Article

Host-Pathogen protein-protein interactions reveal the key mechanisms behind the endosymbiotic association of *Wolbachia* with *Brugia malayi*

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Abstract
Wolbachia are gram-negative endosymbiotic bacteria residing in the nematode host *Brugia malayi* that causes lymphatic filariasis in humans. *Wolbachia* settle in the host environment for their survival, growth and reproduction by maintaining an obligate mutualism relationship. However, the mechanism used by *Wolbachia* to maintain its mutualistic relationship with the nematode is poorly understood. Therefore, to elucidate the host-pathogen interaction (HPI) mechanism of *Wolbachia* and *Brugia malayi*, we used interolog-based approach for identification of host-pathogen protein-protein interactions (HP-PPIs) network and domain-based approach for the validation. The inter-species HP-PPIs network contained 392 proteins (258 *Brugia malayi* and 134 *Wolbachia* proteins) connected by 829 edges. Further, based on interolog approach, we identified 24 pathogen (*Wolbachia*) and 33 host (*Brugia malayi*) proteins involved in HPI. This study also reported 8 hub genes namely bma-atp-1, Bm1_25145, A0A0K0JKQ9, fusA, bma-eef-2, rplB, Bma-rpl-2, and bma-eftu-2 involved in maintaining an endosymbiotic relationship between *Wolbachia* and *Brugia malayi*. Furthermore, we also reported six-pair of host-pathogen interactions containing numerous pairs of shared domains that might be crucial for the establishment of mutualistic relationship between *Wolbachia* and *Brugia malayi*. The functional analysis revealed most of the *Brugia malayi* proteins are involved in the generation of precursor metabolites showing catalytic activity in the intracellular anatomical structures and organelles. The subcellular localization reported host and pathogen proteins were located in mitochondria and cytoplasm respectively. The *Brugia malayi* pathways involved in HPI were metabolic pathway, oxidative phosphorylation pathway, and spliceosome pathways, indicates that for maintaining mutualism energy-yielding pathways are targeted. Overall, this work provides new insights into the mechanism of HPIs between *Wolbachia* and *Brugia malayi*, and will help researchers a deeper understanding of the intracellular pathogenic activity and endosymbiotic relationship of *Wolbachia* with its host *Brugia malayi*.

Keywords *Wolbachia*; *Brugia malayi*; host-pathogen interaction; Interologs; domain-domain interaction; enrichment analysis; Metabolic pathway; network biology.

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1 Introduction

Wolbachia are pathogenic endosymbiotic and alpha-proteobacterias that infect a variety of arthropods and nematodes (Sironi et al., 1995; Werren et al., 1995). These bacteria are gram-negative, obligate and intracellular, and belong to the order Rickettsiales (Harris et al., 2010). *Wolbachia* depict two types of relationships with their hosts one is a reproductive parasite in arthropods, and other is obligate mutualism in filarial nematodes (Bouchon et al., 1998; Kageyama et al., 2002). The pathogen manipulates the host by means of parthenogenesis (P), feminization (F), male-killing (MK), by inducing cytoplasmic incompatibility (CI), and nutritional supplement (Sironi et al., 1995; Werren et al., 2008; Cordaux et al., 2011; Miyata et al., 2017; Beckmann et al., 2017; Perlmutter et al., 2019). It was estimated that *Wolbachia* infection is up to 40-76% in insects (Hilgenboecker et al. 2008; Zug and Hammerstein, 2012; Kajtoch and Kotásková, 2018).

Brugia malayi is a nematode causing lymphatic filariasis in humans (Erickson et al., 2009). *Wolbachia* infects *Brugia malayi* and provides extra fitness to progeny of the host which consequently helps *Brugia malayi* to survive inside humans (Foster et al., 2005; Melnikow et al., 2013). The relationship between *Wolbachia* endosymbiont of *Brugia malayi* (wBm) with its host *Brugia malayi* (Bm) is obligate mutualism in nature (Foster et al., 2005; Grote et al., 2017; Lustigman et al., 2014). *Wolbachia* infecting *Brugia* species belong to supergroup D having relatively smaller genome length with a smaller number of proteome (Foster et al., 2005; Lefoulon et al., 2020; Gerth et al., 2014; Ross et al., 2009; Sharma and Som, 2023).

HPI mechanism using host-pathogen protein-protein interaction (HP-PPI) network is conventionally used to identify the key proteins and their role in pathogenesis. Previously, HP-PPI has been used to explore *Arabidopsis-pseudomonas* relationship (Sahu et al., 2014). In other organisms, HP-PPI methods used for understanding the pathogenesis between host and pathogens were Human-*E. coli* by Bose et al. (2017), and Human-*Mycobacterium* by Verma et al. (2022). HP-PPI has been extensively used to study the mechanism used by pathogen to interact with their host. However, the experimental studies performed to predict the physical interactions between host-pathogen proteins are costly, time consuming and show high rate of false positives. Therefore, it is very important to build computational strategies to predict the HP-PPI on a genome-scale and to find the effector proteins that can be targeted for drug repurposing or to elucidate host-pathogen interaction mechanisms.

Therefore, to describe the HP-PPI mechanism in wBm-Bm, we performed a genome-scale interolog-based prediction that was validated by a domain-domain interaction-based approach. In this work, we tried to identify the key proteins and their mechanisms responsible for maintaining the HP-PPI. The host and pathogen references used were *Brugia malayi* (Bm) and *Wolbachia* (wBm) intra-species interactions respectively to construct HP-PPI network between wBm-Bm. The inter-species HP-PPI network constructed using sequence homology was explored for hub proteins and host-pathogen protein-pairs to decipher the mutualistic mechanism. Further, we performed subcellular localization and functional enrichment analysis to elucidate the essential biological processes, key pathways and site of activity within the host enabling the bacterial pathogenicity. Further, domain-domain interaction-based approach was utilized to validate the results. We believe that the provided evidences in this study will serve as a strong foundation for future experimental validations and a deeper understanding of mechanisms behind mutualistic behavior of *Wolbachia* with its host *Brugia malayi*.

2 Materials and Methods

2.1 Data acquisition

Identification of homologous protein sequences was important to investigate PPI *Wolbachia* and *Brugia malayi*. Accordingly, we downloaded the whole proteome of host *Brugia malayi* and pathogen *Wolbachia*

(wBm) from the UniProt database (https://www.uniprot.org/). As a result, 15168 protein sequences were downloaded for the *host Brugia malayi* (UniProt Id UP000006672), and 805 proteins sequences were downloaded for pathogen wBm (UniProt Id UP000000534). An overview of the methodology used in the study has been given in (Fig. 1).

