The susceptibility of five African Anopheles species to Anabaena PCC 7120 expressing Bacillus thuringiensis subsp. israelensis mosquitocidal cry genes

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Abstract

Background: Malaria, one of the leading causes of death in Africa, is transmitted by the bite of an infected female Anopheles mosquito. Problems associated with the development of resistance to chemical insecticides and concerns about the non-target effects and persistence of chemical insecticides have prompted the development of environmentally friendly mosquito control agents. The aim of this study was to evaluate the larvicidal activity of a genetically engineered cyanobacterium, Anabaena PCC 7120#11, against five African Anopheles species in laboratory bioassays.

Findings: There were significant differences in the susceptibility of the anopheline species to PCC 7120#11. The ranking of the larvicidal activity of PCC 7120#11 against species in the An. gambiae complex was: An. merus < An. arabiensis < An. gambiae < An. quadriannulatus, where < indicates a statistically lower LC50. The LC50 of PCC 7120#11 against the important malaria vectors An. gambiae and An. arabiensis was 12.3 × 10^5 cells/ml and 8.10 × 10^5 cells/ml, respectively. PCC 7120#11 was not effective against An. funestus, with less than 50% mortality obtained at concentrations as high as 3.20 × 10^5 cells/ml.

Conclusions: PCC 7120#11 exhibited good larvicidal activity against larvae of the An. gambiae complex, but relatively weak larvicidal activity against An. funestus. The study has highlighted the importance of evaluating a novel mosquitocidal agent against a range of malaria vectors so as to obtain a clear understanding of the agent’s spectrum of activity and potential as a vector control agent.

Keywords: Malaria vectors, Anopheles, Bacillus thuringiensis subsp. israelensis, Cry proteins, Cyanobacteria, Anabaena sp. PCC 7120, Genetic engineering, Bioassays, Larvicidal activity

Background

Species within the genus Anopheles (Diptera: Culicidae) play a major role in the transmission of malaria in Africa, in particular mosquitoes from the An. gambiae complex and the An. funestus group [1,2]. The An. gambiae complex contains excellent and efficient vectors of malaria (An. gambiae s.s. and An. arabiensis), as well as minor vectors (An. merus) and non-vectors (An. quadriannulatus species A and B) [1]. The An. funestus group contains an important vector of malaria, An. funestus s.s. [2].

Although chemical insecticides have been used successfully in integrated vector control programs [3], many malaria vector control programs are hampered by the development of resistance of the vectors to chemical insecticides [4-6]. In addition to development of resistance, concerns about the non-target effects and persistence of the chemical insecticides have prompted the development of environmentally friendly control agents and control programs [7]. Bacillus thuringiensis subsp. israelensis (Bti) is a Gram-positive, aerobic, spore-forming, bacterium that produces crystalline inclusions that contain crystal (Cry) or cytolytic (Cyt) proteins that are highly toxic to
mosquito larvae [8,9]. Although there is low risk of resistance being developed to Bti [10], there are several disadvantages to using Bti as a control agent [11,12]. These include its low persistence in the field due to inactivation by UV, ingestion of Bti by other aquatic organisms, and the settling of Bti from the mosquito larval feeding zone [11-13]. One strategy to overcome some of the disadvantages of Bti is to clone the cry genes of Bti into aquatic microorganisms that: (1) are not toxic to other organisms, (2) inhabit and persist in the larval feeding zone, (3) are used by mosquito larvae as a food source, (4) express Cry proteins at levels that are mosquitocidal, and (5) have cell walls that reduce inactivation of the Cry proteins by UV [13-15].

Xiaoqiang et al. [15] inserted the Bti cry4Aa, cry11Aa, and p20 genes under the control of two tandem promoters (cyanobacterial constitutive promoter, PpbA, and Escherichia coli T7 early promoter, PAl) into a filamentous nitrogen-fixing cyanobacterium, Anabaena sp. strain PCC 7120 (PCC 7120). The Bti genes are integrated into the chromosome of PCC 7120, resulting in a stable recombinant strain [16]. Laboratory bioassays have shown that the resultant recombinant strain, PCC 7120#11, is a very effective larvicidal agent against Aedes aegypti [13,15,16].

To our knowledge, no studies have examined the larvicidal activity of PCC 7120#11 against several African malaria vectors. The aim of this study was, thus, to evaluate the larvicidal activity of PCC 7120#11 against five African Anopheles species in order to determine if PCC 7120#11 may have potential as a malaria vector control agent.

Methods

Larvicidal activity of PCC 7120#11 was determined by laboratory bioassays against four species in the An. gambiae complex and one species in the An. funestus group. The An. gambiae complex species used in the study were (origin, colony name, and colonisation date provided): An. gambiae s.s. (Ibadan, Nigeria; NAG; 2001), An. arabiensis (Kanyemba, Zimbabwe; KGB; 1975), An. merus (KwaZulu-Natal, South Africa; MAF; 1988), and An. quadriannulatus species A (Sangwe, Zimbabwe; SANGWE; 1998). The species from the An. funestus group that was used in the study was An. funestus s.s. (Maputo, Mozambique; FUMOZ; 2000).

The anopheline mosquito species were obtained from colonies maintained at the National Institute of Communicable Diseases (Johannesburg, South Africa). Since the activity of PCC 7120#11 against A. aegypti had been previously evaluated [15], we included it as a control in the bioassays. The A. aegypti larvae were obtained from the South African Bureau of Standards (Pretoria, South Africa).

