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A NEW SEROVAR OF *BACILLUS THURINGIENSIS* POSSESSING 28a28c FLAGELLAR ANTIGENIC STRUCTURE: *BACILLUS THURINGIENSIS* SEROVAR *JEGATHESAN*, SELECTIVELY TOXIC AGAINST MOSQUITO LARVAE

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ABSTRACT. A novel *Bacillus thuringiensis* strain highly toxic to mosquitoes was isolated from soil samples in Malaysia. This strain was shown to display a new subfraction of the H-28 flagellar antigen determining a new serovar H28a28c, which was designated serovar *jegathesan*. Bioassays indicated that *Culex quinquefasciatus* larvae are the most susceptible to this new isolate, whereas toxicity to *Anopheles maculatus* and *Aedes aegypti* larvae was 10 times lower. The potency of this new serotype is also comparable to most of the Malaysian *B. thuringiensis* H-14 isolates.

Microbial control agents of mosquitoes, *viz.*, *Bacillus thuringiensis* (Berliner) and *Bacillus sphaericus* (Neide), are gaining widespread acceptance in the control of pests and vectors of diseases, especially mosquitoes, principally due to the widespread occurrence of chemical insecticide contamination and the development of resistance in insects.

The World Health Organization has recommended that control agents from indigenous microbial strains are more desirable ecologically and economically (considering feasibility of production) in developing countries (World Health Organization 1982). Therefore, in 1986 a nation-wide screening program to search for potential microbial control agents of mosquitoes was initiated in Malaysia. To date, 876 soil and water samples have been collected from the various ecological habitats in Malaysia. The techniques used to isolate mosquitocidal bacteria from these samples were previously described (Lee and Seleena 1990). A total of 3,922 bacterial colonies were isolated from these samples and screened for larvicidal activity against laboratory-bred *Aedes aegypti* (Linn.), *Culex quinquefasciatus* Say, and *Anopheles maculatus* Theobald larvae.

From these screenings, 29 larvicidal *B. thuringiensis* isolates were obtained, of which 26 belonged to serotype H-14 and one isolate each belonged to serotypes H-7 and H-8a8b.

However, the flagellar (H) antigen of one isolate (no. 367) was not identical to all known *B. thuringiensis* serotypes. This strain, isolated from a soil sample collected from a roadside pool in northern Peninsular Malaysia, was found to share a common antigen with *B. thuringiensis* serovar *monterrey* (H-28a28b), but it possessed a different antigenic structure, as deduced from the values of agglutinating titers observed with the usual procedure (de Barjac 1981). The H antigenic structure for the strain no. 367 was serotyped as H-28a28c at the Pasteur Institute (WHO Collaborating Centre for Entomopathogenic Bacillus). This new strain was subsequently designated *Bacillus thuringiensis* serovar *jegathesan* (*B.t.j.*), and it is the reference strain for this new serovar (strain N° T28A001 in the IEBC Collection). The biochemical characters were as follows: degradation of glucose, starch, gelatin; no degradation of inulin, lactose, mannitol, raffinose, xylose; production of acetylmethyl-carbinol, lecithinase, proteases; no production of β-galactosidase, urease, arginine-dihydrolase. These properties are those already reported for *B. thuringiensis* serovar *israelensis* (*B.t.i.*), with the exception that *B.t.i.* produces arginine dihydrolase (de Barjac and Frachon 1990).

The larvicidal toxicity of this new strain was determined by conducting bioassays using lyophilized powder of the lysed bacteria, according to the WHO protocol (de Barjac and Larget-Thiéry 1982). The isolate grown on nutrient agar at 32°C for 48 h was scraped and suspended in sterile water and further incubated at 32°C for 48 h. The lysed bacterial cell suspension was then lyophilized for 8 h until a dry powder was obtained. A stock solution was prepared by suspending 50 mg of the powder in 10 ml distilled water. Further test dilutions were prepared from this stock. Bioassays were conducted in waxed paper cups containing 150 ml of test solutions in various concentrations. Twenty-five 3rd–4th-instar laboratory-bred larvae of test species were then added to each cup. Each series of bioassays was replicated 3 times on different days. The mortality of the larvae was determined after 24 h and 48 h continuous exposure. A personal

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Table 1. Larval toxicity of *Bacillus thuringiensis* serotype H-28a28c.

