Chapter 2.3

Vector-active toxins: structure and diversity

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Abstract: Bacteria active against Dipteran larvae – mosquitoes and blackflies – include a wide variety of Bacillus thuringiensis and Bacillus sphaericus strains, as well as isolates of Brevibacillus laterosporus and Clostridium bifermentans. All display different spectra and levels of activity correlated with the nature of the toxins produced during the sporulation process. This paper presents an overview of all mosquitocidal strains reported to date and describes the numerous toxins – including both Cry and Cyt proteins, and others – in terms of primary structure and activity against mosquito larvae.

1. INTRODUCTION

Mosquitoes and blackflies (Diptera: Nematocera) are responsible for many important tropical diseases such as malaria, yellow fever, dengue and filarial conditions including onchocerciasis. The widespread use of chemical insecticides to control these insects has led to the development of many resistant populations. Therefore, much attention has been given to more environmentally friendly insecticides, and particularly to the use of microbial control agents that cause disease in the target insects.

Entomopathogenic bacteria, namely Bacillus thuringiensis, have been known from the early 1900’s (see Chapter 2.2), but the control of Dipteran species has been envisaged only since the discovery of B. thuringiensis serovar israelensis. The high activity of Bt israelensis led...
to an increased interest in another bacterium, *B. sphaericus*, for which, initially, only weakly active strains were known. Since that time, several screening programmes, aimed at isolating different mosquitocidal strains, have been developed. These have resulted in the identification of a wide variety of Gram-positive bacteria, including both *B. thuringiensis* and *B. sphaericus* isolates and new bacteria, for which no insecticidal activity had been reported previously: *Brevibacillus laterosporus* and *Clostridium bifermentans*.

All strains isolated have been characterised for their level of mosquitocidal activity and specificity. For most of these bacteria, the factors responsible for the insecticidal activity have then been identified and characterised. As shown below, although not all is known about these mosquitocidal bacteria, many Cry, Cyt, and other toxins – differing in their primary structure, specificity and level of activity – have now been characterised.

2. DIPTERAN ACTIVE BACTERIA

2.1 *Bacillus thuringiensis* strains

The first mosquitocidal *B. thuringiensis* strain was isolated in 1976 from dead *Culex* larvae [39], serotyped as H14 [6], and designated serovar *israelensis* (*Bti*). Composite crystals, produced from stage II of sporulation, are responsible for toxicity. These inclusions show specificity for larval stages of many Culicidae (mosquito) genera: *Culex, Aedes, Anopheles*, with a higher specificity for the two former [39]; crystals are also toxic against Simuliidae [114], Tipulidae [116] and Chironomidae [91] larvae. In contrast, they have no effect on vertebrates and non-target invertebrates [68, 99]. However, upon solubilisation, the crystals display non-specific cytolytic and haemolytic activities [112]. The *Bti* inclusions are composed of four major proteins with molecular weight of 135, 125, 68, and 28 kDa.

Since the discovery of *Bti*, several other *Bt* strains displaying mosquitocidal activity have been isolated [51, 66, 88]. These strains, differing from *Bti* by either their serotype, mosquitocidal activity or polypeptide composition, can be classed into 3 groups (Table 1, page 123): class 1 includes 8 strains with larvicidal and haemolytic activities as well as crystal polypeptides similar to those of *Bti*; class 2 contains 2 strains which are nearly as toxic as *Bti* but produce different polypeptides; class 3 includes 9 strains which synthesise polypeptides different from those found in *Bti* but are only weakly active. On