New insight into phosphoproteome research improves the in-depth understanding of honey bee biology

Saboor Ahmad, PhD cand.[1] and Jianke Li, PhD [1,*]

[1] Institute of Apicultural Research/Key Laboratory of Pollinating Insect Biology, Ministry of Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100093, China;
[*] Corresponding author: Prof. Dr. Jianke Li; Email: apislijk@126.com

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Abstract: Protein phosphorylation is essential in a variety of biological activities. Notably, phosphoproteomics has opened new possibilities for honey bee biological study at the molecular and biochemical levels, considering the advancements in LC-MS/MS apparatus and computational analysis. This review extensively evaluated recent advances in honey bee biology utilizing phosphoproteomics methodologies in terms of progressive physiology, age polytheism, and biological changes in some organs, tissues, and cells. Furthermore, a comprehensive phosphoproteomic is necessary for new insight into honey bee biology, a better understanding of the research subject, and determining prospective future research areas.

Keywords: Honey bee, phosphoproteomics, LC-MS/MS, bioinformatics

Introduction

The honey bees (Hymenoptera: Apidae) are well-known eusocial and beneficial insects, which produce valuable products including honey, royal jelly, propolis, and pollen, and these are used in cosmetic and medicinal industries [1, 2]. In addition, honey bees play an essential part in crop pollination worldwide because of their diverse food, more extended forager range than other pollinators, and ability to discover the discrete area of resources in the larger environment efficiently using scouting [3, 4]. At an earlier stage, most studies on honey bee biology did not go beyond describing them, whether at the colony or individual animal level. As a result, compared to other organisms such as Drosophila, bees are poorly investigated at the molecular, biological, and cellular levels due to a vast variety of knowledge and technical limitations [5]. However, scientists start to investigate honey bee biology at the molecular level at the beginning of the 20th century.

Recently, the honey bee has been well studied in the context of general biology and beekeeping. Despite its long history as a typical organism for studying social performance, the honey bee is only studied at the molecular level in developing biology, neuroscience, immunology, and aging [6]. The situation has drastically changed, with the completion of honey bee genome sequencing, scientific breakthroughs in protein separation, identification, mass spectrometry (MS), and computer stages. After the completion of the honey bee genomic sequence in 2006 [7], it opened a new era of honey bee functional genomic research. However, honey bee genomics is essential for underpinning honey bee biology but not enough to investigate all of its complexity. So, studying gene expression by proteome research is necessary to understand bee biology. Due to tremendous technological advances in the recent decade in terms of MS, protein preparation, and computational algorithms, proteomic research on honey bees has significantly expanded [5]. Proteomics has evolved over time to include more sophisticated liquid chromatography coupled tandem MS (LC-MS/MS) technologies that have substantially increased in speed and quality, such as resolution, sensitivity, and mass accuracy, allowing for large-scale protein analysis [8, 9]. Moreover, in the past few years, proteomics has proven to be a more effective tool for studying post-translational modifi-

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cation (PTMs). This review summarized phosphoproteome research to understand honey bee biology at molecular level.

Phsophoprotemics uncover the wide range of honey bee biologys

Phosphoproteomics is a large-scale investigation that uses mass spectrometry to identify and quantify phosphorylated proteins as well as map phosphorylation sites in a complicated biological sample [10]. It has undergone considerable change since 2000, thanks to advancements in MS-based phosphoproteome methods [10, 11]. Protein phosphorylation such as serine (S), threonine (T), and tyrosine (Y) is a well-known dynamic post-translational alteration in eukaryotes with amazing regulatory and signaling activities [11]. The human phosphoproteome, for example, contains around 30,000 S/T/Y phosphorylation sites [12]. To date, 15 studies regarding S/T/Y phosphorylation on honey bees have been reported (see Figure 1), and detailed description of these studies are mentioned in Table 1. Moreover, using the phosphoproteome technique, new depths have been reached at the molecular and biological level for a wide variety of honey bee biology, including developing biology, physiology, behavior, neuroscience, and immunology (see Figure 2).

