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EVALUATION OF ENTOMOPATHOGENIC BACTERIA AGAINST *AEDES POLYNESIENSIS*, THE VECTOR OF LYMPHATIC FILARIASIS IN FRENCH POLYNESIA

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ABSTRACT. Thirteen strains among 3 species of entomopathogenic bacteria were tested against 3 medically important mosquito species in French Polynesia. Two strains of *Bacillus thuringiensis* were highly toxic to *Aedes polynesiensis, Aedes aegypti,* and *Culex quinquefasciatus.* Six of 7 strains of *Bacillus sphaericus* tested were highly toxic to *Cx. quinquefasciatus* but not to the *Aedes* spp. *Clostridium bifermentans* serovar. *malaysia* was more toxic to *Ae. polynesiensis* than to the other 2 species. Entomopathogenic bacteria merit field testing for larval mosquito control in French Polynesia.

Entomopathogenic bacteria, which act as gut poisons following larval ingestion, may provide environmentally safe mosquito control for French Polynesia in situations where larval control is feasible. Two bacteria, *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus,* have been used safely and effectively in the control of mosquitoes worldwide (Nicolas 1992, Porter et al. 1993). Newly discovered *Bacillus* strains or *Clostridium bifermentans* serovar. *malaysia* (de Barjac et al. 1990) and bioengineered (Porter et al. 1993) mosquitocidal bacteria may provide novel toxins, greater persistence, higher toxicity, or a broader spectrum of activity. *Clostridium bifermentans* serovar. *malaysia* has also been proven innocuous for nontarget arthropods and vertebrates (Thiery et al. 1992a).

The 3 most important mosquito species in French Polynesia are *Aedes polynesiensis* Marks, *Aedes aegypti* (Linn.), and *Culex quinquefasciatus* Say. *Aedes polynesiensis,* rural in distribution and widespread throughout the islands, is the main vector in French Polynesia of *Wuchereria bancrofti,* the causative agent of human lymphatic filariasis, and of *Dirofilaria immitis,* a filarial parasite of dogs. *Aedes aegypti,* a local vector of dengue, and *Cx. quinquefasciatus,* a secondary vector of *W. bancrofti* in French Polynesia, are more urban.

Mosquito control in French Polynesia is based upon insecticides. With increasing agricultural use of pesticides in Polynesia, resistance and environmental pollution are likely to become greater problems in vector control (Failloux et al. 1994). Therefore, we evaluated the toxicities of 13 bacterial strains, most of them novel, against *Ae. polynesiensis.* Because there is no reference strain available for insecticide trials using *Ae. polynesiensis,* susceptibilities were compared with local strains of *Ae. aegypti* and *Cx. quinquefasciatus,* species that have been more thoroughly investigated worldwide.

Larvae of *Ae. polynesiensis* (Raiatea strain, 2nd–4th laboratory generations), *Ae. aegypti* (Tahiti strain, >20 laboratory generations), and *Cx. quinquefasciatus* (Tahiti strain, 1st–2nd laboratory generations) were used. Mosquito larvae of each species were reared at ambient temperature (27 ± 3°C) and 12:12 (L:D) h photoperiod until the late 3rd-instar as described in Failloux et al. (1994).

All *B. thuringiensis* and *B. sphaericus* strains (Table 1) were provided as lyophilized stock powders by The Institut Pasteur, Paris. *Bacillus thuringiensis* strains were cultured in UG medium supplemented with 1% (wt/vol) glucose (de Barjac and Lecadet 1976) and *B. sphaericus* strains were cultured in MBS medium (Kalfon et al. 1983) with shaking at 30°C.

Final whole cultures were centrifuged after 48 or 72 h, when more than 90% of the cells had sporulated, and the bacterial pellets were washed with 1 M NaCl and twice with double-distilled water. Spore counts for both *Bacillus* spp. were performed by plating a series of 10-fold dilutions of final whole cultures, previously heat-shocked, onto solid media. A culture of *C. bifermentans* serovar. *malaysia* was previously fermented under anaerobic conditions and lyophilized at The Institut Pasteur (Thiery et al. 1992b).

Twenty-five late 3rd-instar larvae were placed in 250-ml plastic cups containing 150 ml deionized water and liver powder as a food source (7.5 mg for *Ae. polynesiensis* and *Cx. quinquefasciatus,* and 12.5 mg for *Ae. aegypti*). For each bacterial strain, 6–10 dilutions of bacterial suspension and 50 or 75 larvae per dilution were assayed at room temperature (27 ± 3°C). Mor-

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Table 1. Bacterial strains evaluated for larvicidal activity against *Aedes polynesiensis*.

