Computational Characterization and Comparative Evolutionary Analysis of an Uncharacterized Membrane-Associated Protein LOC410154 in Apis Mellifera

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ABSTRACT

Introduction: Un characterized proteins demonstrate huge knowledge gap in modern genome studies. This gap further widens in insects' studies such as Apis Mellifera which has economic and ecological significance. One third of its predicted proteins don't possess functional annotation. Apis Mellifera largely contributes in production of honey, beeswax, royal jelly and pollen which benefit food, cosmetics and pharmaceutical industries. Despite much research, many proteins associated with honeybees such as Apis mellifera remained unstudied, limiting our knowledge about understanding its survival instincts and evolutionary conservation across Hymenoptera. These proteins may play important role in immunity, sensory perception, cell signaling, transportation or pathogen interaction. LOC410154 in Apis mellifera is a large (1460aa) uncharacterized protein highly found across various honeybees and wasp species but still remained functionally or structurally unstudied. To uncover its biological role, it is necessary to study its placement, predicted localization and functional properties. In silico approach provides powerful opportunity to explore its characteristics and annotate it without needing any laboratory experiment. This study aims to bridge the gap by in silico characterization of LOC410154 using sequence homology, domain prediction, physiochemical properties, subcellular localization and phylogenetic reconstruction

Objectives:

1. Annotation of Apis mellifera protein LOC410154 through sequence-based analysis by utilizing comparative genomic computational tools.
2. Prediction of molecular features such as recognizable functional motifs, transmembrane regions and signal peptides.
3. Determination of subcellular localization using PSORT Bioinformatics tool.
4. Evaluation of conserved region and evolutionary trend among *Apis* and *Bombus* species by using Multiple sequence Alignment tools and reconstructing its phylogenetic tree.
5. To provide basic data for further functional studies on this conserved Hymenopteran protein.

Methodology: The amino acid sequence of protein LOC410154 (1460) of Apis Mellifera was obtained from NCBI. To determine the identical sequences and homology percentage across other species, BLASTp was performed. Another important bioinformatic tool InterProScan was used for identification of motifs, signal peptides, transmembrane helics, and conserved domains. PSORT II and DeepLoc were utilized for prediction of subcellular localization. ProtParam was performed to calculate other physicochemical properties such as molecular weight, pI, aliphatic index, stability index and hydropathy. Multiple sequence alignment and phylogenetic analysis for closely related *Apis* and *Bombus* homologs were obtained by using Clustral Omega. Phylogram-based programed helped tree rendering. All research conducted by using freely available computational tools.

Key Findings: In silico characterization of Apis Mellifera protein LOC410154 reveled exceptional results. BLASTp uncovered its huge conservation and 100% identity with Apis Mellifera and Apis Cerana homologs. Its further expose >85% identity with Bombus species, another vital honeybees' specie. LOC410154 high conservation among these species (Hymenoptera) is clear indication of its preserved biological role. InterProScan indicated a cleavable signal peptide at position 1-23 and transmembrane helices spanning residues at 930-1200. These results suggest that LOC410154 is a multi-pass membrane protein. There was no evidence of the presence of any nuclear, endoplasmic reticulum or mitochondria thus supporting non-organelle and cell surface function.

PSORT II and DeepLoc combinedly predicted the protein's primary localization at cell membrane (probability 0.84). These tools also predicted its secondary distribution possibly towards the endomembrane system. There was no indication of any coiled or known enzymatic motifs which suggests that protein may function as receptor-like, sensing associated membrane component or adhesion-related rather than a catalytic enzyme. ProtParam analysis predicted its molecular weight as 165 kDa, borderline instability index (40.89), and an acidic pI (5.92). Despite highly membrane spanning regions. GRAVY score of -0.109 indicate moderate hydrophilicity, consistent with large extracellular domains.

Clustral Omega phylogenetic reconstruction presents a clear clustering pattern separating Apis and Bombus lineages. It confirms protein conservation but presents evolutionary divergence. Very low branch lengths were observed among Apis species reflecting strong purifying selection acting on this protein.

Conclusion: This study provides first computational annotation of LOC410154 predicting it as a highly conserved protein among Apis mellifera and Apis Cerana and multi-pass protein possibly involved in membrane-associated processes in honeybees. Predicted signal peptide, multiple transmembrane helices and strong evolutionary conservation indicates possible stable and essential biological role. Its potential role established in sensory signaling, immune interaction and intracellular communication. These results establish strong foundation for future structural modeling and paves the way for further research. Although experimental work will be needed to conform results but computational results presented here provide solid starting point.

Keywords: Apis Mellifera, Membrane Protein, inSilico Annotation, Un Characterized Protein, Phylogenetics

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