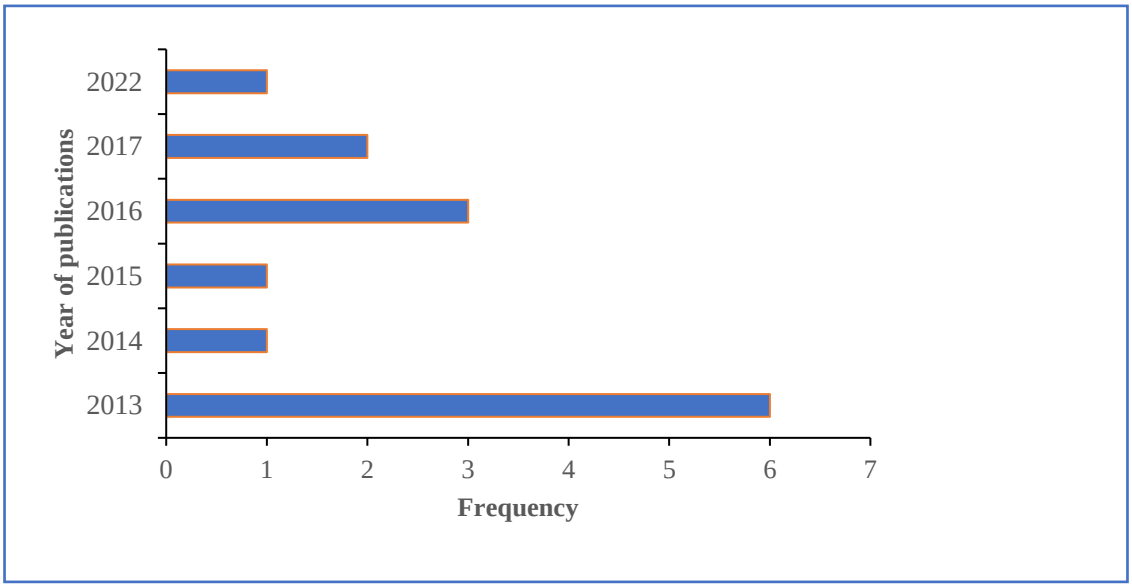

Figure 1: Publications frequency per year

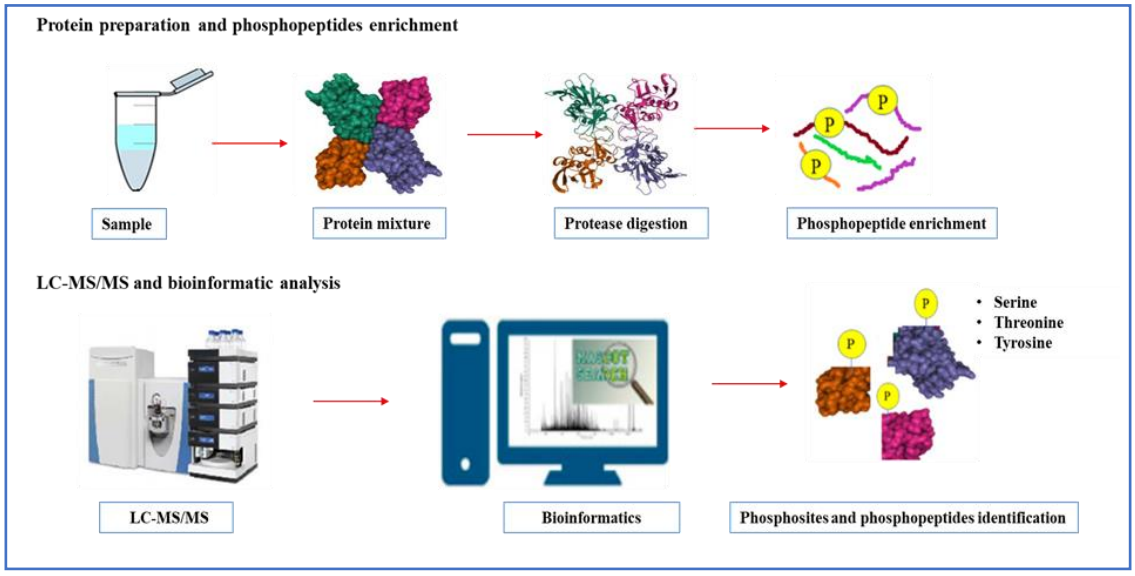

Figure 2: Workflow of phosphoproteomic experiments for honey bee samples.

Table 1: The detailed description of phosphoproteome research to unveil honey bee biology.

Source information	Honey bee species	Honey bee tissue and organ	Peptide enrichment	Phosphoproteomic method	Phosphorylated protein	Phosphorylated peptides	Phosphorylated sites
[13]	*Apis mellifera ligustica*	Salivary glands	CBB stained gels	2DE-ESI-QTOF-MS	36	n. r	n. r
[14]	*Apis mellifera ligustica*	Embryo and larvae	CBB stained gels	2DE-ESI-QTOF-MS	111, 102	n. r	n. r
[15]	ITBs, (*Apis mellifera ligustica*)	Venom	Ti^{4+}-IMAC	LC-MS/MS	3	4	3
[16]	RJBs, (*Apis mellifera ligustica*)	Hypopharyngeal glands	Ti^{4+}-IMAC	LC-MS/MS	6	6	8
[17]	*Apis mellifera ligustica*, *A. m. carnica*	Venom	n. r	LC-MS/MS	2	2	2
[18]	*A. mellifera ligustica*, *A. cerana cerana*	Royal jelly	TiO^2, Ti^{4+}-IMAC	Nano-LC−MS/MS	16, 9	93, 113	67, 71
