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Identification of pheromone-binding proteins of the maize stem borer, *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae)

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Abstract

Pheromone-binding proteins (PBPs) play a significant role in olfaction and mating. The present work was designed to isolate, extract, and purify the pheromone-binding proteins from the antennae of male *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae). The pheromone-binding proteins extracted from the male antennae were found to be 770 µg in 100 mg of sample. Pheromone-binding protein molecular weight was determined as 17 kDa by SDS-PAGE analysis. Identified proteins were purified through gel extraction with a recovery percentage of proteins up to 95%. Purified protein samples are analyzed on native PAGE gels. Relative mobility of proteins was determined as 0.574 nm in the densitometry analysis. These identified pheromone-binding proteins can be used for identification of novel pheromone compounds in in vitro studies. This study can be helpful in designing integrated pest management programs to control the maize stem borer by mass trapping of male moths.

Keywords: *Chilo partellus*, Pheromone-binding proteins, SDS-PAGE, Densitometry

Background

Chilo partellus (Swinhoe, 1885) (Lepidoptera: Crambidae) is a devastating pest, majorly hampering the production of several cereal crops, viz., maize, sorghum, sugarcane, and rice. It infests the crop at all stages and results with a yield loss from 26.7 to 80.4% in Indian maize (Manjunath and Mallapur 2017). There is no permanent solution to control this pest through plant breeding. Usage of chemicals results to negative impact on the environment. However, bio-control agents such as parasitoids, predators, and pathogens suppressed the population outgrowth of *C. partellus* to an extent, but their activity was not adequate to decline the pest populations below the financial damage level (Divya et al. 2010). Integrated pest management (IPM) is one of the best methods for control of the maize stem borer and obtaining the highest yield (Khan et al. 2015).

An insect detects hydrophobic volatile molecules (odorants) present in the environment by hydrophilic antennal lymph to the olfactory receptor by odorant-binding proteins (OBPs) (Garczynski et al. 2013), and thus, OBPs play important roles in the olfaction. OBPs are small, water-soluble, extracellular proteins that are located in the fluid surrounding the sensory dendrite and abundantly found in lymph of insect antennae and other sensory organs (De Biasio et al. 2015). They are reflected to be vital in insect olfactory perception at various levels but the exact mechanism is still unknown.

Pheromone-binding proteins (PBPs) represent a sub-class of insect odorant-binding proteins, particularly related in the detection of sex pheromones (Pelosi et al. 2006). The term pheromone was derived from a Greek word *pherein* which means to carry or transfer and hormone to excite or stimulate. Bombykol is the first identified pheromone released by female silkworms to attract its mating partner (Zhou et al. 2009). PBPs were found in the antennae of females and in sensilla of males which are not pheromone-sensitive (Callahan et al. 2000). Pheromones induce a change in

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the behavior of the receiving individual within the same species (Wyatt 2010). They act as carriers for antagonists and scavengers for pheromone components in female insects (Zhou et al. 2009).

Research is going on to understand the molecular mechanisms by unrevealing the role of OBPs and PBPs in olfactory perception. To address the damage caused by *C. partellus* through molecular level application in IPM programs, an initiative work to extract and purify the pheromone-binding proteins of male *C. partellus* antennae was carried out.

Materials and methods

Sample collection

Chilo partellus male moths were collected from the maize fields by using pheromone traps in the farmer fields in Vaddeswaram, Guntur, India.

Sample protein preparation

Male *C. partellus* moth antenna tissue (100 mg) was taken and homogenized with the help of mortar and pestle using 500 μ l of 1 M Tris buffer (pH 7.5) containing 10% SDS and 0.02 M EDTA. The homogenate was centrifuged at 14,000 rpm for 12 min at 4 °C. Supernatant fraction was carefully collected in fresh autoclaved tubes; the sample was stored at -20 °C until further use. The protein was estimated according to standard protocol suggested by Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Preparation of sample protein for SDS-PAGE

A ratio of 1:1 protein sample and 1.0 M Tris buffer (pH 6.8) containing 10% SDS, 2% β -mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue was added and kept at 90 °C for 5 min (Kasim et al. 2015).

