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BIOINSETICIDA AgMNPV DERIVADO DE CULTURAS CELULARES. LIMITAÇÕES BIOLÓGICAS E SOLUÇÕES DE BIOPROCESSO. CELL CULTURE DERIVED AgMNPV BIOINSECTICIDE. BIOLOGICAL CONSTRAINTS AND BIOPROCESS ISSUES.

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We have studied parameters for optimizing the *Spodoptera frugiperda* (Sf9) cell culture and viral infection for the production of *Anticarsia gemmatalis* multiple nucleopolyhedrosis virus (AgMNPV) polyhedra inclusion bodies (PIBs) in shaker-Schott or spinner bottles and bioreactors. We have assayed the $k_L a$ of the systems, initial cell seeding, cell culture volume, dissolved oxygen (DO), multiplicity of infection (MOI), nutrients consumption, and metabolites production. The medium surface oxygen transfer was shown to be higher in shaker bottles than in spinner ones, which was in direct correlation to the higher cell density obtained. Best quantitative performances of PIBs production were obtained with a SF900II medium volume/ shaker-bottle volume ratio of 15% and MOI of 1 performed at a cell density of 10^6 cells/ml in a medium containing enough glucose and glutamine. Upon infection, a decrease in the cell multiplication was observed to be dependent on the MOI used, and the ?X at the exponential growth phase in infected and non-infected cultures were, respectively, of 0.2832 and 0.3914 (day^{-1}). The glucose consumption and lactate production were higher in the infected cultures (?Glucose and ?Lactate of, respectively, 0.0248 and 0.0089-10^{-8} g/cell x day in infected cultures and 0.0151 and 0.0046-10^{-8} g/cell x day in non infected ones). The glutamine consumption did not differ in both cultures (?Glutamine of 0.0034 and 0.0037-10^{-8} g/cell x day in, respectively, infected and non infected cultures). When a virus MOI of 0.1 was used for infection, a higher concentration of PIBs/mL was obtained. This was in direct correlation to a higher cell concentration present in these cultures, where a decrease in cell multiplication due to virus infection is minimized. When a MOI of 1 was used, a more effective decrease in cell multiplication was observed and a lower concentration of PIBs/mL was obtained, but with a best performance of PIBs/cell. The virulence of PIBs produced in cultures infected at low or high MOI showed comparable DL_{50}. Culture and infection in scaling-up conditions, performed in a bioreactor, reproduced the shaker-Schott findings. For an accurate qualitative control of PIB virulence, hemolymph from AgMNPV infected *Anticarsia gemmatalis* was used as starting material for passages in Sf9 cells. These led to a loss of virulence among the PIBs with an increase in the DL_{50}. The loss of virulence was accompanied by a loss in budded virus titer, a decreased number of PIBs produced and an altered DNA restriction pattern, suggesting the generation of defective interference particles (DIPs). Transmission electron microscopy (TEM) studies revealed that after cell passages, PIBs lacking virions were progressively synthesized. The study described here point out the biological constraints and bioprocess issues for the preparation of AgMNPV PIBs for biological control.

Key words. AgMNPV, bioinsecticide, cell culture

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