

## **426a Heterologous Expression of Aedes Aegypti Densonucleosis Virus Structural Protein Using Pichia Pastoris**

*Rachel Specht, Dan Konet, Jonathan O. Carlson, and Ranil Wickramasinghe*

*Aedes aegypti* densonucleosis virus (AeDNV) like particles have been produced using a *Pichia pastoris* heterologous gene expression system. AeDNV is a mosquito specific parvovirus that shows high infectivity and mortality in *A. aegypti* mosquitoes. The methylotropic yeast *P. pastoris* has previously been utilized to produce several heterologous proteins at concentrations which make them of commercial interest. The structural protein (VP) gene of AeDNV was optimized for codon usage in *P. pastoris* and inserted into the vector pPICZA to form pPICZA-VP. Previous attempts to express the VP gene without codon optimization were unsuccessful. Chromosomal integration of pPICZA-VP into *P. pastoris* downstream of the alcohol oxidase promoter AOX1 was achieved using homologous recombination. The structural protein self assembles in the yeast to form virus-like particles. The recombinant yeast has been cultivated in shaker flasks and in a 3-liter bioreactor. The bioreactor controlled temperature, dissolved oxygen, and pH and showed an increase in protein production compared to the shaker flask. Other structural proteins which have been expressed in *P. pastoris* and generate virus-like particles include dengue virus, hepatitis B virus, and hepatitis C virus. This is the first time a parvovirus-like particle has been expressed using *P. pastoris*. The construction of self assembling recombinant AeDNV particles has the potential to be used in the development of novel biocontrol strategies for mosquitoes.