A preliminary proteomic study of permethrin resistant and susceptible *Aedes aegypti* (L.)

Rosilawati, R.1*, Nabila, R.1, Siti Futri Farahinajua, F.1, Nazni, W.A.1 and Lee, H.L.1
1Medical Entomology Unit, WHO Collaborating Centre for the Ecology, Taxonomy and Control of Vectors of Malaria, Filariasis & Dengue, Infectious Disease Research Centre, Institute for Medical Research, Ministry of Health, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
*Corresponding author e-mail: rosilawati@imr.gov.my
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Abstract. The mechanism of insecticide resistance is traditionally attributed to detoxification enzymes, target site alteration, decreased penetration of insecticides and behavioural resistance. Other form of mechanisms, such as the role of protein(s) in resistance is unknown. In the present study, the protein profiling of both IMR-PSS strain (permethrin-selected) and IMR-LS strain (laboratory-susceptible) 24 hours post exposure period to permethrin was carried out via 1D-gel electrophoresis and liquid chromatography mass spectrometry (LC-MS/MS). The bands which appeared in the gel of 1D-electrophoresis revealed an abundance of proteins. The band pattern of both strains looked macroscopically alike and differed only in band intensity. However, LC-MS/MS analysis revealed that the IMR-PSS strain produced extra 388 peptides that were not found in the IMR-LS strain, indicating that IMR-PSS strain reacted differently from IMR-LS strain as a result of persistent exposure to permethrin. Since the complex banding patterns of 1D-gel electrophoresis were difficult to interpret the significance of the protein difference between IMR-PSS and IMR-LS strain, hence LC-MS/MS analysis is ideally suited for better protein resolution and thus will allow more in-depth comparison of the complex pattern. The findings here provide the first preliminary evidence that insecticide resistance in mosquito induces up regulation of proteins that may be protective to mosquitoes against insecticide and proteins could be another mechanism that contributes to development of resistance.

INTRODUCTION

Proteomic approach in biology and biomedical researches is not a new field. This advanced tool has benefited many scientists to explore cellular function in the perspective of protein expression, protein-protein interaction, and post-translational modifications (Neelam et al., 2014). Availability of this high throughput proteomic analysis leads to discovery of novel biomarker for therapeutic application, for early disease indicator (Ravindran et al., 2009), drug discovery and a lot of scientific exploration that are beyond the reach of genomic and transcriptomic approaches.

In mosquito proteomics research, most of the studies have emphasized the interrelation of proteome and physiological responses of vector against pathogen, characterising proteins in mosquito tissue or organ at different cell state. In Malaysia, Lee et al. (1994) presented the first evidence of various protein bands found in difference stages of development in Malaysian *Aedes aegypti* and *Anopheles maculatus*, hence demonstrated that midgut was the targeted organ for immunisation study. Prevot et al. (2003) showed that 10 proteins that were absent in male mosquito but appeared in female mosquito during feeding were proteins associated with blood digestion. Despite
exploration of proteome in mosquito midgut, others also discovered the importance of proteome characterization in various organs such as salivary glands, hemolymph, peritrophic membrane which were associated with diverse putative function of mosquito response to blood digestion (Paskewitz & Shi, 2005; Kalume et al., 2004 & Dinglasan et al., 2010). In another reported study, Lefevre et al. (2007) showed 12 protein spots in the head of sporozoite-infected malaria mosquito which corresponded with the altered energy metabolisms. The discoveries of protein changes in the saliva and in the head of infected and uninfected mosquito had revealed that proteomic approach anticipates for a promising investigation of the important protein related with regulation in mosquito biological system.

Despite of genomic study, proteomic study has currently become an important research field. The main reason is because protein is the final product in molecular central dogma that plays vital roles in performing diverse function for organisms to live. Though recent advances of proteomic approach have been applied in many areas, yet there has been little scientific research on the association between proteomes and pyrethroid resistance in mosquito. To date, most mosquito resistance researches have been mainly focused on the mosquito genomic aspects, and identification of mutation that influences the affinity binding of insecticide to the targeted-insecticide receptor (Kasai et al., 2011 & 2014; Hemingway et al., 1989; Kawada et al., 2009; Ishak et al., 2015 & Rosilawati et al., 2018).

