864	Tubulin/FtsZ family, GTPase domain;	47-244	4.16e-60
4	PLN00220	PLN00220	tubulin beta chain;	1-430	0e+00
5	PTZ00010	PTZ00010	tubulin beta chain;	1-430	0e+00
6	COG5023	COG5023	Tubulin [Cytoskeleton];	1-429	0e+00
7	Tubulin	pfam00091	Tubulin/FtsZ family, GTPase domain;	4-224	8.24e-59

Table 3: The predicted domain list of β tubulin protein of *P. infestans*

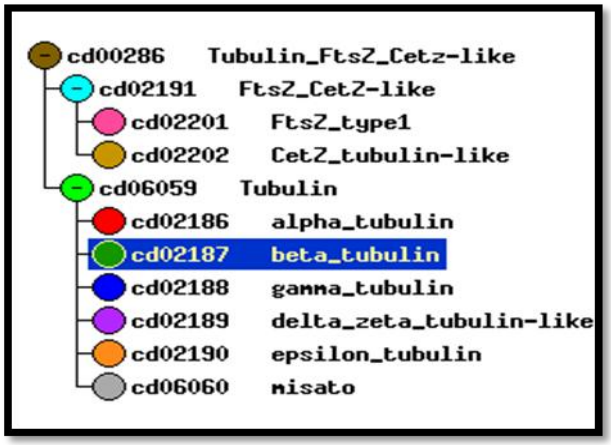

Figure 6: Sub-family hierarchy of *P. infestans* β-tubulin protein

The cd02187 (β-tubulin) was part of a hierarchy of related conserved domain models. The graphical representation (Figure 6) provides details of hierarchy of cd02187 as a member of the superfamily cl10017 (226-232Tubulin_FtsZ_Cetz-like).

IV. CONCLUSION

Microtubules play a critical role in pathogenicity mechanisms of plant pathogens. As they are the targets of fungicides, they are an important area of research in management of these devastating phytopathogens. *In silico* studies of β-tubulin of *P. infestans* carried out in this study reveals important details of its functional partners, structural motifs and functional domains.

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