MOSQUITO HOST RANGE AND FIELD ACTIVITY OF *BACILLUS SPHAERICUS* ISOLATE 2297 (SEROTYPE 25)

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ABSTRACT. The 2297 isolate (serotype 25) of *Bacillus sphaericus* was bioassayed in the laboratory against 8 species of mosquitoes from 3 subfamilies. The most susceptible species were in the genus *Culex* and the least susceptible were the *Aedes* spp. and *Toxorhynchites r. rutilus.* Primary powders of the 2297 and 2362 (serotype 5a5b) isolates were evaluated in the field in natural and simulated habitats against *Culex* spp. The larvicidal activity of the two isolates was similar, with longer residual activity observed for both preparations in shaded shallow clear water. Larvicidal activity was curtailed in organically enriched and unshaded habitats. Isolate 2297 provided effective control for at least 1 week in an organically enriched habitat and for over 5 weeks in clear water in a shaded habitat when applied at the rate of 0.25 kg/ha.

INTRODUCTION

Since the isolation of the K strain of *Bacillus sphaericus* Neide by Kellen et al. (1965), numerous other strains have been discovered with far greater mosquito larvicidal activity. Most of the isolates of *B. sphaericus* with the highest larvicidal activity are in the 5a5b serotype (de Barjac et al. 1985). Several studies have elucidated the host range and field activity of these isolates (reviewed by Davidson 1985). In some situations *B. sphaericus* is more efficacious against *Culex* and *Psorophora* species than the commercially produced *Bacillus thuringiensis* var *israelensis* (Lacey et al. 1986, Mulla et al. 1985). The other advantages of *B. sphaericus:* persistence, ability to recycle and selectivity, have been summarized by Davidson (1985). Its main disadvantage is its relatively narrow mosquito host range; it is virtually inactive against *Aedes aegypti* (Linn.) and certain other *Aedes* spp. Accordingly, continued search for new strains is germane to the improvement of host range and efficacy.

The 2297 isolate from Sri Lanka demonstrated promising larvicidal activity against *Culex quinquefasciatus* Say larvae (Wickremesinghe and Mendis 1980) and *Culex pipiens* Linn. (de Barjac and Charles 1983). This isolate is unique in that it represents a new serotype (H-25) and apparently a new toxin, and its sporangia contain conspicuously larger parasporal inclusions than those reported for other serotypes (Yousten and Davidson 1982, de Barjac and Charles 1983). Preliminary investigations, unfortunately, indicate that it lacks significant activity towards *Ae. aegypti* and certain other *Aedes* spp. (de Barjac and Charles 1983, Gardner et al. 1986). This study was initiated to elucidate the host range of 2297 and to document its field activity compared to that of the 2362 isolate (serotype 5a5b).

METHODS AND MATERIALS

A lyophilized preparation of the 2297 isolate, produced at the Pasteur Institute, was bioassayed against 8 species of mosquitoes from three subfamilies using the procedures described by Lacey (1983, 1984). Forty-eight-hr-old larvae of *Anopheles quadrimaculatus* Say, *Anopheles albimanus* Weidemann, *Ae. aegypti, Aedes taeniorhynchus* (Weidemann), *Aedes triseriatus* (Say), *Cx. quinquefasciatus, Culex salinarius* Coquillett and *Toxorhynchites r. rutilus* (Coquillett) were obtained from colonies at the Insects Affecting Man and Animals Laboratory (IAMARL). With the exception of the tests conducted using *Tx. r. rutilus,* the bioassay procedure consisted of exposing second instar larvae to 5–7 concentrations of the primary powder for 48 hrs. at 27°C in waxed paper cups containing 100 ml of deionized water. Three cups of 20 larvae were used for each concentration and control. Prior to the addition of serially diluted suspensions of 2297, the larvae were provided with 50 mg of debittered brewer's yeast/cup. Mortality was assessed after the 48 hr exposure period. At least 3 repetitions of the tests were run on separate dates for each species. The bioassays conducted with *Tx. r. rutilus* differed from the aforementioned protocol in that the larvae were individually placed in 30 ml cups containing the appropriate concentration of 2297 and ca. 20 late

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1st or early 2nd instar *Ae. aegypti* that had been exposed to the bacterium for 30 min prior to adding the *Tx. r. rutilus*. Ten *Tx. r. rutilus* larvae/concentration and control were utilized for each of the 3 tests. Mortality was determined after 48 hours.