Not all proteins are expressed at the same time in a living cell and changes of protein due to alternative splicing and post-translation modification (Sudhakaran et al., 2012) has therefore prompted us to examine the differences of proteomes produced by susceptible and resistant Aedes aegypti. This study reported the first scientific evidence on the involvement of protein changes in pyrethroid-resistant Aedes aegypti mosquito in Malaysia. On that account, we believe these findings would contribute in expanding the exploration for the possible novel proteins, and establishment of reliable and accurate protein biomarkers for detection of insecticide resistance in mosquito in the near future.

MATERIALS AND METHODS

Mosquitoes rearing
Adult Aedes aegypti mosquitoes were reared in colony room at the insectarium of Medical Entomology Unit, Institute for Medical Research (IMR), Malaysia at 27±2°C and 75±5% relative humidity. A constant photoperiod cycle of 12 hours of light and 12 hours of darkness was applied to allow optimum and uniform mosquito development. Initially, Aedes aegypti eggs were hatched in a container containing seasoned water (water held overnight to obtain chlorine free condition). In this experiment, L1 and L2 larvae were fed with ox liver powder, while L3 and L4 larvae were fed with half-cooked fresh ox liver. Development of larvae to pupae was observed daily where pupae were collected individually and transferred into plastic bowl following by placing the bowl into an adult cage. The emerged adult was provided with solution containing 10% sugar and vitamin B complex at ad libitum.

Mosquitoes strains
Two strains of Aedes aegypti, which were laboratory-susceptible strain (IMR-LS), F1068; and permethrin-selected strain (IMR-PSS), F15, were used in this study. The IMR-PSS used in the present study was derived from the same population used in the study reported by Rosilawati et al. (2018), while the IMR-LS was originated from the colony that was continuously maintained in the Insectary of Medical Entomology Unit for more than 1000 generation. The susceptibility status of both strains was reassessed by following the protocol described by the World Health Organization (WHO) (WHO, 2016). The female adult mosquitoes; aged 3 to 5 days, were used during evaluation of mosquito insecticide susceptibility status. Whilst, those alive IMR-PSS strain mosquitoes at post 24 hours exposure to permethrin were collected into 1.5mL
Development of IMR-PSS strain via selection pressure against permethrin

Selection pressure of permethrin was applied to every succession generation of IMR-PSS strain, begins from filial 1 and currently has reached to filial 22. The IMR-PSS strain was selected with permethrin at lethal concentration (LC$_{50}$) that caused 50% mortality against 3$^{rd}$ instar larvae. Larvae that survived at post 24 hours exposure were reared until adult and their generation was maintained and again was selected with permethrin at LC$_{50}$ concentration.

Establishment of serial concentration of permethrin

The LC$_{50}$ of permethrin for each generation of IMR-PSS strain was determined by establishing serial concentrations of permethrin that caused wide range of larval mortality to the respective generation of IMR-PSS strain. The larval mortality was conducted via employing WHO larval bioassay protocol (WHO, 2016). The LC$_{50}$ was determined by analyzing the larval mortality of tested serial concentrations with SPSS statistical analysis software (version 10.0) using Probit Analysis Statistical method.

Determination of insecticide susceptibility status of IMR-LS strain and IMR-PSS strain via WHO adult bioassay

Adult bioassay was employed (WHO 2016) to determine the susceptibility status of both IMR-PSS and IMR-LS strains. A total of 500 sugar-fed adult female mosquitoes aged 3-5 days were used for the test. Four replicates consisted of 20 adult mosquitoes per replicate were exposed to the permethrin, deltamethrin, cyfluthrin, lambdacyhalothrin and malathion dosage, respectively. Other four replicates of the same number and same batch of adult female mosquitoes were exposed to the insecticide-free paper which served as the control. The exposure period was one hour as recommended by the WHO (2016). Consequently, all the tested mosquitoes (live and knocked down) were transferred into clean paper cups and supplied with 10% sugar solution through cotton balls. After 24 hours recovery period, the adult mortality was recorded. Result interpretation of adult mortality based on WHO criteria indicates that more than 98% means mosquitoes are susceptible; 90% to 98% means possibility of resistance development, and less than 90% means developed resistance.