[19]	RJBs, (*Apis mellifera ligustica*)	Hypopharyngeal glands	TiO^2, Ti^{4+}-IMAC	LC-MS/MS	1967	3559	3469
[20]	ITBs, (*Apis mellifera ligustica*)	Embryo	TiO^2, Ti^{4+}-IMAC	LC-MS/MS	1354	n. r	2829
[21]	ITBs, RJBs (*Apis mellifera ligustica*)	Brain	Ti^{4+}-IMAC	LC-MS/MS	519	n. r	1378
[22]	ITBs, (*Apis mellifera ligustica*)	Brain	Ti^{4+}-IMAC	LC-MS/MS	1962	6344	6133
[23]	ITBs, RJBs (*Apis mellifera ligustica*)	Brain	Ti^{4+}-IMAC	LC-MS/MS	297	672	577
[24]	ITBs, RJBs (*Apis mellifera ligustica*)	Mandibular glands	Ti^{4+}-IMAC	LC-MS/MS	5014, 6306	n. r	n. r
[25]	ITBs, (*Apis mellifera ligustica*)	Brain	Ti4+-IMAC	Nano-LC−MS/MS	1058	22,656	n. r
[26]	ITBs, (*Apis mellifera ligustica*)	Brain	Ti4+-IMAC	Nano-LC−MS/MS	916	n. r	n. r
[27]	*A. mellifera ligustica*, *A. cerana cerana*	Antennal lobes	Ti4+-IMAC	Nano-LC−MS/MS	1265	2812	2971

Phosphoproteomics of honey bee glands unveils the castes and age-dependent differences