Comparative bioassays were also conducted with *An. quadrimaculatus, Cx. quinquefasciatus,* and *Tx. r. rutilus* using the two protocols described above and a lyophilized preparation of the 1593 isolate of *B. sphaericus* (RB-80, serotype 5a5b, also produced at the Pasteur Institute).

Sod-lined, cement potholes (1.8 m^2) located at the IAMARL were used for exposing natural populations of *Cx. quinquefasciatus* to primary powders of the 2297 and 2362 isolates of *B. sphaericus* (produced by H. T. Dulmage, USDA, Brownsville, TX). The 2362 (Serotype 5a5b) isolate of *B. sphaericus* was used for comparative field trials due to the availability of sufficient primary powder that was prepared in an identical manner to that of the 2297 primary powder. The 2362 isolate is comparable to the 1593 isolate in terms of larvicidal activity and host range. Samples were taken before application of aqueous suspensions of the primary powders and at 2, 4, 7, 10 and 14 days posttreatment. Sampling of each pothole consisted of 10 dips taken with a standard 250 ml capacity mosquito dipper; eight in the corners and two on the sides of each pothole. Each of the powders was applied to 3 potholes at the rate of 0.25kg/ha with a CO_2-pressurized sprayer equipped with a flat fan nozzle. Three potholes were left untreated as controls.

Tests were also conducted in plastic wading pools against natural populations of *Cx. quinquefasciatus* larvae. Nine pools (1.5 m in diameter, 1.8 m^2, 17–26 cm water depth depending on rainfall) were placed in a cypress dome in a rural setting near Jacksonville, Fl and 9 were placed in a mesic hammock of mixed hardwoods in Gainesville, Florida. The pools were shaded for most of the day. The bottom of each was covered with approximately 8 cm of soil and leaf mold from the vicinity and flooded with well water to within 7–10 cm from the rim of the pool. After 1 week floating debris was removed from the pools and additional water was added. Pretreatment larval samples (10 dips/pool) were taken the following day. Three pools at each site were treated with aqueous suspensions of primary powders of either the 2297 or 2362 isolate and 3 pools were left untreated as controls. At the Gainesville site, posttreatment samples were taken 2, 4 and 7 days after treatment and twice a week thereafter for another 1.5 weeks. In Jacksonville samples were taken 3, 7, 10 and 14 days after treatment and twice weekly thereafter for the next 3 weeks.

Comparative field trials were also conducted against *Culex nigripalpus* Theobald in small plots in full sunlight in a waste water lagoon (effluent from an orange juice processing plant) near Ft. Pierce, Florida. Nine 1 m^2 enclosures (3 for each treatment and controls) were made using pieces of thick plastic sheeting (1.2 m high; 4 m long) attached to 4 polyvinylchloride pipes (7.5 cm) diam; 1.5 m high) with staples. The enclosures were placed in the lagoon in shallow water (60 cm deep) that was free of vegetation. The pipes were driven into the bottom of the lagoon with a mallet until they were firmly anchored and the sheeting was several cm below the surface of the substratum. In this manner, larvae were restricted from entering or leaving the enclosures. Samples were taken as in the pothole studies before treatment, at 48 hr after treatment and at weekly intervals for 2 weeks.