Protein extraction

Proteins were extracted via Trizol method. A total of 15 mosquito samples were pooled and were homogenized; and the protein were precipitated via TRIzol® Reagent (Invitrogen, UK). This ready-to-use reagent is a monophasic solution, consisting 2 major components of phenol and guanidiumisothiocyanate used to solubilise and homogenise mosquito samples. After solubilisation, chloroform was added into the homogenate and 2 distinct phases which were organic phase and aqueous phase were observed after 15 minutes of 12,000g centrifugation at 4°C. The protein content together with DNA in the organic phase was then used and therefore the aqueous phase was discarded. Subsequently, absolute ethanol was added into the mixture, and a phenol-ethanol supernatant product was produced which was transferred into a new tube. The protein was further isolated via addition of isopropanol and finally the protein pellet was formed at the bottom of the tube. This protein pellet was then washed with 0.3M guanidine hydrochloride in 95% ethanol, followed by absolute ethanol. The samples were resuspended in 1% SDS and 8M urea in Tris-HCl 1M, pH 8.0 at the ratio of 1:1 and were incubated for 1 hour in a 50°C heat block, followed by 3 minutes of bath sonication. The samples were then stored at -20°C.

Protein quantification

Proteins were quantified based on Bradford (1976), using a 1:4 dilution of Bio-Rad Protein assay dye concentrate which contained Coomassie® Brilliant Blue G-250 dye (Bio-Rad, Hercules, CA) in water. Several concen-
trations of Bovine Serum Albumin (BSA) at 1 mg/ml, 0.8 mg/ml, 0.4 mg/ml, 0.6 mg/ml and 0.2 mg/ml were prepared and subsequently 100 µl of each standard protein concentrations were mixed with 5 ml of protein assays. The mixture was allowed to react for 5 minutes and the protein constituents were read at 595nm wavelength via spectrophotometer (Bio-Spectrometer, Eppendorf, US).

**Determination of unknown protein concentration**
The OD values of 5 known concentrations of BSA were plotted. The equation of standard curve was constructed and used for determination of unknown protein concentration of extracted protein from IMR-LS and IMR-PR strain. Prior to that, the samples were prepared by mixing 10 µl of each protein samples with 500 µl of protein assay in a cuvette. The protein constituents were read at 595nm wavelength via spectrophotometer (Bio-Spectrometer, Eppendorf, US).

**Polyacrylamide gel electrophoresis (SDS-PAGE)**
Protein profiling was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% resolving gel and 4% stacking gel, following the procedures of Laemmli (1970). 10 µl of the prepared sample, which consisted of 5 µl protein samples, 4.5 µl 2x SDS-PAGE sample buffer and 0.5 µl beta-mecarptoethanol, was separated by electrophoresis at a current of 350 mA and 200V for 40 minutes. The broad range of SeeBlue® Pre-Stained Protein Standard (Thermo Fisher Scientific, US) was used to determine the molecular weight of protein extracted from resistant and susceptible *Aedes aegypti*.

**Liquid chromatography-mass spectrometry (LC-MS/MS)**
Peptides were dissolved in 0.1% FA and 2% ACN, directly loaded onto a reversed-phase analytical column (75 um i.d. x 150mm, packed with Acclaim PepMap RSLC C18, 2 um, 100Å, nanoViper). The gradient comprised solvent B (0.1% FA in 80% ACN) in increasing concentrations from 5% to 50% over 40 min, and climbing to 90% in 5 min, then holding at 90% for another 5 min. All were conducted at a constant flow rate of 300 nl/min. The MS analysis was performed on Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, US). The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q ExactiveTM (Thermo) coupled online to the UPLC. Intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were selected for MS/MS using NCE setting as 27; ion fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans was applied for the top 20 precursor ions above a threshold ion count of 1E4 in the MS survey scan with 30.0s dynamic exclusion. The electrospray voltage applied was 2.0 kV. Automatic gain control (AGC) was used to prevent overfilling of the ion trap; 1E5 ions were accumulated for generation of MS/MS spectra. For MS scans, the m/z scan range was 350 to 1800 m/z. Fixed first mass was set as 100 m/z.

**Protein Data analysis**
Protein identification were performed with MASCOT software (Matrix Science, USA) by searching Uniprot_ *Aedes aegypti* (The Uniprot Consortium, 2016).

**RESULTS**
**Insecticide Resistance Screening Via WHO adult assay**
According to WHO (2016) insecticide bioassay criteria, the IMR-PSS strain was strongly resistant to pyrethroid insecticide, but susceptible to organophosphate insecticide (Figure 1). On the other words, this IMR-PSS strain showed a complete absence of adult mortality at post 24 hours exposure against permethrin and demonstrated a very low mortality percentage against deltamethrin, lambda-cyhalothrin.
and cyfluthrin, with 6%, 4% and 34% adult mortality, respectively; in contrary, a complete mortality against malathion was revealed (Figure 1). In comparison, the laboratory-susceptible strain was confirmed susceptible to all tested pyrethroids, with complete mortality at post 24 hours exposure (Figure 2).