2.2 Ortholog-based protein-protein interaction identification

The STRING database (https://string-db.org/) was used to collect the intra-species template interactions needed to build a background library. This phase involved downloading the intra-species interactomes for the host (Bm) and pathogen (wBm) separately. The STRING database contains all varieties of interaction, including text mining, homology-derived, and experimental. Hence, in order to reduce the possibility of false positives, we only considered PPIs that were experimentally reported. Furthermore, in order to lower the false discovery rate, PPIs were retrieved with the confidence score >0.7 for both the host and the pathogen.

2.3 Host (Bm) and pathogen (wBm) protein-protein interactions

Sequence homology detection approach was used to find intra-species template interactions as well as inter-species protein-protein interactions (i.e., HP-PPI). Here, we used BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the proteomes of the pathogen wBm and the host Bm, which were both compiled using proteins with experimentally reported interactions. However, there is no consensus in the scientific community on the parameters to be used for homolog screening, Sahu et al. (2014) used a set of parameters to identify inter-species homologs such as an e-value >1e-4, sequence identity >50%, and query coverage >80%. In a different investigation, Bose et al. (2017) employed the parameters like e-value below 1e-10, identification percent greater than 30%, and query coverage greater than 80% to detect HP-PPI. In this study, we considered expected value (E-value) ≤1e-10, query coverage (Qc) ≥60%; and sequence identity (Pi) ≥40%.

2.4 Subcellular localization of the proteins

It is essential to understand where proteins are located within the cell in order to detect HP-PPIs. Therefore, computational prediction of subcellular localization of proteins is a crucial step to understanding how pathogen proteins work within the host environment. PSORTb v3.0.3 (https://www.psort.org/psortb/) was used to identify the subcellular location of *Wolbachia*, as PSORTb utilizes the probabilistic approach to identify the most probable place of subcellular localization. The online webserver, DeepLoc v2.0 (Thumuluri et al., 2017) was used for the identification of subcellular localization of *Brugia malayi*.

2.5 HPI network analysis and hub identification

The predicted inter-species HPIs were used to construct a PPIs network between the host (Bm) and pathogen (wBm). This was achieved by the incorporation of intra-species background libraries of host and pathogen as templates for the construction of HPIs. Cytoscape v3.8.2 (https://cytoscape.org/) was utilized for the visualization of networks (Shannon et al., 2003). The topological analysis of a network helps in the identification of the most influential node, known as the hub gene/protein (Som et al., 2010; Narad et al., 2017; Yang and Zhang, 2022). The hub genes/proteins are known to be most conserved at the sequence level and therefore show a high degree of robustness (Chaturvedi and Som, 2022; Barabási and Oltvai, 2004). In this study, we utilized three parameters for the identification of hubs: Betweenness, closeness and degree centrality. CytoHubba plugin of Cytoscape was used for hub-gene identification by calculating the node scores for the centrality measures (Chin et al., 2014). The common proteins amongst all three centralities were identified as the hub proteins.

2.6 Functional enrichment analysis: Gene ontology and pathway analysis

The common function/pathway regulated by proteins of the host and pathogen might point out the interdependency on each other and therefore justify the mutualistic relationship between the Bm host and wBm

pathogen. Hence, it is necessary to functionally annotate the proteins involved in HPI. Gene ontology (GO) analysis using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) was performed for the pathogen interacting host proteins for all enriched terms: biological processes (BP), molecular functions (MF), cellular compartments (CC). Pathway analysis of the proteins involved in HPI might help in understanding probable mechanisms related to the survival of the host. Identification of enriched pathways for the pathogen interacting host proteins was performed using ShinyGo v0.77 (Ge et al., 2020). Significantly enriched terms for pathway enrichment analysis were identified based on the false discovery rate (FDR) < 0.05.

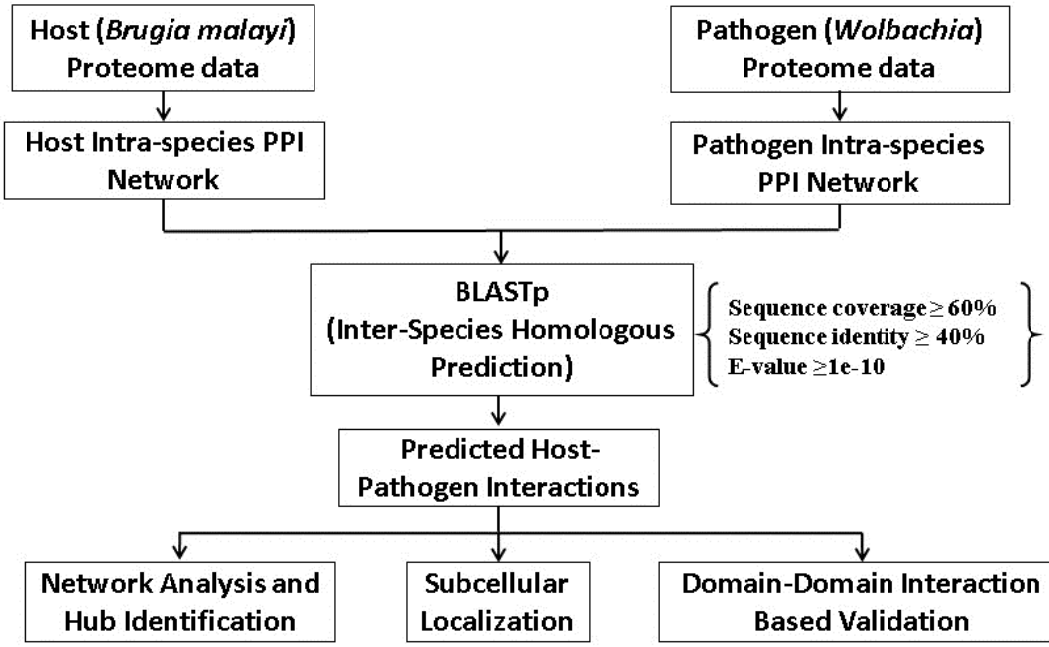

Fig. 1 Workflow of the methodology used in this study.

3 Results

3.1 Host-pathogen interaction between *Wolbachia* (wBm) and *Brugia malayi*

Wolbachia pathogenic and endosymbiotic bacteria exhibit two kinds of lifestyle with their hosts: reproductive parasitism and obligate mutualism. It has been observed that both lifestyles were indecently evolved with respect to their host. In this work, we have tried to understand the mutualistic behavior of *Wolbachia* (wBm) with its host *Brugia malayi*.

