

## Improved Genetically Engineered Mosquitocidal Bacteria for Vector Control

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### Abstract

There is an urgent need for new agents and strategies to control mosquito vectors of diseases such as malaria, filariasis, and virus encephalitides. The larvicidal bacteria *Bacillus sphaericus* (Bs) and *B. thuringiensis* subsp. *israelensis* (Bti) hold promise, but use is limited by their high cost. Moreover, mosquito resistance has evolved rapidly to Bs where used intensively. Toxicity of these bacteria is due, respectively, to a single binary protein (BsB) in Bs, and four proteins in Bti, Cry4A, Cry4B, Cry11A, and Cyt1A. Cyt1A is of particular importance because it synergizes Cry toxicity and delays resistance to these. Additionally, Cyt1A suppresses high levels of resistance to Bs and expands its target spectrum. Recently, we used *cyt1A* promoters and a 5' mRNA stabilizing (STAB-SD) sequence to synthesize high levels of the Bs2362 binary toxin in acrySTALLIFEROUS (4Q7) and crystalliferous (IPS-82) strains of Bti. The BtiIPS-82/BsB recombinant ( $LC_{50} = 0.37$  ng/ml) was the most toxic of these against fourth instars of *Culex quinquefasciatus*, a vector of West Nile virus, making it 21 times as toxic as BtiIPS-82 ( $LC_{50} = 8.1$  ng/ml), and 32 times as toxic as Bs2362 ( $LC_{50} = 11.9$  ng/ml). Moreover, BtiIPS-82/BsB completely suppressed extremely high levels of resistance (>100,000-fold) to Bs2362 in *Cx. quinquefasciatus*. Against *Aedes aegypti* and *Anopheles albimanus*, BtiIPS-82/BsB was approximately twice as toxic as BtiIPS-82. The latter two species are not sensitive to the Bs Bin toxin, and this likely accounts for the lower levels of improvement over the wild type bacteria. However, these recombinants should prove highly effective against species such as *An. gambiae* and *An. arabiensis*, which are sensitive to Bs Bin. In general, these and other new recombinant bacterial insecticides should be highly effective against *Culex* and *Anopheles* vectors and much less prone to resistance due to their high toxicity and endotoxin complexity combined with Cyt1A's synergistic and resistance-delaying properties.