Mosquito species	LC_{50} (mg of dry powder/liter) (95% CL) 24 h	48 h
Aedes aegypti Bora-bora	0.047[1] (0.042–0.052)	0.027 (0.019–0.038)
Culex quinquefasciatus	0.005 (0.004–0.006)	0.004 (0.003–0.006)
Anopheles maculatus	0.059 (0.048–0.07)	0.032 (0.025–0.042)

[1] The L_{50} value for IPS82, the international standard for *B. thuringiensis* serovar *israelensis*, to *Ae. aegypti* Bora-bora is 0.0036 (0.0033–0.0088).

computer programmed with probit analysis as described by Raymond (1985) was used to analyze the data. For comparison purposes IPS82, the standard *B. thuringiensis* H-14, was also produced in the same manner as *B.t.j.* and bioassayed against *Ae. aegypti* larvae.

The larval toxicity of *B.t.j.* is presented in Table 1. *Culex quinquefasciatus* larvae were the most susceptible with *Ae. aegypti* Bora-bora and *An. maculatus* larvae being about 10 times less susceptible than *Cx. quinquefasciatus* larvae. The trend of susceptibility of the mosquito larvae to *B.t.j.* is similar to Malaysian larvicidal *B. thuringiensis* H-14 isolates (Lee and Seleena, unpublished data). The potency of this new serotype is also comparable to most of the Malaysian *B. thuringiensis* serotype H-14 isolates (Table 2), a serotype among the Malaysian isolates that shows a higher preferential toxicity to mosquito larvae than other *B. thuringiensis* serotypes. *Bacillus thuringiensis* serovar *jegathesan* (1,150 ITU) is about 10 times less toxic to *Ae. aegypti* larvae than is IPS82 (15,000 ITU).

Table 2. Susceptibility of indigenous *Aedes aegypti* Bora-bora larvae to Malaysian isolates of *Bacillus thuringiensis* serovar *israelensis* (H-14).

Isolate[1]	LC_{50} (mg of dry powder/liter) (95% CL) 24 h exposure
IMR-BT-1	0.0063 (0.0055–0.0069)
IMR-BT-2	0.018 (0.016–0.022)
IMR-BT-3	0.152 (0.14–0.16)
IMR-BT-4	0.0015 (0.0014–0.0017)
IMR-BT-7	0.0062 (0.0057–0.0067)
IMR-BT-10	0.025 (0.024–0.028)
IMR-BT-11	0.01 (0.009–0.011)
IMR-BT-15	0.04 (0.036–0.044)
IMR-BT-25	0.039 (0.034–0.042)
IMR-BT-27	0.066 (0.062–0.07)
IMR-BT-29	0.039 (0.035–0.043)
IPS82[2]	0.0036 (0.0033–0.0088)

[1] *B. thuringiensis* serovar *israelensis* isolated from soil samples collected from the various ecological habitats in Malaysia.
[2] International standard for *B. thuringiensis* serovar *israelensis* potency.

The possible production of thermostable exotoxin by *B.t.j.* was examined on housefly (*Musca domestica* Linn.) larvae, using the filter paper technique (Ohba et al. 1981), but it was not detected as there was no abnormality in the emerged adult houseflies.

The 24-, 48-, and 72-h final whole cultures of strain N° 367 in nutrient broth (Difco) were fed to German cockroach (*Blattella germanica* Linn.) and *M. domestica* adults. No mortality was observed even after 10 and 3 days of continuous feeding, respectively. Thus, *B.t.j.* is similar to *B.t.i.* and *B. thuringiensis* serovar *morrisoni*, strain PG14, in that both serotypes are toxic to mosquito larvae but do not exhibit any toxicity to other insects such as the housefly and cockroach.

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