The intra-species networks of the wBm and *Brugia malayi* host were seperately derived using the STRING database with a confidence score>0.7. The PPI network of wBm contained 252 nodes with 2120 edges. Alternatively, the PPI network of *Brugia malayi* contained 1329 nodes with 6625 edges. Eventually, both the PPI networks were used as template libraries for the construction of inter-species PPI. Interolog-based approach was used to predict inter-species HPIs between proteins from wBm and *Brugia malayi*. Hence, we performed homology identification of protein from both the host and pathogen using BLASTp alignment. After aligning the 252 proteins of wBm against 2287 proteins of *Brugia malayi*, 35 homologous pairings of HPI were discovered involving 24 wBm proteins and 33 host proteins. The list of the pathogen (wBm) and host (Bm) proteins involved in inter-species PPI has been given in Table 1. The predicted homologous pairings were used to construct an inter-species PPIs network between the host and the pathogen (Fig. 2).

Table 1 List of interacted pathogen (wBm) and host (*Brugia malayi*) proteins.

wBm Acc. (Pathogen)	*Brugia malayi* Acc. (Host)	Query coverage (%)	Sequence Identity (%)	E-value
Wbm0108	A0A5S6PFF8	73	42	3.70E-62
Wbm0448	A0A0J9XWZ6_Bm1_17330	99	56	0
	A0A0K0IMU2_Bm1_17325	99	54	0
Wbm0474	A0A0K0J4J0_Bma-nuo-1	98	64	0
	A0A5S6PGP5_Bm1_14150	93	65	0
Wbm0600	A0A1P6BM73_Bm1_17690	90	59	1.28E-105
Wbm0708	A0A0J9XLN3	88	40	1.11E-76
	A0A0J9YG30_Bm1_14510	85	40	7.82E-73
	A0A0J9Y634_bma-laf-1	90	40	2.98E-72
	A0A0J9Y3N8_Bma-ddx-23	86	40	9.26E-67
	A0A5S6PJL4	86	40	9.29E-67
Wbm0756	A0A0K0JPY6_Bm1_11355	99	43	1.47E-11
Wbm0759	A8Q1H5_bma-iscu-1	97	70	3.90E-59
	A0A0I9N5E9_bma-hsp-1	95	40	5.05E-104
	P27541_HSP70	84	40	3.35E-99
Wbm0774	A0A0K0IZY1_Bm1_26800	98	40	1.08E-34
Wbm0738_acpP	A0A0K0J1K2_Bm1_19045	61	51	5.98E-12
Wbm0314_atpA	A0A0K0IZ73_bma-atp-1	98	57	0
Wbm0553_clpP	A0A0H5S7U2_bma-clpp-1	89	45	1.36E-43
Wbm0785_dnaJ	A0A5S6PKC1_Bm1_44845	95	40	3.57E-65
Wbm0495_dnaK	A0A0I9N5E9_bma-hsp-1	88	53	0
	P27541_HSP70	88	53	0
Wbm0344_fusA	A0A0K0JE88_bma-stc-1	60	40	2.17E-71
	A0A0H5S693_bma-eef-2	76	40	4.28E-21
	A0A1U7F448_bma-eftu-2	70	40	1.00E-16
Wbm0350_groL	A0A0K0JMP9_bma-hsp-60	92	45	3.68E-146
Wbm0443_guaA	A0A5S6PVE9_Bm1_44235	99	48	4.82E-81
Wbm0527_guaB	A0A1I9FZY8_Bm5924	90	40	1.97E-95
Wbm0125_nuoD	A0A0K0JGT8_Bm1_25145	99	61	0
Wbm0471_nuoI	A0A0K0JKQ9	99	70	5.32E-79
Wbm0358_prfA	A0A0K0IZS0_Bm1_54480	90	40	4.31E-59
Wbm0169_pyrG	A0A1P6BLY8_Bma-ctps-1	98	43	3.93E-127
Wbm0338_rplB	A0A1I9G6N5_Bma-rpl-2	73	40	5.92E-30
Wbm0531_rpsP	A0A0J9XNC5	60	41	7.67E-10
Wbm0519_sucD	A0A1P6BM13_Bm1_00350	98	59	9.22E-122

