# PRODUCTION OF LdNPV IN CELL CULTURE BIOREACTORS

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The development of bioreactor production methods for Gypchek would provide a means of production that can be scaled to a large capacity (production of 50,000 acre equivalents + per year), that is potentially at a lower cost than the current larval based production method, and would generate a product completely free of bacteria, fungi, and other viruses. The methods developed would be applicable to production of any baculovirus (e.g., TM-biocontrol, browntail moth virus) for use in insect pest control programs. We are currently focused on development of methods for production of Gypchek (LdNPV Isolate 203-Wild-type) in 3, 7, and 14 liter cell culture bioreactors. Briefly, the production process entails the preparation and addition of insect cell culture medium into the bioreactor. The medium is sterilized by filtration as the medium is pumped in the bioreactor. Ld652Y cells are then added that were grown to the proper density in a shake flask. Once the cells reach the appropriate density, budded virus is added to begin the infection process. Polyhedra are isolated by centrifugation at the end of a production run. The final product is nearly pure polyhedra, and lacks bacterial, viral, and fungal contaminants. Recent studies have focused on determination of the maximal cell density that can be achieved, analysis of spent media, and determination of the optimal ratio of budded virus:host cells to use for production of polyhedra. The amount of polyhedra produced in bioreactors is dependent upon the number of cells present. Maximum polyhedra production will be achieved when an infection is performed using the maximum possible number of cells per liter of medium. To determine the maximum density to which Ld652Y cells can be grown, cells were seeded into the 2 and 5 liter bioreactors and grown until cell growth nearly stopped. A cell density of approximately 1 x 107 cells/ml was achieved, when using an agitation speed of 75 rpm and a dissolved oxygen concentration of 50% of saturation. Depletion of a medium component, such as glucose or an amino acid, during the cell growth phase of polyhedra production could cause cell growth to stop and hence negatively impact polyhedra production. An analysis of medium was performed after a cell density of about 1 x $10^7$ cells/ml was achieved. No amino acid, vitamin, or glucose was found to be depleted. A high utilization of aspartic acid, glutamine, tyrosine, and serine was found. From 27% to 38% of the starting amount of these amino acids were utilized during the cell growth phase. Approximately 54% of the riboflavin present was used. Cholesterol may be limiting cell growth since it was depleted from the medium. There was no build up of the metabolic byproducts ammonia and lactate that are generated by the metabolism of glutamine and glucose, respectively. These byproducts can be inhibitory to cell growth if their levels become too high. Polyhedra production was found to be significantly greater when using a multiplicity of infection (m.o.i.) of 0.005 virus particles per cell compared to using a m.o.i. of 0.01. Polyhedra production levels of 4.8 x$10^{11}$ and 5.8 x $10^{11}$ polyhedra per liter have been achieved to date in the 2 and 5 liter bioreactors, respectively. Preliminary results suggest that the sparging rate and cell growth rate can impact polyhedra production. These parameters are under investigation.