Bacterial species	H serotype	Subspecies	Strain
B. thuringiensis	14	*israelensis*	1884
	30	*medellin*	163–131
	10a, 10b	*darmstadiensis*	73E10-2
	28a, 28c	*jegathesan*	367
	3a, 3d, 3e	*fukuokaensis*	Bang 302.3
B. sphaericus	5a, 5b		2362
	5a, 5b		1593
	5a, 5b		1691
	5a, 5b		1601
	5		Mal
	3		IAB 881
	25		2297
C. bifermentans		*malaysia*	CH18

tality was recorded after 48 h of exposure to bacterial suspensions. Bioassays with greater than 10% mortality in the controls (with no bacterial suspension) were discarded. Values for LC_{50} and LC_{90} (spores/ml) were determined at 48 h using the log-probit program of Raymond (1993). Results from at least 3 bioassays were used to calculate mean values for LC_{50} and LC_{90}.

The toxicities of *B. thuringiensis* strains and *C. bifermentans* serovar. *malaysia* against *Ae. polynesiensis* (LC_{50} and LC_{90} at 48 h, plus susceptibilities relative to *Ae. aegypti* and *Cx. quinquefasciatus*) are listed in Table 2. *Bacillus thuringiensis* subsp. *israelensis* showed the highest toxicity toward *Ae. polynesiensis*. However, 3 other *B. thuringiensis* strains (*medellin, darmstadiensis,* and *jegathesan*) were also very toxic. *Aedes polynesiensis* was 3 times more susceptible (LC_{50} at 48 h) than *Ae. aegypti* to *B. thuringiensis* subsp. *israelensis, medellin,* and *darmstadiensis*. By contrast, *Ae. polynesiensis* was less susceptible than *Cx. quinquefasciatus* to 3 *B. thuringiensis* strains (Table 2). None of the 3 mosquito species tested was susceptible to *B. thuringiensis* subsp. *fukuokaensis* (data not shown). Interestingly, *Ae. polynesiensis* was susceptible to the novel bacterium *C. bifermentans* serovar. *malaysia* (2.5 times and 11 times more susceptible than *Ae. aegypti* and *Cx. quinquefasciatus,* respectively, Table 2).

Aedes polynesiensis, like *Ae. aegypti,* was not susceptible to 6 of the 7 *B. sphaericus* strains tested ($LC_{50} > 10^4$ spores/ml). *Bacillus sphaericus* strain 1593 was moderately toxic ($LC_{50} = 2.1 \times 10^3$ spores/ml) to *Ae. polynesiensis,* an activity 500 times lower than against *Cx. quinquefasciatus.* All *B. sphaericus* strains were highly toxic against the Tahitian strain of *Cx. quinquefasciatus,* consistent with data published for other geographic strains.

Table 2. Toxicity of bacteria against *Aedes polynesiensis*.

Bacterial subspecies	Strain	*Ae. polynesiensis* LC_{50} 48 h[1]	*Ae. polynesiensis* LC_{90} 48 h[1]	LC_{50} 48 h *Ae. aegypti* ÷ LC_{50} 48 h *Ae. polynesiensis*	LC_{50} *Cx. quinquefasciatus* ÷ LC_{50} *Ae. polynesiensis*
B. thuringiensis					
israelensis	1884	24 ± 2.2[2]	65 ± 12	3.0	0.5
medellin	163–131	47 ± 10	200 ± 72	1.5	0.5
darmstadiensis	72E10-2	82 ± 19	170 ± 51	3.7	0.8
jegathesan	367	270 ± 33	530 ± 37	3.4	1.1
C. bifermentans					
malaysia	CH18	250 ± 63	1,100 ± 540	2.5	10.8

[1] Spores/ml.
[2] Mean ± SE.

Table 1. Extended.

Origin	Reference or isolator
Israel	Goldberg and Margalit (1977)
Colombia	Orduz et al. (1992)
Japan	Padua et al. (1980)
Malaysia	Lee (1991, unpublished data)
Central African Rep.	Institut Pasteur (1990, unpublished data)
Nigeria	Weiser (1984)
Indonesia	Singer (1973)
El Salvador	Singer (1977, unpublished data)
Canada	Chung (1989, unpublished data)
Malaysia	University of Malaysia (1986, unpublished data)
Ghana	Thiery et al. (1992c)
Sri Lanka	Wrickremesinghe and Mendis (1980)
Malaysia	de Barjac et al. (1990)

Several larvicides based upon *B. thuringiensis* subsp. *israelensis* are commercially available and could be field tested against *Ae. polynesiensis*. Interestingly, other strains that produce novel toxins, particularly *B. thuringiensis* subsp. *medellin* (Orduz et al. 1994) and *C. bifermentans* serovar. *malaysia* (Nicolas et al. 1993), are also active against *Ae. polynesiensis*. These could provide additional tools for larval control if products based upon these novel toxins or bacterial strains are developed either as native strains or recombinant bacteria.

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