Separation of PBPs by SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight distribution of insect proteins as described by Nagnan-Le et al. (1996). To study the SDS-PAGE protein profile, one-dimensional SDS-PAGE (12% separating gel and 4% stacking gel) was carried out in a small vertical system. To 10 μ g of protein, 10 μ l of lamellae buffer (loading dye) was mixed and samples were incubated at 95 °C for 5 min in water bath and then immediately load the protein sample into the wells. The loading buffer containing bromophenol blue acts as a tracking dye. A medium range marker (NEX-GEN blue spray prestain protein ladder) was also loaded into the well for the determination of molecular weight through protein bands. The gel was run at constant voltage of 80 V for 3–4 h followed by staining with Coomassie brilliant blue R-250 overnight.

The relative mobility of protein bands was determined and the zymogram was constructed.

Extraction and purification of proteins from SDS-PAGE

After SDS-PAGE electrophoresis, proteins were extracted and purified according to Nagnan-Le et al. (1996). The visualized bands are removed from the gel using a surgical blade and washed using 2 ml of 250 mM Tris buffer/250 mM EDTA, pH of 7.4, thoroughly for three times then finally washed with autoclaved distilled water. The water was completely removed from the gel slices and homogenized with 1 ml of Tris buffer (pH 7.4). The samples were subjected to sonication then centrifuged at 12,000 rpm for 10 min at 4 °C. The protein sample was equilibrated with 20 mM Tris buffer, pH 7.4, containing 0.1% SDS. The partially purified protein aliquots were run in the native PAGE; it is used for the densitometric analysis and determination of the recovery percentage.

Densitometry analysis

To identify the universal expression pattern of existing protein spots on gel, densitometry analysis was employed. It is majorly used to measure protein expression levels and the intensities of specific bands by using an online software tool (Gelquant.NET). In this study, the relative amounts of protein were quantified by using a computational program from a TIFF image assisted with a commercial scanner. Relative mobility (Rf) of each stained band, its concentration, and area occupied by it with reference to total protein present in the lane can be calculated by gel perfect program explained by Bozzo and Retamal (1991). SDS-PAGE gels of purified proteins were digitized, and peak areas of both purified and crude protein bands were compared. The relative concentration of areas provides us the percentage recovery.

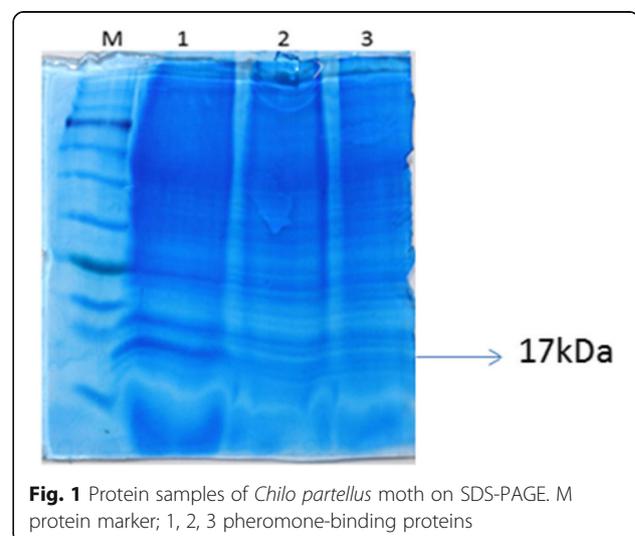
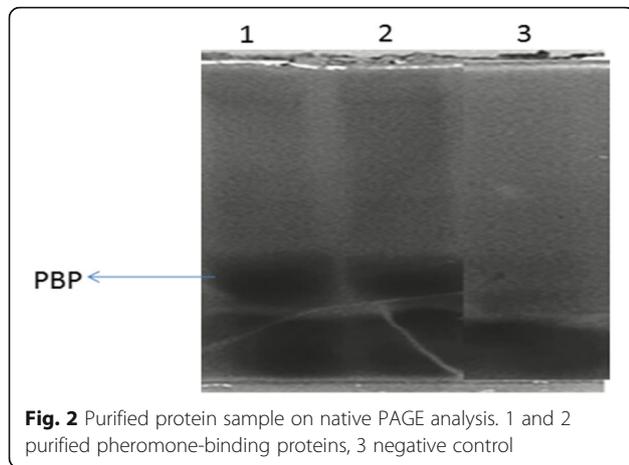


Fig. 1 Protein samples of *Chilo partellus* moth on SDS-PAGE. M protein marker; 1, 2, 3 pheromone-binding proteins



Results and discussion

Separation of PBPs by SDS-PAGE

Extracted proteins from the sensory organs were quantified by using Lowry's method and the protein concentration was found to be 0.77 $\mu\text{g}/\text{mg}$. Molecular weight of the extracted proteins was determined using SDS-PAGE (12% resolving gel and 4% stacking gel). Usually, molecular weight of PBP was of 17 kDa and found to be the same in our results (de Santis et al. 2006) using a protein marker (NEX-GEN blue spray pre-stain protein ladder) (Fig. 1).