Different glands which are required for the honey bee to perform their biological task have been studied through phosphoproteome research such as hypopharyngeal glands (HGS) [16, 19], mandibular glands (MGs) [24] and salivary glands (SGs) [13]. HGs are the unique characteristics of Hymenopteran insects, especially in the honey bee that produces royal jelly in nurse bees and digestive enzymes in forager bees. The HGs have a wide range of genes and proteins that cause age-related physiological changes. According to time-resolved proteome profiling, proteins generated in the HGs of worker bees fit age-dependent function occurrence. Furthermore, the phosphoproteomics analysis of HGs at each worker stage reveals that a considerable fraction of cellular protein is phosphorylated, suggesting that phosphorylation may play an essential role in HG activity [19]. Both phosphoproteome and non-phosphoproteome are required to support the HGs' distinct age-dependent physiology by complementing biological tasks. In phosphoproteome analysis of HGs across the worker ages, the majority of proteins are regulated by phosphorylation independently to their expression level, and comparable protein and phosphoprotein expression profiles are required for the unique secretory physiology of HGs [19]. Furthermore, MGs are a pair of sack-like glands situated inside the head. The secretions of MGs have crucial caste-specific roles linked to honey bee social evolution [28]. The molecular origins of caste-specific MGs activities have been demonstrated by phosphoproteome research. The phosphoproteome reveals the ontogeny and functionality of MGs at each stage. In MGs, mainly phosphorylated proteins are associated with energy, fatty acid metabolic process and energy metabolism [24].

The salivary system shows functional flexibility across the age of bee workers [29]. The salivary glands (SGs) of the honey bees have two parts: cephalic SG inside the head and thoracic SG inside the thorax [30]. The fatty acid esters that makeup brood pheromones and regulation of juvenile hormones, which regulate young bee behavior and encourage pollen gathering, are stored in the larval SGs [13]. However, the secretion of the adult female is different from the larvae. The primary function of the adult cephalic SG is to produce an oily ingredient which involves wax manipulation, mouth lubrication, and release of the pheromones to locate the food sources [31, 32]. The thoracic SG produces the secretion involved in honey and sugar digestion, and pollen and wax moistening [33]. In addition, the thoracic SG expresses more proteins linked to glucose and energy metabolism, protein metabolism and folding, cellular homeostasis, and the cytoskeleton, to promote the gland's efficient honey processing through synthesis and secretion of saliva into nectar [13]. In short, phosphoproteome research is critical to determine the molecular insight how the honey bee SGs obtain the multiple biological mission for efficient management of bee colonies. Thus, changes in protein and phosphoprotein expression levels among cephalic SG and thoracic SG are the primary motivators for worker bees to carry out their various tasks following their physiological status. Further research is required to understand the development and functionality of bee glands by using new phosphopeptide enrichment techniques and the latest LC-MS/MS technologies.

Molecular basis neurology underlies the honey bee biology

The brain performs higher-order mental processes such as processing sensory signals and modifying functioning dynamics. In honey bee workers, the age-related behavior transition is nearly associated with alteration in the brain's structure, gene expression, and protein synthesis [34]. The alteration in gene expression in the bee brain can be utilize to forecast natural behavior [35]. In addition, the honey bee brain proteome is also linked to the age-related change from hive worker to forager [36]. To this end, multiple phosphoproteome studies on honey bee brains contribute to a better knowledge of the molecular foundation of neurology in honey bees that activate various social behaviors. In nurse bees, phosphoproteins are involved in brain maturation, signal transduction, development, and the olfactory learning processes during the initial stage of worker life to increase the nursing activities [25]. For instance, phosphatidylinositol signaling and arachidonic acid metabolism are important in RJB nurses' enhanced olfaction sensation in response to larval pheromone stimulation, and richer signal processing pathways in RJB foragers are to encourage a stronger inclination in pollen gathering [23]. In addition, phosphorylated proteins responsible for wide range of pathways depending on age, for instance, AGE/ RAGE, glycolysis/ gluconeogenesis, phosphorylation in nurse bees while ATP metabolic process, metal ion transport, phototransduction in foraging bees [22]. Thus, phosphorylation

is critical for fine-tuning protein activity to regulate brain function in bees performing nursing and foraging tasks.