**Polyacrylamide gel electrophoresis (SDS-PAGE)**

Using laboratory-susceptible strain as reference strain to determine protein changes in permethrin-selected strain, the SDS-PAGE gel revealed similarity on the pattern of protein bands between protein extracted from permethrin-selected and laboratory-susceptible strain (Figure 3). Generally, there were an abundant protein bands observed from 98kDa to as low as 3kDa. It was worth noted that, there were two intense bands of IMR-PSS strain falling within the range from 28 kDa to 17 kDa and at 6 kDa, while a faint protein band was found at 3 kDa.

**HPLC-MS protein analysis**

A total of 1519 and 1131 proteins were identified in IMR-PSS strain and IMR-LS strain, respectively. Of all these proteins only 1131 proteins were common in both strains, while additional of 388 proteins were only identified in IMR-PSS strain but absent in IMR-LS strain. Among these 388 proteins, 275 proteins were identified as uncharacterized proteins, meanwhile those remaining 113 proteins were identified as functional proteins (Table 1). The optimum protein concentration of mosquitoes for 1D-gel electrophoresis was approximately 2.0 mg/L.

**DISCUSSION**

Proteomic was introduced in 1977 to make an analogy with genomics. The word proteomic is the word abbreviation of the words protein and genomics (Cellis J., 1998). Proteomic analysis permits protein identification of the entire protein extracts in any biological sample. Not all proteins are expressed at the same time in a living cell and continuously changes are happening. Therefore, proteomic analysis is shown to have the ability to display, quantify and identify thousands of proteins in a single gel and subsequently can be used to detect quantitative changes and expression levels of the differences in cell states.

A range of proteomic technologies are widely available for proteomic exploration with each technology possesses unique application. In this present study, we utilized gel based approaches; SDS-PAGE and Mass spectrophotometry with LC-MS/MS. The gel based approached allows separation of protein sample according to the size, subsequently assist in estimation of protein molecular weight. In the present study, it is

![Figure 1. Resistance status of permethrin-selected *Aedes aegypti* strain to 0.25% for permethrin; 0.05% for deltamethrin; 0.15% lambda-cyhalothrin; 0.05% cyfluthrin; and 0.8% malathion.](image-url)
Figure 2. 24 hours adult mortality of laboratory-susceptible *Aedes aegypti* strain against 0.25% for permethrin; 0.05% for deltamethrin; 0.15% lambda-cyhalothrin; 0.05% cyfluthrin; and 0.8% malathion.

Figure 3. Comparison of SDS-PAGE electrophoresis of protein samples extracted from permethrin-selected (IMR-PSS) and susceptible *Aedes aegypti* (IMR-LS). Two replicates of IMR-PSS strain were placed in lane 2 (L2) and 4 (L4); while, replicates for IMR-LS strain were placed in lane 3 (L3) and 5 (L5), respectively. M indicates protein marker. The protein concentration of samples that were loaded in L2 to L5 ranged 2.08mg/L to 1.60mg/L.

It is challenging to spot the differences of bands size between total protein extracted from resistant and susceptible *Aedes aegypti* sample, respectively. The reason is because the set of protein bands after SDS-PAGE is similar for both study samples. However, this preliminary comparison performed by SDS-PAGE revealed different protein expression in view of the level of intensity of the bands (Figure 3). The difference in protein intensity of the protein bands here may be correlated with the possible protein(s) that might correspond to pyrethroid resistance. However, the second approach utilising Liquid Chromatography – tandem mass spectrophotometry (LC-MS/MS) helped in providing a significant characterization of the total cell proteins for both samples. The LC-MS/MS is currently a widely used proteomics techniques and is highly...
Table 1. List of functional proteins which are only present in IMR-PSS strain