Extraction and purification of proteins from SDS-PAGE

The identified proteins were further purified through gel extraction and recovery of protein varied from 80 to 95%. Purified samples were run on native PAGE gel with negative control (Fig. 2). Few samples are homogenized and purified using Tris buffer (20 mM, pH 7.4) without SDS, while recovery percentage was observed to be slightly lower when compared to SDS. Our results illustrate that it was not critical to use a large quantity of starting material to achieve an adequate amount of purified protein either to improve an immunoassay or to characterize a protein chemically.

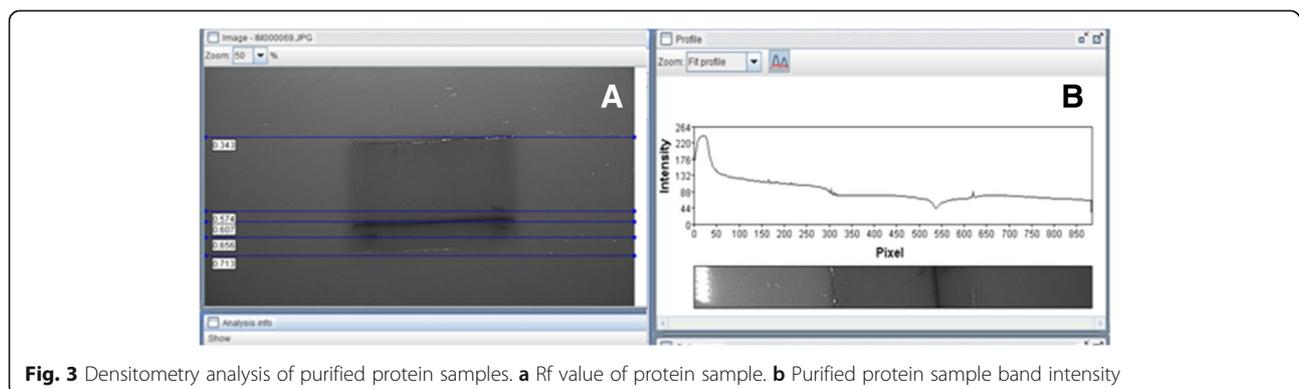
Densitometry analysis

Through densitometry analysis, the electrophoretic mobility was calculated, using molecular weights of proteins with reference to protein marker standards (Priya et al. 2013). In this analysis, the perfect gel picture on relative mobility of protein was observed to be 0.574 nm (Fig. 3a) and the band intensity was approximately 250 units as shown in (Fig. 3b).

Results of this study may serve as preliminary information to identify olfaction-based approaches which interfere with insect host location and mating behavior through a strategy called push-pull pest control. PBPs are the ideal targets for understanding the olfactory code of insects as well as for interfering with their communication system. These proteins are specifically expressed in the antennae of male moths. Insects survive by possessing a specialized sensory system to monitor various odors present in the environment. Thus, sense of smell often plays a crucial role for detecting food, mating partner, and choosing for oviposition site or even protecting themselves from predators and pathogens to fit among various existing ecological lifestyles (Cande et al. 2013). Considering the importance of PBPs of male moths, they play a vital role in the detection of sex pheromones. Leal (2013) stated that male moths detect sex pheromones with tuned odorant receptors, which confer specificity to peripheral odorant receptor neurons, with the assistance of other olfactory proteins.

Conclusions

In conclusion, the pheromone-binding protein receptors are present in the male and female antennae of *C. partellus* moth. Male-specific PBPs are important for sexual mating. In this study, PBPs were extracted and purified from the antenna tissue. Extracted PBPs can be used for further protein sequence analysis and molecular docking studies. These PBPs can be used in identification of novel pheromone compound screening, which can be used in integrated pest management for sustainable agriculture.



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Authors' contributions

DPK carried out the SDS-PAGE studies. RS participated in Native PAGE studies. PS participated in rearing of moths. MSRK conceived of the study and participated in design and coordination. UL performed densitometry analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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