The production of phosphoproteins in common biological pathways and kinase activities in the forager brains is critical for maintaining central neural activity during foraging. The phosphoproteins of the forager's brain are strongly involved in the genetic pathway of inositol phosphate metabolism, phosphatidylinositol signaling system, phototransduction, Wnt and glycerophospholipid metabolism, and activated kinases such as JNK, p38, CLK, and PKA are involved brain maintenance, olfactory learning processes, the signal transduction to increase the foraging activities performance [26].

Furthermore, the majority of phosphoproteins discovered in A. cerana are involved in protein metabolism and transport, with the bulk of phosphoproteins in the mTOR signaling pathway being increased in the antennal lob of A. cerana. In addition, phosphorylated proteins increased A. cerana's protein synthesis-dependent synaptic plasticity, making it easier for the species to process more complex floral olfactory hints in mountain foraging locations [27]. The phosphoproteome-driven brain modulation of honeybee olfactory activities could be relevant for future neurobiological research in honeybees and other insects.

Molecular insight into biochemistry and function of honey bee products

Phosphoproteome study of honey bee products such as royal jelly and venom is critical to exploring their biochemical and functional properties. Royal jelly is queen food for the entire life and is high in defensive components linked to collective immunological defenses [37]. The phosphoproteome analysis of royal jelly between honey bee species showed diverse biological characteristics. The phosphosites, peptide quantity, and antibacterial action of the phosphorylated royal jelly proteins have evolved significant variations between these two bee species. The abundance level of phosphorylated proteins and phosphorylated peptides from the royal jelly of Apis cerana showed stronger anti-microbial and anti-fungal properties. In both species, MRJP abundance levels are better explained by their biological needs for existence and development. For instance, the abundance level of MRJPs is linked with the support of their large body size in western honey bees. While in eastern honey bees, the large number of phosphorylated peptides balances the low level of MRJPs by maintaining life and development [18].

In addition, honey bee venom is created by the female worker and is also known to contain a variety of compounds such as peptides (apamin, melittin, and adolapin etc.), enzymes (phospholipase A2 and hyaluronidase), proteins, amino acids and volatile ingredients. These chemicals show promise in treating inflammation and central nervous system illnesses such as Alzheimer's, and amyotrophic lateral sclerosis [38]. Toxins from honey bee venom cause immune, metabolic, and neurological responses in victims.

Phosphoproteins studies of honey bee venom explores the depth of understanding of biochemical ingredients and their functions. A comparative proteome and phosphoproteome analysis of honey bee venom reveal three novel phosphorylated venom proteins, which may trigger diverse immune responses by identifying the antigenic determinants [15]. The discovery of three novel phosphorylation sites on venom proteins adds to our knowledge of the biochemical nature of bee venom. In addition, melittin and icarapin were phosphorylated in Africanized and European honey bees. Melittin is the major toxin of bee venom and, was phosphorylated in all venoms at the 10 T and 18 S residues. The phosphorylation of icarapin occurred at the 205 S residue near its known antigenic location [17]. In short, more research is needed to identify novel phosphorylated proteins in honey bee products such as honey, royal jelly, and venoms, as well as further evaluation in other mechanistic investigations of honeybee biology and health-promoting action in humans.

Conclusions and future research direction

Honey bee phosphoproteomics research is progressing in the academic community, and substantial progress has been achieved in understanding the molecular foundation of honeybee biology. In various domains of honey bee biology, including developmental biology, physiology, and neurobiology, for instance, numerous expression-based phosphoproteomic modifications have been reported, reaching new depths at the molecular and biological levels. However, additional research is needed into the specialized involvement of phosphoproteins in related specific biochemical pathways in development and behavioral physiology. Further studies are required to examine the function of

phosphoproteins that regulate the honey bee ontology and behavioral physiology using advanced LC-MS/MS methodologies.

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Conflicts of Interest

The authors state no conflict of interest.