<table>
<thead>
<tr>
<th>No.</th>
<th>Protein Name (Accession Number)</th>
<th>Protein Name (Accession Number)</th>
<th>Protein Name (Accession Number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaline phosphatase</td>
<td>Putative zinc finger ceh domain</td>
<td>UDP-galactosyltransferase</td>
</tr>
<tr>
<td>2</td>
<td>Alpha-manosidase</td>
<td>Putative nuclear transport receptor</td>
<td>UTF-1 glucosyltransferase 2</td>
</tr>
<tr>
<td>3</td>
<td>Apolipoprotein B</td>
<td>Putative oxidase-reductase</td>
<td>Carboxypeptidase</td>
</tr>
<tr>
<td>4</td>
<td>E3 ubiquitin-protein ligase</td>
<td>Putative protein hydrolase</td>
<td>Carboxypeptidase</td>
</tr>
<tr>
<td>5</td>
<td>Eukaryotic translation initiation factor 3</td>
<td>Putative nuclear transport receptor</td>
<td>C elegant complex subunit 1</td>
</tr>
<tr>
<td>6</td>
<td>Eukaryotic translation initiation factor 3</td>
<td>Putative receptor mediating netrin-dependent axon guidance</td>
<td>C elegant complex subunit 1</td>
</tr>
<tr>
<td>7</td>
<td>Eukaryotic translation initiation factor 3</td>
<td>Putative receptor mediating netrin-dependent axon guidance</td>
<td>C elegant complex subunit 1</td>
</tr>
<tr>
<td>8</td>
<td>Eukaryotic translation initiation factor 3</td>
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<td>C elegant complex subunit 1</td>
</tr>
<tr>
<td>9</td>
<td>Eukaryotic translation initiation factor 3</td>
<td>Putative receptor mediating netrin-dependent axon guidance</td>
<td>C elegant complex subunit 1</td>
</tr>
<tr>
<td>10</td>
<td>Ferritin</td>
<td>Putative salivary C type lectin</td>
<td>Cytochrome c oxidase subunit 2</td>
</tr>
<tr>
<td>11</td>
<td>Flagellar motor 1</td>
<td>Putative serpin</td>
<td>Mitochondrial ATP synthase F chain</td>
</tr>
<tr>
<td>12</td>
<td>Galectin</td>
<td>Putative thioredoxin</td>
<td>Mitochondrial import membrane translocase subunit TIM8</td>
</tr>
<tr>
<td>13</td>
<td>Histone H2B</td>
<td>Putative vacuolar protein</td>
<td>Mitochondrial import membrane translocase subunit TIM1</td>
</tr>
<tr>
<td>14</td>
<td>Isocitrate dehydrogenase [NADP]</td>
<td>Spiroplasma salivary C type lectin</td>
<td>Putative ATP synthase β chain</td>
</tr>
<tr>
<td>15</td>
<td>Laminin gamma 1 chain</td>
<td>Spiroplasma salivary C type lectin</td>
<td>Putative ATP synthase β chain</td>
</tr>
<tr>
<td>16</td>
<td>Spiroplasma salivary C type lectin</td>
<td>Spiroplasma salivary C type lectin</td>
<td>Putative ATP synthase β chain</td>
</tr>
<tr>
<td>17</td>
<td>Serine/threonine-protein kinase ROC1</td>
<td>Spiroplasma salivary C type lectin</td>
<td>Putative ATP synthase β chain</td>
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<tr>
<td>18</td>
<td>Serine/threonine-protein phosphatase</td>
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<td>Putative ATP synthase β chain</td>
</tr>
<tr>
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<td>DNAJ chaperone</td>
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<tr>
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<td>Dihydrophosphorosylase</td>
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<td>21</td>
<td>Dorsal isomerase-1-S</td>
<td>Glutathione peroxidase</td>
<td>Salivary secreted peptide</td>
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<tr>
<td>22</td>
<td>Dynamin heavy chain</td>
<td>GTP-AMP phosphotransferase</td>
<td>Vacular protein sorting-associated protein 25 (Fragment)</td>
</tr>
<tr>
<td>23</td>
<td>Putative apoptosis-promoting ma-binding protein p134-514 zymogen family</td>
<td>GTP-AMP phosphotransferase</td>
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<td>Metalloproteinase</td>
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<td>Vacular protein sorting-associated protein 25 (Fragment)</td>
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<td>Microtubule-associated protein</td>
<td>Tubulin alpha chain</td>
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<td>Microtubule-associated protein</td>
<td>Tubulin alpha chain</td>
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<td>Tubulin alpha chain</td>
</tr>
<tr>
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<td>Putative myosin class II heavy chain</td>
<td>NADH dehydrogenase subunit 4</td>
<td>Tubulin-alpha-depolymerizing enzyme</td>
</tr>
<tr>
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<td>Putative serine protease inhibitor</td>
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<td>Ubiquitin-dependent protease</td>
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</table>
suggested for characterizing a complex samples with overlapped peptide masses.