The data from the laboratory bioassays of the 2297 and 1593 primary powders were subjected to probit analysis using SAS mainframe software (SAS User's Guide: Statistics. 1982, SAS Institute Inc., Cary, NC). Since there were no significant differences among the separate bioassays of each isolate against each species of mosquito, these data were combined for probit analysis; each separate test date was regarded as a replicate. The 95% fiducial limits for the LC_{50} values generated from the probit analysis for each isolate against each of the mosquito species tested, were compared for overlap. The LC_{50} values were considered significantly different from one another if their fiducial limits did not overlap. Analysis of variance and Duncan's New Multiple Range test were performed on all field trial data after correcting for check mortality using Abbott's formula and arcsine transformations of percentages.

RESULTS

Comparative susceptibility of the 8 species tested to the 2297 isolate and the calculated LC_{50} and LC_{95} values of the 2297 and RB-80 (1593) preparations are presented in Table 1. Application of the formula of Bourgouin et al. (1984) to the LC_{50} values generated for the two isolates bioassayed with *Cx. quinquefasciatus* enables the determination of a relative toxicity rating of 250 toxic units/mg for the 2297 preparation. Using the same procedure with the data obtained from bioassays of both isolates against *An. quadrimaculatus* results in a toxicity rating of 1218 toxic units/mg. The RB-80 preparation has an arbitrarily assigned rating of

Table 1. Comparative larvicidal activity of lyophilized preparations of the 2297 (H-25) and 1593 (H-5a5b) isolates of *Bacillus sphaericus* toward 8 species of mosquitoes (48 hr exposure, 27°C, second instars).

Species	LC_{50} and LC_{95} (mg/liter)[1]			
	2297		1593	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}
Culex quinquefasciatus	0.016a	0.736	0.004a	0.016[2]
Culex salinarius	0.059b	2.908		
Anopheles albimanus	0.275c	7.510		
Anopheles quadrimaculatus	0.620cd	9.484	0.755b	5.828
Aedes triseriatus	2.232d	>500		
Aedes taeniorhynchus	60.721e	>180		
Aedes aegypti	not susceptible f[3]			
Toxorhynchites r. rutilus	not susceptible f		0.273c	4.722[2]

[1] LC_{50} values in the same column followed by the same letter have overlapping 95% fiducial limits.
[2] LC_{50} values for each isolate in this row have 95% fiducial limits that do not overlap with one another.
[3] LC_{50} and LC_{95} values for this species range from over 2.7×10^5 to over 5.0×10^9 mg/liter.

1000 toxic units/mg (Bourgouin et al. 1984). The *Aedes* spp. were considerably less susceptible to 2297 than the other culicines and both anophelines. Larvae of *Ae. aegypti* and *Tx. r. rutilus* were not susceptible to the 2297 toxin.

Under field conditions the 2297 and 2362 primary powders performed in a similar manner in terms of efficacy and duration of larvicidal activity. Results of the comparative field trials that were conducted in sod-lined potholes are presented in Table 2. Data are only presented for the first 10 days of the test. After that time pronounced decline in larval density in the control plots did not permit further assessment of treatment effects.

The results of tests that were conducted in the woodland pools are presented in Table 3. Although sampling was continued after 1 and 2 weeks in the Gainesville and Jacksonville sites, respectively, only data through these dates are included in the table due to the precipitous drop in larval numbers in the control plots. From 1.5 to 2.5 weeks posttreatment, reduction of control larvae in the Gainesville pools ranged from 84.5 to 97.8%. Concomitantly, 96.5–97.9% and 93.9–94.9% reduction of larvae was observed in the pools treated with 2297 and 2362, respectively. From 2.5 to 5 weeks posttreatment at the Jacksonville site, reduction in control populations ranged from 47.2 to 97.4% while plots that had received both treatments remained devoid of larvae.

The results of tests conducted in the waste water lagoon are presented in Table 4. The reduction of residual larvicidal activity for 2297 is somewhat pronounced relative to the other experimental sites. Sampling was discontinued after 13 days due to an abrupt reduction of control populations.

