

Antimicrobial Activity of Hemolymph from Larvae *Spodoptera frugiperda* (Lepdoptera)

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Introduction: In the last decades, protein molecules have shown antimicrobial potential, being candidate for new biocide drugs, more effective and selective, against pathogenic microorganisms. In insect, the defense system induces the synthesis of defense proteins in hemolymph. Therefore, insect hemolymph is a rich source to investigate new antimicrobial molecules. **Objectives:** Here, we evaluate the biocide action of protein molecules from hemolymph of *Spodoptera frugiperda* (Lepdoptera) larvae. **Materials and Methods:** Hemolymph was collected from *S. frugiperda* in 5th Instar larval stage, kept at 27°C and 12 h photophase. It was centrifuged at 10,000 rpm for 5 min, and the supernatant used to purify the antimicrobial proteins as following. The protein amount was determined by Bradford method. Hemolymph sample (5 mg of protein) was applied onto a Prep-C18 column in a HPLC system, with linear acetonitrile gradient (5-95%) add 0.1% TFA, flow rate of 2.5 mL/min and 280 nm monitoring. The antimicrobial activity of chromatography fractions was evaluated by broth microdilution antibacterial assay against human pathogen bacteria, being monitoring at 595nm, in each 30 minutes over 18 hours. SDS-PAGE analysis revealed the proteins molecular weight profile. **Results and Discussion:** Hemolymph proteins purified from *S. frugiperda* showed a profile with bands in a range of 38-17KDa in reducing gel electrophoresis. HPLC fractions 10 and 11 eluted with 55 and 60% of acetonitrile, respectively, showed antimicrobial activity toward Gram negative and positive bacteria species. Fractions 10 and 11 showed OD decrease of *Escherichia coli* growth after 3 hours, resulting in a 100% inhibition. While for *Staphylococcus epidermidis*, fraction 10 promoted same inhibition after 7 hours and fraction 11 after the 3 hours. **Conclusion:** However, proteins from *S. frugiperda* hemolymph demonstrated expressive antimicrobial potential against human pathogens and can be used as a template to new drugs development.

Key words: Insect, Antimicrobial agents, Hemolymph.

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