In order to address the mechanisms of resistance development, two existing methods for determining resistance mechanisms in mosquitoes were established; quantification of detoxification enzyme activities, and molecular detection of mutation gene in voltage gated sodium and Acetylcholinesterase (AcHE) enzyme, respectively (WHO, 1998 & WHO, 2016). Both established methods are well described in many published research and by the World Health Organization, and these approaches are well known to complement and supplement WHO larval and adult bioassay test. In the present study, the resistance mechanisms of IMR-PSS were previously reported by Rosilawati et al. (2018). IMR-PSS was resistant to pyrethroid and showed susceptible to organophosphate; the resistance conferred due to MFO enzyme activity, and presence of knockdown resistance mutation in position V1023G and S996P (Rosilawati et al., 2018).

Insecticide resistance in mosquito is not confined to enzyme detoxification only, but also includes insensitive insecticidal targeted receptor site. The enzyme assays provides quantitative information associated with enzymatic activity and metabolic resistance. Enzyme assays can be conducted in the laboratory (WHO, 2005) as well as in the field using rapid enzyme test kits (Lee et al., 1998). Lee (1998) in Malaysia was one of the earliest to detect resistance enzymes using a rapid method. Brogdon et al. (1997) described the optimum conditions for simple and rapid microplate assay, and Nazni et al. (2000) developed a modified method to detect oxidase activity related with insecticide resistance.

In addition, genomic study which explores the targeted gene responsible for resistance development has been widely reported. Genomic findings provide valuable information on distribution of resistance alleles existing in vectors by detecting point-mutation or knock-down resistance gene (kdr) in para-type or voltage-gated sodium channel (VGSC), hence confers pyrethroid resistance in mosquitoes (Shinji et al., 2011; Soderlund et al., 2003).

Molecular application such as DNA sequencing technique allows massive exploration of mutation in voltage-gated sodium channel that were reported as the contributing factors responsible for providing protective effect against pyrethroids. The high-throughput approaches to detect kdr mutation in mosquitoes such as pyrosequencing and high-resolution melt (HRM) genotyping assays were also employed (Ishak et al., 2015; Wuliandari et al., 2015).

Beside genomic study, transcriptome analysis such as serial analysis of gene expression (SAGE) or microarray allowed understanding on how transcriptome expressed genetic variants of multiple detoxification genes. Unfortunately, study on mRNA transcription had been found not always parallel to the protein expression level, where proteome which consists of considerable more protein than expected by direct transcription is due to alternative splicing and post-translation modification (Audagnotto and Dal Peraro, 2017).

Molecular approaches provide insightful information on resistance mechanisms in mosquitoes at DNA and RNA aspect. Unfortunately, there is paucity of literature on proteomic approaches especially in exploring insecticide resistance in mosquito at proteomic level. Therefore, after genomics and transcriptomics, proteomics should be the next step in the study of biological system such as insecticide resistance, which is believed to complement the genomic findings (Wasinger et al., 1995).

In the literature, CYP6AA9 and CYP6AA1 were reported to be associated with pyrethroid resistance in Culex pipiens (Weijie W. et al., 2015) and Anopheles funestus (Sulaiman L. et al., 2018), respectively. To date, there are no published pyrethroid resistance-related proteins studies especially in pyrethroid resistant Aedes aegypti especially for Malaysia strain. Of all 113 functional proteins that were produced in pyrethroid resistant Aedes aegypti in the present study, the most significant related proteins that functions to protect and
defence for the survival against insecticides pyrethroids are alkaline phosphatase, apolipophorin II, E3 ubiquiton-protein ligase, heat shock protein HSP70, serine protease IV, serine threonine related protein, DNAJ chaperone and cytochrome c oxidase subunit. While, the remaining proteins expressed in pyrethroid resistant *Ae. aegypti* are proteins that functions for transportation, storage, midgut proteins and regulation of tissue and organ (Table 1).

In conclusion, the proteomic analysis in this present study provides a preliminary finding that there are protein variations between permethrin resistant and susceptible strain. Understanding and examining mechanisms of insecticides in mosquitoes are crucially important for strategy to manage insecticide resistance, and to search for new target sites in the development of novel insecticides. Future research is required to further determine the possible targeted protein(s) which could possibly be exploited as a novel protein marker for rapid detection of insecticide resistance and probably also providing new counter measures in addition to development of insecticides with novel mode of action.

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