

Development of Techno-Industrial Platform for Large Scale Production of Biotechnology Based Product for Dengue Control.

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Abstract

Nowadays, Dengue fever is one of the major epidemic diseases in urban, semi-urban, and rural areas in tropical and subtropical countries. This mosquito transmitted viral disease is considered as fastest transmitted mosquito disease and spread in over 100 countries. Based on WHO report in 2010, Southeast Asia (SEA) and Western Pacific (WP) regions bear nearly 75% of current disease population. This disease is transmitted using two species of mosquitoes, the main vector *Aedes aegypti* (L.) and the less competent vector *A. albopictus*. For many years, controlling dengue disease is carried out through killing the mosquito larvae. This is usually carried out by using chemical methods which cause many environmental problems and human and animal toxicity. Another approach was developed by using the biological control agent *Bacillus thuringiensis* var *israelensis* as specific and safe larvicidal agent. However, after many years application, some resistance varieties were developed and thus decrease the agent efficiency. More recently, based on the many years and innovative research in University of Florida in USA, a newly discovered protein called Trypsin Modulating Oostatic Factor (TMOF) showed strong larvicidal activity in aquatic Environment. TMOF consist of 10 amino acid sequence of the hormone (YDPAPPPPPP) known as mosquito decapeptide. The hormone synthesized by the ovaries after the blood meal, circulates in the hemolymph, binds to gut receptor and stops trypsin biosynthesis. A unique primary and stable amino acid from gut proteolytic enzymes that can interferes with digestion of mosquito larvae, leading to their starvation by termination of trypsin biosynthesis in their midgut. No harm to humans or the environment is expected from use of TMOF as a pesticide active ingredient. The genes for making TMOF have been inserted into yeast cells (*Pichia pastoris*) so the yeast cells make large amounts of the protein. The yeast cells are then killed by exposure to extremely high temperatures. Killed yeast cells containing TMOF are applied to bodies of water to control mosquito larvae. Once eaten by mosquito larvae, TMOF interferes with the production of

trypsin, a critical enzyme needed by the larval digestive system. Exposed larvae are unable to digest food and therefore starve to death.

Since late 2008, Institute of Bioproduct Development (IBD) at Universiti Teknologi Malaysia (UTM) is working in long term strategic partner for EntoGenex Co. (the TMOF technology owner) for technology transfer, bioprocess development, large scale production, and final product formulation. The IBD bioprocessing microbial platform is a complete integrated upstream/downstream platform to complete the process and production chain from microbe vial to finished product. IBD is currently the first and biggest bioprocessing facilities among Malaysian universities and the currently the only approved facility by Genetic Modification Advisory Committee (GMAC) under the Biosafety Act 2007 for running living modified organism (LMO) for large scale production up to 1500 L fermentation capacity.

The current presentation, will present the successful story of cooperation between IBD and biotechnology companies to produce highly demanded high technology product to solve big health problem in National, regional, and international agenda. At first, IBD was worked as technology transfer hub and cell banking of the recombinant strain and improved the process of large scale production of TMOF by recombinant *P. pastoris*. Second, a new process for high cell mass production for *Bacillus thuringiensis var israelensis* was developed to be used as complementary active ingredient in the final product formula. Third, IBD acted as final product formulation laboratory for the production of wettable powder form and rice husk based formula for field trial. After all these many years successful cooperation, we act as large scale production platform for the company to cope with the world wide growing demand of the product and we work closely with the company to establish a larger scale production facility for bioactive ingredient production and formulation to cope with the high demand of both local and international markets.