DISCUSSION

Isolate 2297 displayed larvicidal activity against most of the species tested similar to or less than that of the serotype 5a5b isolates reported here and elsewhere (Davidson 1985). Al-

Table 2. Efficacy of primary powders of the 2297 (H-25) and 2362 (H-5a5b) isolates of *Bacillus sphaericus* applied at the rate of 0.25 kg/ha against mostly later instars of *Culex quinquefasciatus* in sod lined potholes. Gainesville, Florida, May 21, 1985.[1]

Treatment	% reduction ± s. e. (days posttreatment)[2]			
	2	4	7	10
2297	75.6 ± 11.9a	96.7 ± 3.3a	97.1 ± 2.3a	72.2 ± 21.6a
2362	97.4 ± 2.6a	99.6 ± 0.4a	88.5 ± 8.8a	52.5 ± 28.4a
Control	48.4 ± 10.4b	0[3]b	0[3]b	0b

[1] Mean pretreatment larval density for all potholes: 22 larvae/dip; mostly late instars; water temp. 27–31°C, clear water in full sunlight.
[2] Means in the same column followed by the same letter are not significantly different from one another ($P < 0.05$).
[3] Increase over pretreatment larval density. Mean larval densities in control plots at pretreatment and 2, 4, 7 and 10 days posttreatment were 30, 16, 48, 31 and 30 larvae/dip respectively.

though *An. quadrimaculatus* was more susceptible to 2297 than to 1593, it is still considerably less susceptible to both serotypes than are *Culex* larvae. Similar observations were made by de Barjac and Charles (1983) for bioassays of the 2297 isolate against *Anopheles stephensi* Liston and *Cx. pipiens*. Compared to *B. thuringiensis* (H-14), 2297 demonstrates far less larvicidal activity against anophelines. Unlike the serotype 5a5b isolate, 2297 did not display larvicidal activity against *Tx. r. rutilus*. Several other species in the genus *Toxorhynchites* are not susceptible to serotype 5a5b isolates of *B. sphaericus* (Lacey 1983). This phenomenon may provide some advantage if the bacterium and predator were integrated into a common control strategy.

Under field conditions both isolates demonstrated residual activity similar to that reported by other investigators for 2362 and other serotype 5a5b isolates. In the woodland pools the prolonged control that was obtained was probably due to the accessability of toxin in the relatively shallow clear water. Although recycling of *B. sphaericus* is possible under certain conditions (Davidson et al. 1984, Charles and Nicolas 1986, Nicolas et al. 1987), the larva to larva contact that would be necessary for sustained recycling would have diminished shortly after treatments were made due to the apparent decline in oviposition at both sites. Settling of spores and other toxic moieties and inactivation of toxin by sunlight are two possible factors that limited the duration of control in the pothole and waste water lagoon settings. Similar observations on settling and solar inactivation of *B.*

Table 3. Comparative larvicidal activity of aqueous suspensions of primary powders of the *Bacillus sphaericus* 2297 (H-25) and 2362 (H-5a5b) isolates applied at the rate of 0.25 kg/ha against natural populations of *Culex quinquefasciatus* under simulated field conditions in Gainesville and Jacksonville, Florida.

Location/treatment	% reduction ± s. e. (time after treatment)[1]			
Gainesville[2]	48 hr	4 days	1 wk	
2297	99.1 ± 0.3a	99.3 ± 0.4a	99.3 ± 0.4a	
2362	99.9 ± 0.1a	100a	100a	
Control	20.4 ± 5.0b	52.0 ± 14.1b	65.1 ± 12.3b	
Jacksonville[3]	72 hr	1 wk	10 days[4]	2 wks
2297	100a	98.5 ± 0.8a	100a	100a
2362	100a	96.5 ± 3.8a	100a	100a
Control	46.6 ± 6.7b	0[5]b	59.4 ± 9.9b	25.5 ± 13.2b

[1] Means in the same column at the same site followed by the same letter are not significantly different ($P < 0.05$).