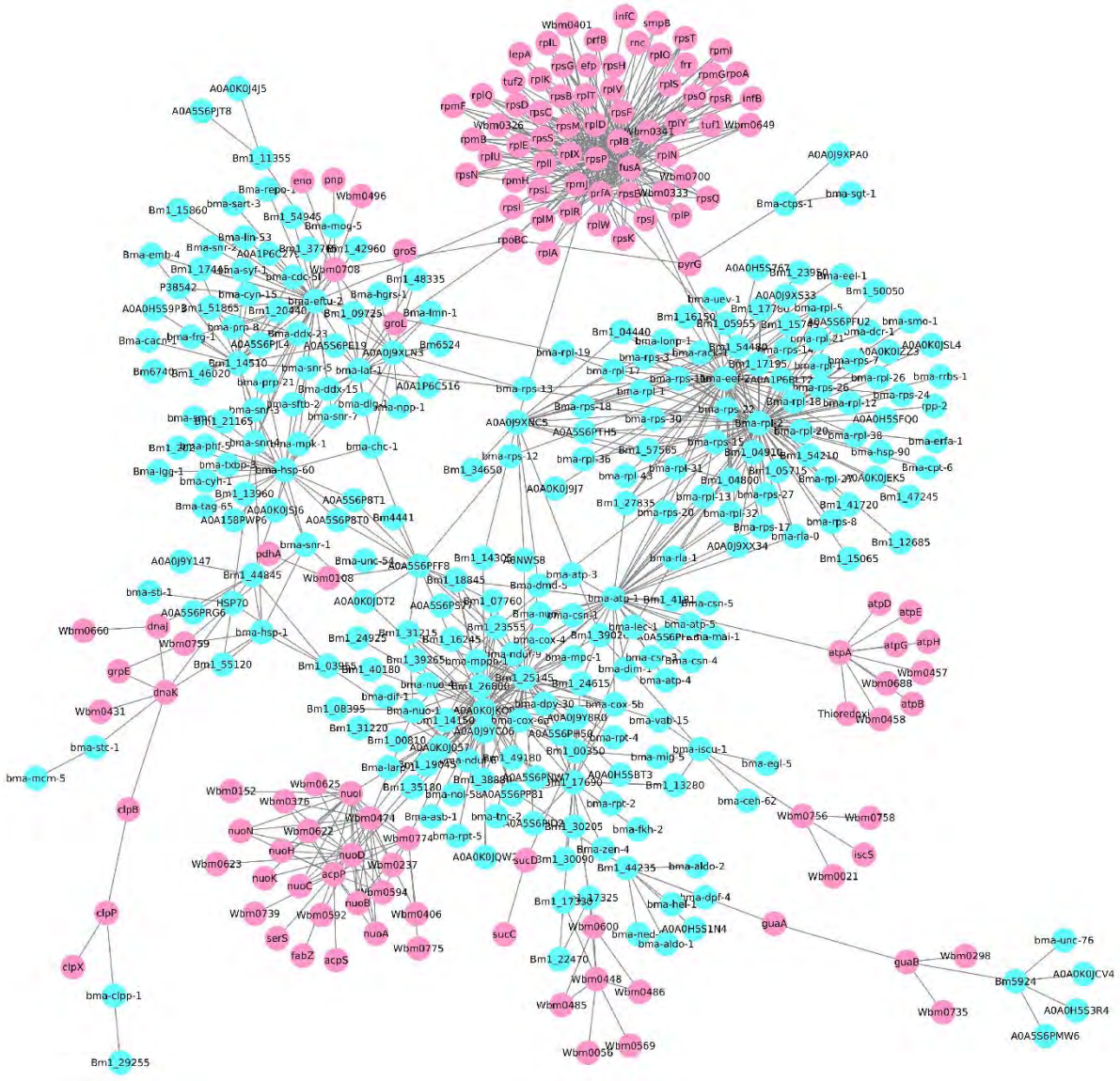

Fig. 2 Core host-pathogen interaction network. The pink nodes represent the pathogen (wBm) proteins and the cyan-colored nodes represent the host (Bm) proteins.

3.2 Subcellular localization of *Brugia malayi* proteins targeted by the wBm proteins

The homologous proteins involved in the formation of the inter-species HP-PPI network were further explored for their subcellular localization. Gram-negative bacteria have evolved a wide array of secretion systems to transport proteins into the extracellular space or target cells (Costa et al., 2015). *Wolbachia* however has been known to rely on type I-IV secretion systems for interacting with their host environment (Lindsey et al., 2020). Therefore, identifying the subcellular localization of host and pathogen homologous proteins might help us to understand their interaction mechanism responsible for the pathogen's endosymbiotic behavior. We found that most of the pathogen, wBm proteins were localized in the cytoplasm and cytoplasmic membranes, and the host, *Brugia malayi* proteins were localized in mitochondrion and cytoplasm (Fig. 3). If the targeted *Brugia malayi* proteins are located in cellular compartments that are similar to pathogens' location, they are very likely to be involved in HPI. Therefore, the pathogen proteins located in the cytoplasm were predicted to be interacting with the host proteins located in the cytoplasm and mitochondrion. The majority of pathogen proteins such as

rplB, fusA, dnaK, nuoD, and nuoI were found in cytoplasm, while wbm0448 and wbm0600 were present in cytoplasmic membrane, whereas only wbm0774 present in periplasmic space. Alternatively, several host proteins were found in cytoplasm such as A0A1I9G6N5, A0A1I9FZY8, and P27541. However, proteins A0A0K0J1K2, A0A0J9XNC5, A0A1P6BM13 were found in mitochondria. Only A0A0K0JE88 was located in endoplasmic reticulum and a few other host proteins were found in nucleus of the host cell as well.

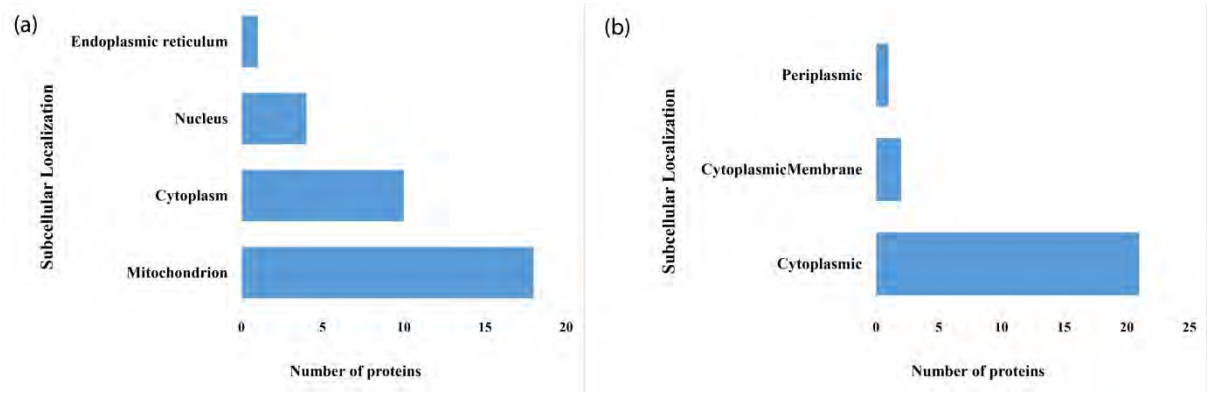

Fig. 3 Subcellular localization of (a) *Brugia malayi* (host) proteins, and (b) *wBm* (pathogen) proteins found in the HP-PPI network.

3.3 Identification of hub proteins in the inter-species HPI network

PPI network analysis has been extensively applied for the investigation of significant nodes in the network. A few nodes that are highly connected with the rest of the network nodes are known as hubs and therefore provide a high level of robustness to the network (Barabási and Oltvai, 2004; Ghosh and Som, 2020). Hub proteins are an intrinsic part of the HPI network that are involved in key biological processes, molecular functions and biological pathways, therefore tracing the path traversing the hub nodes might enhance the understanding of the infection mechanism of the pathogen. In this study, three centrality measures have been used to investigate the protein hubs involved in the HP-PPI: betweenness, closeness and degree centralities. Betweenness centrality represents important nodes connected through the shortest path as compared to others, and practically no other path can be presumed for interaction between the nodes in concern (Singh and Som, 2020). The average betweenness centrality of host proteins was 0.279 while for the pathogen proteins were 0.062. The top 10 proteins in the HPI network based on highest betweenness centralities were bma-snr-1, bma-atp-1, Bm1_25145, A0A0K0JKQ9, fusA, bma-eef-2, rplB, Bma-rpl-2, bma-eftu-2, and bma-hsp-60.

