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EXPRESSION OF AN ANTIVIRAL RECOMBINANT PROTEIN OBTAINED FROM *LONOMIA OBLIQUA* HEMOLYMPH IN A BACULOVIRUS/CELLS

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Introduction. The control of viruses, especially those induced by influenza virus is of great interest to the public health area. Several studies have been conducted that show the presence of pharmacologically active substances in the hemolymph. Recently we have demonstrated the existence of a potent antiviral in hemolymph of *Lonomia obliqua* caterpillar. This purified protein reduced virus production by more than 157 fold (from $3.3\pm1.25x10^7$ to $2.1\pm1.5x10^5$) to measles virus, 61 fold to polio virus ($2.8\pm1{,}08x10^9$ to $4.58\pm1.42x10^7$) and 64 fold to H_1N_1 influenza virus (Antiviral Research 84, 84-90, 2009). Thus, this study aim to build recombinants bacmids containing sequences encoding this antiviral protein in baculovirus/SF-9 cell system.

Metodology. To synthesize cDNA, RNA of *L. obliqua* was extracted with Trizol reagent and used in polymerase chain reactions using reverse transcriptase polymerase (RT-PCR) with primers specific for the antiviral protein. Restriction sites were inserted in the cDNA for connection to the donor plasmid pFastBac1™ (Invitrogen). The recombinant plasmid was selected in *Escherichia coli* DH5α and subsequently used in the transformation of DH10Bac *E. coli*, to obtain the recombinant bacmid. This bacmid was used for expression of this protein in baculovirus/insect cells system. Samples of the supernatant of infected cultures were collected daily, concentrated and subjected to SDS-PAGE chromatography. A protein band around 20 kDa was observed. Studies on the activity of the recombinant protein were performed with the supernatant of infected culture applied in cultures infected with influenza virus. These studies showed that the protein was capable of reduced virus production.

Results. The results are presented below. Figure 1 shows the agarose gels to confirm amplification of the antiviral protein sequence (1a), the release of the insert after digestion with enzymes used for cloning (1b) and the amplification of the protein sequence (1c). Figure 2 is presented the colonies containing the recombinant bacmid after transformation into E. DH10Bac ™ coli. Figure 3 presents the electrophoresis gels of infected cells with bacmids (3a) and an SDS-PAGE electrophoresis of infected culture supernatant of recombinant baculovirus infected with recombinant the antiviral protein. Figure 4 shows an electron microscopy of recombinant baculovirus. Figure 5 shown the effect of antiviral recombinant protein in EMC virus replication. Control cell (a) infected cells with EMC virus (b) and the protective effect of recombinant protein in a culture infected with the virus (c).

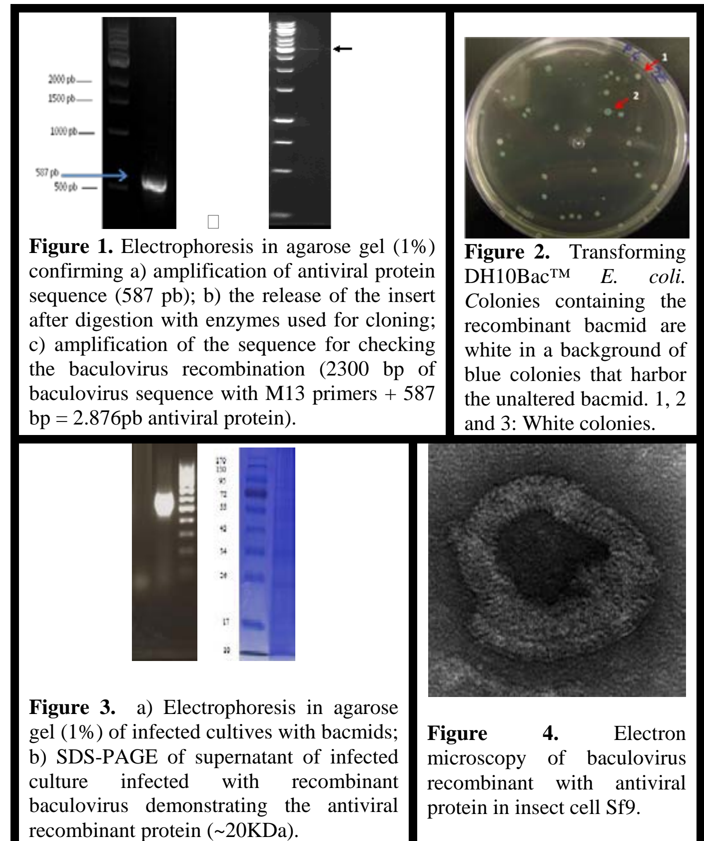

Figure 1. Electrophoresis in agarose gel (1%) confirming a) amplification of antiviral protein sequence (587 pb); b) the release of the insert after digestion with enzymes used for cloning; c) amplification of the sequence for checking the baculovirus recombination (2300 bp of baculovirus sequence with M13 primers + 587 bp = 2.876pb antiviral protein).

Figure 2. Transforming DH10Bac™ *E. coli.* Colonies containing the recombinant bacmid are white in a background of blue colonies that harbor the unaltered bacmid. 1, 2 and 3: White colonies.

Figure 3. a) Electrophoresis in agarose gel (1%) of infected cultives with bacmids; b) SDS-PAGE of supernatant of infected culture infected with recombinant baculovirus demonstrating the antiviral recombinant protein (~20KDa).

Figure 4. Electron microscopy of baculovirus recombinant with antiviral protein in insect cell Sf9.

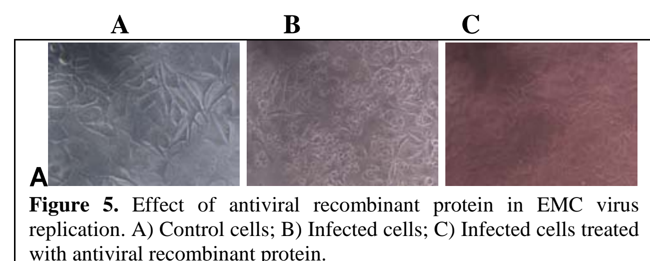

Figure 5. Effect of antiviral recombinant protein in EMC virus replication. A) Control cells; B) Infected cells; C) Infected cells treated with antiviral recombinant protein.

Conclusión:

In this study, recombinant bacmid containing the sequence encoding this antiviral protein was produced and expressed in baculovirus/SF-9 cell system. The recombinant protein obtained was able to block the replication of EMC virus replication.

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Bibliography. Antiviral Research 84, 84-90, (2009).