[2] Mean pretreatment density (all plots): 70.5 larvae/dip; age structure: 38.6% 1st instar; 30.7% 2nd instar, 15.8% 3rd instar; 14.9% 4th instar; 11 June 85, 22.5–25°C; Partially to fully shaded wading pools with natural substrate. Mean larval densities in control plots at pretreatment and 2, 4 and 7 days posttreatment were 115, 91, 55 and 40 larvae/dip, respectively.

[3] Mean pretreatment larval density (all plots): 12.7 larvae/dip; age structure: 26.9% 1st instar, 23.1% 2nd instar, 13.8% 3rd instar and 36.2% fourth instar; 22–26°C, 7–21 June; partially to fully shaded wading pools with natural substrate. Mean larval densities in control plots at pretreatment and 3, 7, 10 and 14 days posttreatment were 21, 11, 31, 9 and 16 larvae/dip, respectively.

[4] Pools overflowed due to rainfall.

[5] Increase over pretreatment larval density.

Table 4. Reduction of *Culex nigripalpus* in a highly turbid and organically enriched waste water lagoon after application of aqueous suspensions of *Bacillus sphaericus* isolates 2297 (H-25) and 2362 (H-5a5b) at 0.25 kg/ha in 1 m^2 plots. Ft. Pierce, Florida, July 3–16, 1985.[1]

Treatment	% reduction ± s. e. (days posttreatment)[2]		
	2	6	13
2297	99.1 ± 0.7a	96.0 ± 2.5a	50.4 ± 26.1ab
2362	99.4 ± 0.3a	99.7 ± 0.3a	98.9 ± 0.7a
Control	0[3]b	44.3 ± 13.2b	51.9 ± 12.6b

[1] Age structure: 4.7% 1st instar; 9.0% 2nd instar; 28.8% 3rd instar; 48.3% 4th instar; 9.2% pupae; mean pretreatment larval density (all plots): 32 larvae/dip; water temperature at treatment time, 26°C.

[2] Means in the same column followed by the same letter are not significantly different ($P < 0.05$).

[3] Increase over pretreatment larval density. Mean larval densities in control plots at pretreatment and 2, 6 and 13 days posttreatment were 47, 48, 26 and 22 larvae/dip, respectively.

sphaericus were made by Mulligan et al. (1980), Mulla et al. (1984) and others. Reduction of residual larvicidal activity of *B. sphaericus* in organically enriched habitats was also reported by these authors. In our studies in the waste water lagoons the spores and inclusion bodies were probably rendered even less accessable due to settling into the rich muck of the substratum. Conversely, research conducted by Nicolas et al. (1987) on the persistence of larvicidal activity of the 2362 isolate in a highly polluted habitat demonstrated prolonged residual activity. The authors attributed this activity to very slow settling of the spores.

In most of the trials, especially those conducted in the woodland pools, conditions in the habitat changed sufficiently to bring about significant to complete decline in control larval populations. This decline may have been due to increased predation, decreased oviposition, decreased availability of larval food or other changes in the environment that affected larval survival. Under operational conditions, a decrease in habitat suitability that overlaps with the residual larvicidal capabilities of the *B. sphaericus* isolate utilized, will result in effective prolonged abatement of larval populations. Detailed background knowledge of habitat and climatic conditions and target species susceptibility to *B. sphaericus,* as well as precise timing of bacterial applications would be critical if maximum advantage of this strategy is to be realized.

Although the host range of 2297 appears to be slightly narrower than that of 1593, its greater activity toward the anopheline species warrants further attention. A number of other important aspects of its activity remain to be thoroughly investigated. Most notably, additional field studies under a variety of conditions are required in order to determine the degree of residual activity and recycling potential of this isolate.

The lack of susceptibility of the *Tx. r. rutilus* larvae to the 2297 toxin and their concomitant susceptibility to the serotype 5a5b isolate may help provide some additional clues to differences in their respective modes of action. An additional benefit that may be afforded by this isolate is the more effective purification of toxin that might be permitted by the exceptionally large toxin containing parasporal inclusions.

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