Closeness centrality reveals how fast information flows from one node to another based on the normalized inverse of the sum of the topological distances between the nodes. The average closeness centrality of host proteins was 8.26E-03 and 0.492 for the pathogen proteins. The top 10 proteins in the HPI network based on highest closeness centralities were bma-atp-1, Bm1_25145, A0A0K0JKQ9, fusA, bma-eef-2, rplB, Bma-rpl-2, bma-eftu-2, rpsP, and Bm1_23555.

Degree centrality measures the number of links attached to a particular node and a higher degree is correlated with the stability of the network. The average degree of host proteins was 9.97 and 16.82 for pathogen proteins. Eventually, we identified 30 significant genes based on all three parameters, where 8 genes were found to be common between all the three parameters and were identified as hub proteins and are

important for establishing the host-pathogen relationship (Fig. 4). bma-atp-1, Bm1_25145, A0A0K0JKQ9, fusA, bma-eef-2, rplB, Bma-rpl-2, and bma-eftu-2 were identified as hub proteins in the intra-specific HP-PPI network (Table 2). The hub proteins of the host and pathogen were found to be interacting with each other as well, for instance, in the HPI network few hub proteins such as rplB from wBm pathogen interacted with bma-rpl-2 from host Bm, justifying both are homologs to each other. The interaction network showing hub interactions and their homologs has been shown in (Fig. 5). On further exploring the interaction between host-pathogen in this intra-specific HPI network using hub proteins, we identified six pairs of host-pathogen interactions involving 8 hub proteins. We believe that these six pairs of interactions between host-pathogen proteins might be crucial for the establishment of an obligate mutualistic relationship between wBm and *Brugia malayi*.

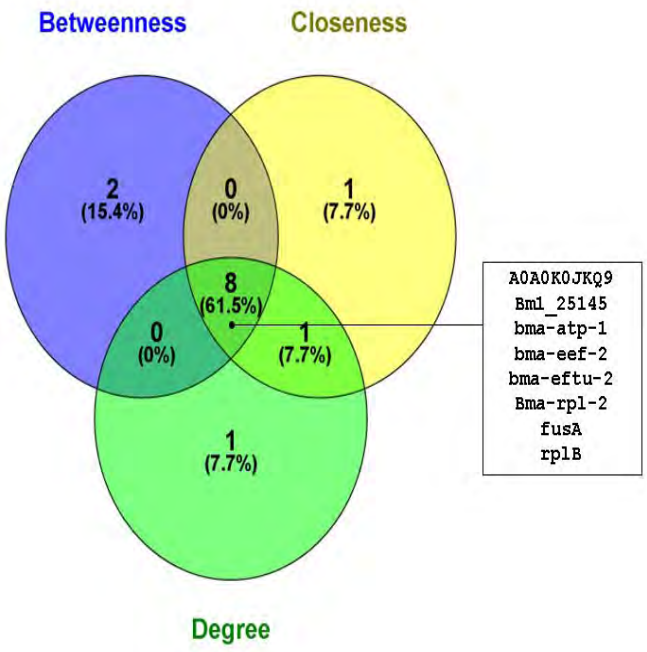

Fig. 4 Ven diagram shows the top 10 proteins based on betweenness, closeness and degree centralities. The box represents hubs based on the three measures.

Table 2 Details of the hub proteins associated with the HP-PPI network.

Proteins	Host/Pathogen	Acc. No	Function	Reference
bma-atp-1	Host	A0A0K0IZ73	Produces ATP from ADP in the presence of a proton gradient across the membrane.	Takeda et al. 1986
Bm1_25145	Host	A0A0K0JGT8	Catalysis of an oxidation-reduction (redox) reaction in which NADH or NADPH acts as a hydrogen or electron donor and reduces a hydrogen or electron acceptor.	Ghedin et al. 2007
Bm5876	Host	A0A0K0JKQ9	Catalysis of an oxidation-reduction (redox) reaction in which NADH or NADPH acts as a hydrogen or electron donor and reduces a hydrogen or electron acceptor.	Ghedin et al. 2007
fusA	Pathogen	Wbm0344	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation.	Foster et al. 2005
bma-eef-2	Host	A0A0H5S693	GTPase activity	Ghedin et al. 2007; Ofolue et al. 1991
rplB	Pathogen	Wbm0338	One of the primary rRNA binding proteins. Required for association of the 30S and 50S subunits to form the 70S ribosome, for tRNA binding and peptide bond formation.	Foster et al.2005
Bma-rpl-2	Host	A0A1I9G6N5	Protein of the ribosome and the signal recognition particle (SRP) where it induces elongation arrest of nascent presecretory and membrane proteins until the ribosome becomes associated with the rough endoplasmic reticulum or the prokaryotic cytoplasmic membrane.	Ghedin et al. 2007
bma-eftu-2	Host	A0A1U7F448	GTPase activity	Ghedin et al. 2007

