

NOVEL STRAINS SHOWING INSECTICIDAL, NEMATICIDAL, AND MOLLUSCIDAL ACTIVITY

From the laboratory of Samuel Singer, who retired in 1997 from Western Illinois University, comes a collection of environmental bacterial isolates demonstrating activity against a variety of invertebrate species. Included in the target ranges are the zebra mussel (*Dreissena polymorpha*), parasitic nematodes of animals (*Trichostrongylus colubriformis*) and plants (*Heterodera glycines*), and disease-vector mosquitoes (*Aedes*, *Anopheles*, and *Culex*) and snails (*Biomphalaria glabrata*). There are few available data about the nature of the toxins produced by these bacteria or about their mechanism of action. The toll exacted by these invertebrate pests on our public health, economy, and environment is reason enough to make this strain collection worthy of further investigation. Table 1 (next page) summarizes these bacteria and their known activities against target organisms. It is important to note that these activities seem significantly lower than those shown by *Bacillus thuringiensis* subsp. *israelensis* against mosquitoes, for example. The potential for enhancing the toxicity to more useful levels has so far been untested.

Bacillus circulans—The two *Bacillus circulans* strains are fairly typical of the isolates in the collection. They were isolated in Singer's laboratory from dead insects collected in Burma. When tested, they did display a low but significant level of activity against mosquito larvae. Later experiments revealed higher toxicities against nematodes and the veliger (larval) form of snails and zebra mussels (7).

Brevibacillus brevis—The collection includes three strains isolated from insects collection in Ghana and a fourth from the Wellcome Research Laboratories. All of them are molluscicidal, and at least two are nematicidal as well. *B. brevis* is well-known for producing small peptide antibiotics, the gramicidins. Assays indicate that these antibiotics themselves have some molluscicidal activity (7).

Paenibacillus alvei—Among the four *Pb. alvei* strains in the collection are isolates active against dipteran insects, nematodes, and mollusks (2, 5-7). Interestingly, diluted cultures of strain 2771 (our strain 33A3) caused digestive tissue damage in zebra mussels. Digestive tubule epithelia vacuolized and eventually sloughed off. Damage was apparently due to the action of a toxin rather than to infection (2).

Brevibacillus laterosporus—The species *Brevibacillus laterosporus* has long been known to include strains toxic to certain invertebrate organisms. Favret and Yousten (1) found that over half of the 29 isolates they tested were toxic larvae of the mosquito, *C. quinquefasciatus*. Activity was also detected against larvae of the black fly (*Simulium vittatum*) and the yellow-fever mosquito (*A. aegypti*). The toxic activity—which was heat-stable and UV-resistant—began to accumulate during stationary phase at a stage when the cells contained no endospores but did show oval, electron-dense areas near the cell membrane. Rivers *et al.* (4), studying essentially the same strain collection, detected activity against coleopteran (but not lepidopteran) larvae as well. Orlova obtained two mosquitocidal *B. laterosporus* isolates that produced large parasporal crystals reminiscent of those found in *B. thuringiensis* (3). The strains from Singer's lab show toxicity against certain coleopteran, nematode, and mollusk pests (6, 7). The nematicidal toxin appears to be small, proteinaceous, and heat-stable (6).

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Table 1. Demonstrated Pesticidal Activities for Strains from the Samuel Singer Collection.

Adapted from Singer, S. 1996. The Utility of Morphological Group II *Bacillus*. *Adv. Appl. Microbiol.* 42:219-261

BGSC	Strain Designation	Insecticidal			Nematicidal		Molluscicidal		
		Mosquito Larvae	Biting Blackflies	Cigarette Beetle	Indian Meal Moth	Animal Nematodes	Plant Nematodes	Snails	Zebra Mussels
16A2	<i>Bacillus circulans</i> 42G1	+				++	++	+++	
16A3	<i>Bacillus circulans</i> 46J3	++				+++	++	++	
26A2	<i>Brevibacillus brevis</i> SS86-3						+++		
26A3	<i>Brevibacillus brevis</i> SS86-4					+++	+++	+++	
26A4	<i>Brevibacillus brevis</i> SS86-5						+++		
26A5	<i>Brevibacillus brevis</i> 2904					+++	+++	+++	
33A1	<i>Paenibacillus alvei</i> III3DT-1A		+++			+++	+++	+++	
33A2	<i>Paenibacillus alvei</i> III2E	++		+	+				
33A3	<i>Paenibacillus alvei</i> 46C3	++				+++			
33A4	<i>Paenibacillus alvei</i> 2771					+++	++	+++	
40A7	<i>Brevibacillus laterosporus</i> 64	++		+		+++	+++	+++	
40A8	<i>Brevibacillus laterosporus</i> 1111	++		++		-	+++	+++	++
40A9	<i>Brevibacillus laterosporus</i> 1645	++		+		-			
40A10	<i>Brevibacillus laterosporus</i> 1647	++		+		+++	+++	+++	++

^a Dilution of the final whole culture killing 50% of the test organisms: +++, high ($>10^{-3}$); ++, moderate (10^{-2} - 10^{-3}); +, low ($<10^{-2}$)