Characterization of Beta-1,3-glucanases in Aedes aegypti larvae

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Introduction. Insect β-1,3-glucanases are involved in digestion of fungal cells and hemicellulose. Recently, these enzymes were pointed as part of the innate immune system of moths and termites. Insect beta-1,3-glucanases and beta-1,3-glucan binding proteins are homologous proteins, belonging to Family 16 of Glycoside Hydrolases. We investigated the role of GH16 genes in A. aegypti larvae, and its possible participation in digestion of fungi ingested by these detritivorous insects.

Material and Methods. GHF16 sequences were analysed using programs Bioedit and MEGA. Glucanase activity was measured by releasing of reducing groups from laminarin, and molecular masses were measured by gel filtration chromatography. Semi quantitative RT-PCR was performed using cDNA from larvae and specific primers for each gene. Larvae were raised hatching eggs in water containing live Ustilago sp. cells or cat food, and the development of insects was monitored daily.

Results and Discussion. The genome of A. aegypti contains six genes coding for GHF16 proteins (Aae GH16.1 – Aae GH16.6), containing 2-9 exons. Phylogenetic analysis suggest that Aae GH16.4 is related to beta-1,3-glucan binding proteins, which have no catalytic activity. Aae GH16.1,2,3,5,6 contain catalytic residues and are related to glucanases, which apparently suffered duplications in Diptera Nematocera genomes. A. aegypti larvae contain beta-1,3-glucanase activity in head (0,38±0,01 mU/animal; 0,83±0,09 mU/mg; optimum pH 6-9), gut (0,39±0,03; 0,30±0,03; 5-9) and carcass (1,6±0,2; 3,38±0,07; 5-10). Gut activity is not acquired from food. These activities have molecular masses of 142 kDa (head), 41/133 kDa (gut) and 150 kDa (carcass). All GH16 genes showed expression in the larval head, gut and carcass. A. aegypti develop well from egg to adults feeding only on live yeasts, with developmental parameters (larvae/pupae/male/female weight, rates of pupation/emerging) similar to controls. Conclusions. A. aegypti larvae are able to nourish from fungi, and several genes from GHF16 are probably involved in this process.

Keywords: Aedes aegypti, beta-1,3-glucanase, digestion, gene expression

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