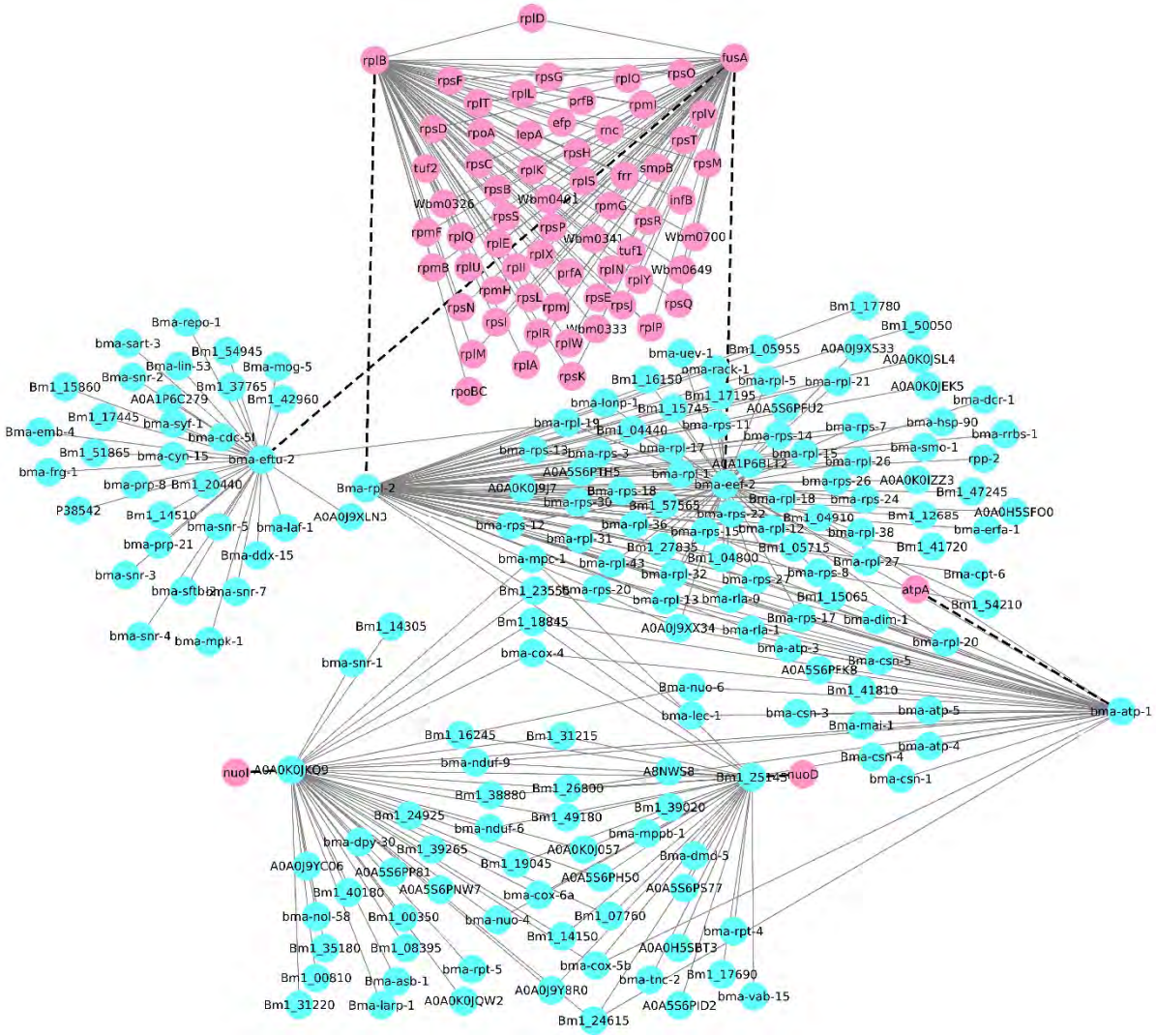

Fig. 5 wBm-Bm PPI network showing hub proteins. The dashed line shows the interaction of hub proteins with pathogen proteins.

3.4 Domain-Domain interaction

The host-pathogen proteins are believed to interact if at least one pair of domains from two proteins interacts (Huo et al., 2015; Zhang and Xin, 2020). Hence, the six pairs of host-pathogen interacting proteins crucial for the establishment of an obligate mutualistic relationship between host and pathogen that were identified using interolog-based approach and were further validated using the domain-based interaction approach. The proteins were assigned to the domain using the InterPro (https://www.ebi.ac.uk/interpro/) database and we found that both host-pathogen proteins in all six pairs had more than one common domain (Table 3). For example, the bma-rpl-2 and rplB pair of host-pathogen interaction had similarities between RNA-binding and the C-terminal domain. It is already evident that these pairs of proteins are homologous and have been found to be interacting on the basis of subcellular localization results as well.

Table 3 List of host-pathogen hub protein pairs along with domain information.

Host (Bm) protein	Bm protein-domain	Pathogen (wBm) protein	wBm protein-domain
bma-rpl-2	RNA binding C-terminal domain	rplB	RNA binding C-terminal domain
efTu	Elongation factor Tu GTP binding domain Elongation factor G C-terminus Elongation factor Tu domain 2 Elongation factor G, domain IV 116 kDa U5 small nuclear ribonucleoprotein component N-terminus	fusA	Elongation factor Tu GTP binding domain Elongation factor G C-terminus Elongation factor Tu domain 2 Elongation factor G, domain IV Elongation Factor G, domain III
eef-2	Elongation factor Tu GTP binding domain Elongation factor G C-terminus Elongation factor Tu domain 2 Elongation factor G, domain IV Elongation Factor G, domain III	fusA	Elongation factor Tu GTP binding domain Elongation factor G C-terminus Elongation factor Tu domain 2 Elongation factor G, domain IV Elongation Factor G, domain III
bma-atp-1	nucleotide-binding domain C terminal domain beta-barrel domain	atpA	nucleotide-binding domain C terminal domain beta-barrel domain
A0A0K0JKQ9	4Fe-4S diclusterdomain	nuoI	4Fe-4S dicluster domain
bma1_25145	Respiratory-chain NADH dehydrogenase, 49 Kd subunit	nuoD	Respiratory-chain NADH dehydrogenase, 49 Kd subunit

3.5 Functional enrichment analysis: gene ontology and pathway analysis

Analysis of the gene ontology and functional pathways might elucidate the functional relevance of the host and pathogen proteins involved in host-pathogen interactions. Gene ontology enrichment analysis was performed to identify enriched biological processes, cellular compartments and molecular function of the host proteins (Fig. 6). We found that the majority of the host proteins were located in mitochondria and cytoplasm and highly enriched in molecular functions such as catalytic activity, heterocyclic compound binding, organic compound binding, etc, and biological process terms such as generation of precursor metabolites and energy, tricarboxylic acid cycle (TCA), electron transport chain (ETC), aerobic respiration, etc. Further, we performed pathway enrichment analysis of the host proteins and we identified 13 significantly enriched (FDR<0.05) pathways such as metabolic pathways, oxidative phosphorylation pathways, carbon metabolism, TCA, and spliceosome (Fig. 7).

Pathway analysis results indicated that most of the proteins found in the HPI network were involved in pathways related to energy thereby suggesting the role of metabolic pathways responsible for the mutualistic relationship between Bm-wBm host-pathogen interactions.