PCC 7120 and PCC 7120#11, were cultured in BG-11 medium [15], at 30°C under continuous illumination (2000 lux) with constant agitation [13,16]. The PCC 7120 and PCC 7120#11 cells were harvested by differential centrifugation and the cell concentration was determined by haemocytometer counts. Two millilitres of the appropriate dilution (covering an in-cup concentration range of 1.00 × 10⁴ to 3.20 × 10⁷ cells/ml) of either PCC 7120 or PCC 7120#11 was added to 130 ml plastic cups that contained 98 ml sterile distilled water and 20 third-instar mosquito larvae. In the case of An. merus, a sterile 5 M saline solution was used instead of sterile distilled water.

Larvicidal activity was determined 24 hours post-inoculation, with larvae presumed dead if they did not move when prodded. An untreated control (sterile distilled water) was included in the bioassays. If mortality in the controls (PCC 7120 and untreated) exceeded 5%, the test was discarded and repeated. Each bioassay was repeated in triplicate on different days. The lethal concentration (LC₅₀ and LC₉₀) for each species was determined by probit analysis [17]. For each species, probit analysis was based on the mortality data obtained from five PCC 7120#11 concentrations.

Results and discussion

The concentration-mortality data for the mosquito species are summarised in Table 1. The heterogeneity factors for the different mosquito species evaluated were all less than one, indicating a good fit of the concentration-mortality data to the probit model [18].

The concentration-mortality regression slopes indicate the variability in response to a toxin within the vector population being examined [19]. In this study, An. arabiensis had a significantly steeper slope than the other species evaluated, suggesting that An. arabiensis had lower response variability or reduced heterogeneity in its population compared to the other mosquito species examined. The shallower slopes of the concentration-mortality regression lines obtained for An. gambiae and An. quadriannulatus mean that there are larger differences between the LC₅₀ and LC₉₀ values for these species than for the other anopheline species evaluated.

Although slight variation in LC₅₀ values between studies may be expected due to differences in experimental conditions such as rearing conditions of the larvae and natural variations in the larval populations [20], the susceptibility of A. aegypti larvae to PCC 7120#11 in this study was comparable to that (LC₅₀ of 0.9 × 10⁵ cells/ml) previously reported by Xiaoqiang et al. [15]. However, the LC₅₀ value of PCC 7120#11 against A. aegypti larvae was significantly lower than those of the anopheline species evaluated (Table 1). The decreased susceptibility of anopheline larvae compared to A. aegypti larvae.
may be due to differences in feeding behaviour [21]; Anophelinae larvae (surface feeders) may ingest fewer PCC 7120#11 cells than An. funestus larvae (surface feeders) may ingest fewer PCC 7120#11 cells than Anopheles adults and the larvae of other An. funestus adults and the larvae of other malaria vectors. However, before PCC 7120#11 can used in vector control programs, its effects on non-target organisms and its persistence in aquatic environments would have to be comprehensively evaluated.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Conceived the idea: GB. Performed the experiment and analyzed the data: IK and GB. Contributed reagents/materials/analysis tools: GB. Wrote the manuscript: IK. Clarified the manuscript: GB. All authors read and approved the final manuscript.

Acknowledgements
The authors thank M. Coetzee and L.L. Koekemoer (Vector Control Reference Unit, National Institute for Communicable Diseases of the National Health

Table 1 Probit analysis of concentration-mortality data for Anabaena PCC 7120#11 against third instar mosquito larvae

| Species          | LC50 (10⁵ cells/ml)* | LC90 (10⁵ cells/ml)* | Slope ± SE†  | Heterogeneity‡ |
|------------------|----------------------|----------------------|---------------|-----------------|
| A. aegypti       | 1.42 (1.12-1.76)*    | 8.21 (6.05-12.3)*    | 1.70±0.15*    | 0.75            |
| An. merus        | 3.90 (3.58-4.17)b    | 9.30 (8.0-11.4)b     | 3.37±0.31b    | 0.62            |
| An. arabiensis   | 8.10 (7.62-8.56)c    | 14.3 (12.8-16.5)c    | 5.18±0.46c    | 0.19            |
| An. gambiae      | 12.3 (11.4-13.3)d    | 35.1 (29.5-44.9)d    | 2.81±0.24d    | 0.49            |
| An. quadriannulatus | 15.7 (14.3-17.3)e  | 43.0 (35.9-54.9)e    | 2.93±0.26e    | 0.92            |
| An. funestus     | N.D.                 | N.D.                 | N.D.          | N.D.            |

* Values in brackets show the 95% fiducial limits (FLs). Values in a column followed by the same letters are not significantly different (overlapping 95% FLs).
† Slope ± standard error. Values followed by the same letters are not significantly different (p > 0.05).
‡ Heterogeneity factor = χ² / d.f. (degrees of freedom).
§ Not determined. Less than 50% mortality was obtained even at concentrations as high as 3.2 × 10⁷ cells/ml.

The authors thank M. Coetzee and L.L. Koekemoer (Vector Control Reference Unit, National Institute for Communicable Diseases of the National Health...
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Cite this article as: Ketseoglou and Bouwer: The susceptibility of five African Anophelinae species to *Anabaena* PCC 7120 expressing *Bacillus thuringiensis* subsp. *israelensis* mosquitocidal cry genes. *Parasites & Vectors* 2012, 5:220.