Original Article

Proteomic Analysis of the Peritrophic Matrix from the Midgut of Third Instar Larvae, *Musca domestica*[*]

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Abstract

Objective To better comprehend the molecular structure and physiological function of the housefly larval peritrophic matrix (PM), a mass spectrometry approach was used to investigate the PM protein composition.

Methods The PM was dissected from the midgut of the third instar larvae, and protein extracted from the PM was evaluated using SDS-PAGE. A 1D-PAGE lane containing all protein bands was cut from top to bottom, the proteins in-gel trypsinised and analysed via shotgun liquid chromatography- tandem mass spectrometry (LC-MS/MS).

Results In total, 374 proteins, with molecular weights varying from 8.225 kD to 996.065 kD and isoelectric points ranging from 3.83 to 11.24 were successfully identified, most identified proteins were mainly related to immunity, digestion, nutrient metabolism and PM structure. Furthermore, many of these proteins were functionally associated with pattern binding, polysaccharide binding, structural constituent of peritrophic membrane and chitin binding, according to Gene Ontology annotation.

Conclusion The PM protein composition, which provides a basis for further functional investigations of the identified proteins, will be useful for understanding the housefly larval gut immune system and may help to identify potential targets and exploit new bioinsecticides.

Key words: *Musca domestica*; Peritrophic matrix; Proteome

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INTRODUCTION

In many insects, the midgut epithelium is generally lined with an extracellular, semi-permeable structure referred to as the peritrophic matrix (PM), PM and mucous secretions are somewhat similar, but there are big differences too. The PM is essential for insect digestive physiology, as it protects the midgut epithelium from abrasion by food particles and toxins, serves as the first biophysical barrier that alters the temporal kinetics of host immune responses to pathogens ingested during feeding, and increases digestive efficiency by compartmentalizing of digestive processes[1-6]. The insect PM is composed of chitin and glycoproteins, exhibiting characteristic

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chitin-binding activity[7]. PM proteins are classified into four categories on the basis of extractability under different conditions, and most of these proteins play significant roles in the functions of the PM[7]. Studies of the red flour beetle *Tribolium castaneum* have demonstrated that specific individual PM proteins may regulate PM permeability, and that a gradient of PM barrier function is essential for survival[8]. The PM not only plays important roles in facilitating food digestion and protecting the gut epithelium, but also may act as a significant structural target for insect control[9-10].

The housefly (*Musca domestica* L.; Diptera: Muscidae) is a major domestic, medical, and veterinary pest that causes more than 100 human and animal diseases, including bacterial, protozoan, helminthic, viral, and rickettsial infections[11-12]. Although insecticides have been widely used to control insect pests, the housefly has shown a remarkable and rapid ability to evolve resistance[13-14]. Therefore, effective novel strategies of housefly control are vital for limiting the spread of disease, the evolution of resistance, and the economic losses associated with reduced production[15]. Because bacteria are both nutritional and developmental requirement of housefly larvae[16], adult flies associate with microbe-rich animal waste or septic substrates throughout their life cycle[17], but rarely show signs of disease, indicating the presence of efficient systems for gut defense in conjunction with biophysical barriers against microbes[18]. Bacteria do not pass through the PM and are sequestered therein by size exclusion, in addition, some bacterial species appear to be immobilized within the PM by an unknown mechanism[19].

Housefly larvae play a vital role in ecosystems as decomposers of organic waste[14]. Adaptation to different ecological environments may have led to the evolution of a housefly defense response. Therefore, a comparison of the PM proteome from *Musca domestica* with those from species exposed to different niches and microorganisms, such as *Anopheles* and *Drosophila,* may be very significant, and will provide insights into housefly survival maintenance while in close contact with many pathogens[12,20]. The housefly niches is unique relative to that of other insects (e.g., *Anopheles gambiae*[21], *Helicoverpa armigera*[22] *and Glossina morsitans morsitans*[23], *Bombyx mori*[9,24]), for which the PM composition have been reported. Increasing evidence suggests that the housefly gut is a primary site of pathogen replication after oral infection[25-26]. Therefore, the importance of the PM as a crucial component of the local intestinal immune system merits further research. However, few studies have investigated the housefly' PM, and therefore, the available information with which to understand its biological function is very limited. For a better understanding of how the PM performs these functions, a thorough revision of the molecular architecture of the housefly PM is required.

In this study, we attempted to achieve a comprehensive identification of proteins in the PM of the housefly, *Musca domestica.*

MATERIALS AND METHODS

Housefly Larval Rearing Conditions and Isolation of Larval PM

House flies were reared at the Department of Parasitology, Guizhou Medical University (Guiyang, China)[27]. Larvae were raised in a climate-controlled room at 25 °C with a relative humidity of 75%-85% and were provided, medium comprising wheat bran (500 g), heat-inactivated yeast (30 g), and water (1500 mL) until pupation. After eclosion, adult flies were fed water, sugar, and milk powder. Flies were maintained at 25 °C under a 12 h light /12 h dark cycle (LD12:12).

The PM was isolated from the midgut of the third instar larvae. The larval midgut was dissected, and the PM allowed slid to out when the end of the midgut was cut transversely, the PM was subsequently washed with a 0.75% NaCl solution until no food debris remained. Approximately 200 PMs were pooled and stored at -80 °C until further use.

Sample Preparation and Gel Electrophoresis

The PM was homogenized on ice for 5 min, and total proteins were extracted with 20 µL of lysis buffer (2.5% SDS, 10% glycerin, 5% β-mercaptoethanol, and 50 mmol/L Tris-HCl, pH 8.8) per mg of sample weight[24]. PMs were disrupted after incubation with the lysis buffer, mixed several times during a 1 h incubation at 4 °C, and centrifuged at 13,000 rpm and 4 °C for 20 min, after which the supernatant was recovered and recentrifuged. Protein concentrations were measured according to the Bradford method[28]. Each sample was subsequently boiled for 10 min and