Optimization of growth conditions of *Bacillus thuringiensis* isolates from various sources in Kenya and toxicity assays of their delta-endotoxin against *Chilo partellus*

Daniel Anyika Kwalimwa

A Thesis submitted in partial fulfilment for the Degree of Master of Science in Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2012

ABSTRACT

Chemical pesticides have disadvantages such as high production costs, short persistence, comparative low efficacy, development of resistance to toxin and causing ecological damage. In order to obtain cheaper biopesticides, many locally available and inexpensive agricultural/industrial byproducts have potential as culture media for *Bacillus thuringiensis* Crystal protein production. In this study, cost-effective media were developed, based on locally available raw materials namely legumes, potato, and whey. Optimization studies indicate that pH 6.4-7.5 was best for sporulation and OD 600 was highest at 37 °C for all isolates tested. The optical density, protein concentration yield, sporulation and *Chilo partellus* larvicidal action were studied by growing bacterial strains in these waste product and in comparison with the conventional medium (NYSM). Protein concentration yield of 27.60 µg/ ml, spore count of 5.60×10^8 and *Chilo partellus* larvicidal activity (LC_{50}) of 78 µg/ l against first-instar larvae were obtained with a 72 h culture of this bacterium. Based on media comparison between NYSM and other media, the legumes produced the highest spore counts, followed by potato and then whey; and, differences between media treatments were significantly different ($P \leq 0.05$). The SDS-PAGE profiles indicated that spore-crystal product from each treatment consisted of proteins with molecular weights of approximately 110-120 kDa and 60-70 kDa, suggesting the presence of bacterial insecticidal protoxins. Therefore the investigation suggests that legume, potato and whey-based culture media are more economical for the industrial production of *Bt* Insecticidal Crystal Proteins.