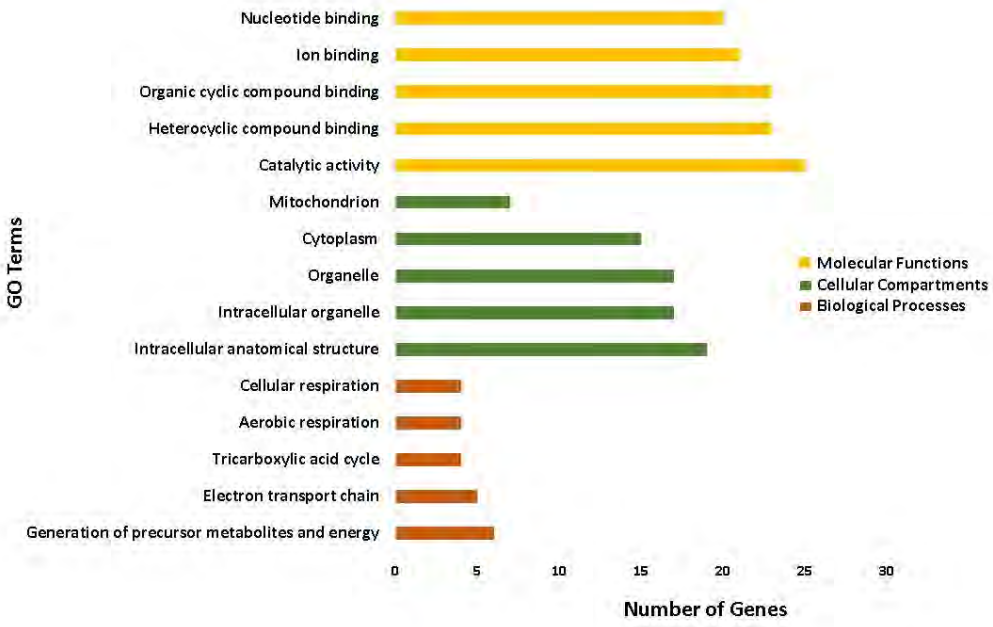

Fig. 6 *Brugia malayi* GO terms found in HP-PPI. Biological process (BP), cellular component (CC), and molecular function (MF).

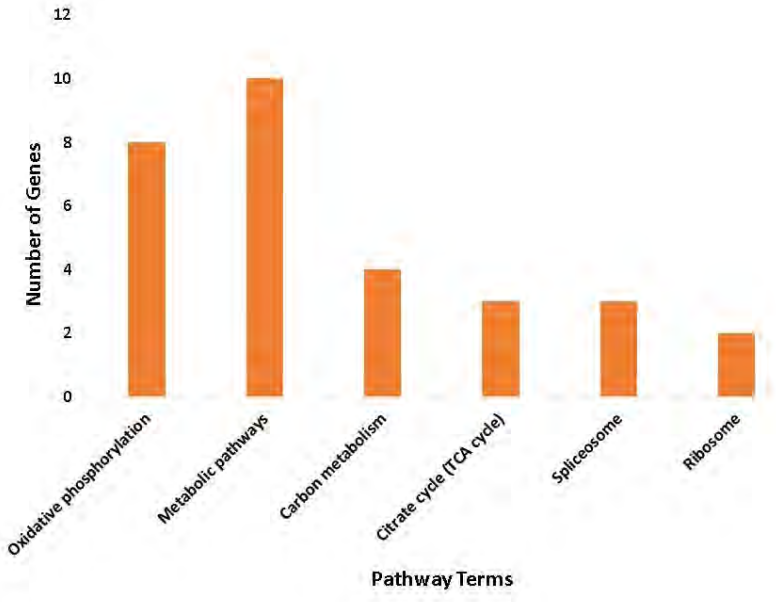

Fig. 7 The major pathways of the *Brugia malayi* proteins involved in HP-PPI.

4 Discussion

The interaction between the *Wolbachia* endosymbiont of *Brugia malayi* (wBm) and its host *Brugia malayi* (Bm) is an obligate mutualism relationship.Obligatory endosymbiotic bacteria that are located in the cytoplasm of the host egg or sperm, as well as around the mitochondria of eukaryotic cells rely on the host's intracellular environment as their principal source of metabolites, energy, nutrients, amino acids, and/or fatty acids (Matthews et al., 2001). *Wolbachia* have been linked structurally and functionally to host organelles such as the endoplasmic reticulum, mitochondria, and intracellular vesicles in filarial worms. In an established infection, *Wolbachia* are primarily intracellular, residing within host-derived vacuoles that are associated with the endoplasmic reticulum (Nevalainen et al., 2023). However, the mechanism of *Wolbachia*'s endosymbiotic behavior with its host *Brugia malayi* is poorly understood. Therefore, to understand this mutualistic association of wBm with its host Bm, we have established a HP-PPI network and identified the crucial proteins that were involved in HP-PPI. Further, we also analyzed the host and pathogen proteins sub-cellular localization, GO and functional pathways of host proteins involved in HP-PPI to understand the role of pathogen interacting host proteins in the survival or maintaining mutualism between Bm-wBm.

We predicted interologs on a homology-based approach and identified 24 wBm proteins showing homology with 33 Bm proteins (Table 1), that were used to establish an inter-species HP-PPI network (Fig. 2). The idea behind interologs, also known as the homologous PPI technique, is that homologous proteins retain their capacity to interact. Lately, it has been used to identify protein interactions between hosts and pathogens in addition to PPIs inside a single organism (Matthews et al., 2001; Wang et al., 2012; Schleker et al., 2012; Krishnadev and Srinivasan, 2011). Interologs represent the conserved interaction between a pair of proteins that have interacting homologs in another organism (Matthews et al., 2001). For instance, if X and Z are interacting proteins of an organism and are homologous to X' and Z' interacting proteins, respectively in another organism then the X-Z protein pair of the host is interolog of the X'-Z' protein pair of the pathogen. Therefore, we established 35 pairs of interologs on homology-based prediction. Most of the proteins identified in the analysis are well established for their-role in HPI. For instance, the host protein Bma-hsp-1 participates in various pathways such as spliceosome, longevity regulating pathway, MAPK signaling pathway, protein processing in the endoplasmic reticulum, and endocytosis (Dorion and Landry, 2002; Kotlajich et al., 2009; Stetler et al., 2010; Will and Luhrmann, 2011; Jiménez et al., 2021). This is an important protein targeted by wBm for its survival and maintaining mutualism with *Brugia malayi*.

Pathogens invade the cytoplasm of the host cells and from there they are transported to many subcellular locations where they manipulate the host environment to enable their own growth and reproduction. The knowledge of subcellular localization of the Bm proteins targeted by the predicted wBm proteins can be helpful in deciphering the mechanism of host-pathogen interactions. The expected outcome will support host-pathogen interactions if the targeted Bm proteins are very relevant to the invasion of the pathogen or very likely to be involved in interactions with the pathogen. To have a better understanding of the location of interactions in the host, we explored the subcellular localization of the Bm and wBm proteins. The host proteins were mostly found in their cytoplasm and mitochondrial membranes, and most of the pathogen proteins were located in their cytoplasm and periplasm (Nevalainen et al., 2023).

