PRODUCTION OF ANTI-VgR (VITELLOGENIN RECEPTOR)

Dohanik, VT; Paula, AT; Silveira, WF; Oliveira, LL; Serrão, JE

Department of General Biology of the Federal University of Viçosa, Minas Gerais, Brazil

The vitellogenesis is a key process that controls reproduction in insects. This process is over the control of the juvenile and / or ecdizona hormone, which are the major inducers of vitellogenin synthesis and its uptake into the oocyte via vitellogenin receptor-mediated endocytosis. The objective of this study is the production of antibodies for immunolocalization of vitellogenin receptor in the cells of the ovary in bees. To obtain an anti-VgR antibody, we first cloned N-terminal portion of vitellogenin receptor (predicted honey bee VgR, XP_001121707). The recombinant protein was expressed in E. coli strain C41. Briefly, colonies transformed by heat shock were placed to grow in LB medium with ampicillin for 4 h until an OD 600 nm = 0.6, then IPTG was added to induce expression of the recombinant. The cells were collected by centrifugation, the supernatant was discarded and the pellet resuspended with Tris buffer + Triton X-100 and sonicated. The cell extract was centrifuged and the supernatant was used for purification on nickel column. The presence of recombinant protein was confirmed using gel electrophoresis (SDS-PAGE) which showed a band of 36 kDa as expected. The purified recombinant protein was injected into five Balb/C mice for the production of antibodies, 10 µg of protein was injected into two doses at 15-day intervals. After 10 days of the last immunization the hyperimmune serum was obtained from animals. The presence of specific anti-VgR was evaluated with Western blotting, which showed positive reaction to the anti-vitellogenin receptor antibody. The antibody produced is specific to the purified protein vitellogenin receptor, the next step is to identify this protein in ovarian bees

Key words: antibody, Hymenoptera, protein