Gene ontology results in our study reported that cytoplasm and mitochondria are the most prominent site for the activity of pathogen proteins inside the host. Hence, it was hypothesized that as *Wolbachia* is found near mitochondria and inside the cytoplasm of the host, and also maternally inherited (Poole et al., 2014; Nevalainen et al., 2023), hence there is a high possibility that pathogen proteins are interacting with host protein near these subcellular locations for their survival. According to the biological process results, the host proteins are involved in the generation of precursor metabolites and energy, electron transport chain, TCA,

cellular respiration, aerobic respiration, etc. The molecular functions performed by host proteins were catalytic activity, ion binding, iron and sulfur binding, heterocyclic compound binding, carbohydrate derivative binding, etc.

Based on the results of this study and earlier reports (Voronin et al., 2016; Currin-Ross et al., 2021), it was hypothesized that pathogen-interacting proteins in the host must be observed in energy-yielding and metabolic pathways. We found that 10 genes of HP-PPI were found in various metabolic pathways such as oxidative phosphorylation, carbon metabolism, citrate cycle (TCA cycle), etc. In our results, we found 10 host proteins that are found to be interacting with wBm were involved in these metabolic pathways, namely, Bm1936, Bma-nuo-1, Bm4943, Bm5876, Bm10316, Bm13824, Bm13837, Bma-sdhb-1, Bma-cyc-1, and Bm14732 (Ghedin et al., 2009). These results indicated that metabolic pathways are mostly targeted pathways by *Wolbachia* for maintaining an endosymbiotic relationship with its host. Eight proteins were involved in oxidative phosphorylation which is an energy-producing pathway in the host. Host proteins found to be involved in this pathway were Bm1936, Bma-nuo-1, Bm4943, Bm5876, Bm13837, Bma-sdhb-1, Bma-cyc-1, and Bm14732 and were also found located in mitochondria based on GO results. Other enriched pathways were carbon metabolism that involved four host proteins, Bm1936, Bm10316, Bm13824, and Bma-sdhb-1, spliceosome pathway involved Bma-hsp-1, Bm13697, and Bma-eftu-2 host proteins and this pathway assists in a splicing mechanism where pre-mature RNA is processed to mature RNA, and citrate cycle (TCA pathway) that involved Bm1936, Bm13824, and Bma-sdhb-1 host proteins, again, this pathway helps the host to maintain its energy requirement. Pathogen protein interacts with host proteins, and these proteins are involved in energy production in host indicating that the mutualism between *Brugia* and *Wolbachia* is maintained. Jiménez et al. (2021) reported that the interaction of *Wolbachia* and its host is observed in glycolysis and TCA pathway to maintain the symbiotic relationship.

We identified 8 hub proteins based on betweenness, closeness and degree centralities (Table 2). These hub proteins can be used for identifying crucial biological processes, and functional pathways or for the identification of potential drug targets. fusA is known to catalyze the GTP-dependent ribosomal translocation step during translation elongation (Foster et al., 2005). rplB, one of the most prominent proteins known to be involved in *Wolbachia* pathogenicity for the association of the 30S and 50S subunits to form the 70S ribosome, for tRNA binding and peptide bond formation (Foster et al., 2005). Bma-atp-1 host protein is involved in the production of ATP from ADP in the presence of a proton gradient across the membrane and atpA was found to be interacting with this host protein suggesting their role in a metabolic pathway (Takeda et al., 1996; Voronin et al., 2016).

We further explored the interaction of these hub genes in the HP-PPI network and identified six pairs of host-pathogen interactions that might be crucial to the mutualistic relationship between host and pathogen. These six-pair of host-pathogen interactions were further validated using domain-domain interaction approach and we found several similar domains for each pair of host-pathogen interactions (Table 3).

The pathogen protein rplB interacted with bma-rpl-2 of the host and both are ribosomal proteins with two common C-terminal and RNA-binding domains. It was evident from subcellular localization results that the activity site of both the host and pathogen proteins is cytoplasm which suggests their high probability of establishing a mutualistic relationship. The fusA protein was found to interact with two host proteins efTu2 and bma-eef-2 with shared domains namely, elongation factor Tu GTP binding, elongation factor G C-terminus, elongation factor Tu domain 2 and elongation factor G-domain IV. Both the host proteins are involved in GTPase activity and the ribosomal translocation step during translation elongation. atpA pathogen protein interacts with bma-atp-1 three common domains, namely, nucleotide-binding domain, C terminal domain and beta-barrel domain, and produces ATP from ADP in the presence of a proton gradient across the

membrane which again suggests that *Brugia* and *Wolbachia* share common pathway of energy production required for survival of both the organisms. It is already known that *Wolbachia* depends on its hosts metabolic products such as glucose and pyruvate for their survival and growth (Huo et al., 2015). The findings by Zug and Hammerstein who postulated that strains of *Wolbachia* that have coevolved with their respective hosts do not stimulate an immune response (Zug and Hammerstein, 2015). This has been found supported by results of this study as well, since we did not find any of the host immune response proteins involve in HP-PPI. Therefore, we rule out the involvement of any host immune response by *Brugia malayi* thereby helping in establishing an obligate mutualism relationship.

5 Conclusions

In this study, we have primarily reported 24 pathogen (*Wolbachia*) and 33 host (*Brugia malayi*) proteins constituted 35 pairs of HPI identified from the host-pathogen inter-species protein-protein interaction network. Further, through network biology insights, we reported 8 hub proteins that included two pathogen proteins (rplB and fusA) and six host proteins (bma-atp-1, Bm1_25145, A0A0K0JKQ9, bma-eef-2, Bma-rpl-2, and bma-eftu-2). Subsequently, GO and pathway analysis indicated the role of the reported HPIs in various metabolic processes such as oxidative phosphorylation, the TCA cycle to gather energy for their own survival and growth. Alternatively, evidence of mitochondrial association and maternal inheritance of *Wolbachia* with its host has been suggested for its pathogenicity. Also, based on our results, there is no evidence of host immune response by *Brugia malayi*, making the host environment habitable for *Wolbachia*. Furthermore, we found six-pair of host-pathogen PPIs along with their role in the energy-yielding process responsible for pathogens' growth and survival. These pair of host-pathogen interactions were also validated using the domain-domain interaction approach, where these proteins shared more than one domain. Overall, this work provides new insight into the mechanism of HPIs between *Wolbachia* and *Brugia malayi*, and will help researchers a deeper understanding of *Wolbachia-Brugia malayi* mutualistic association mechanism. Furthermore, the reported hub proteins can be targeted for the treatment of filariasis.

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