

RECOVERY OF *BACILLUS THURINGIENSIS* AND RELATED SPORE-FORMING BACTERIA FROM SOIL AFTER APPLICATION FOR GYPSY MOTH CONTROL

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ABSTRACT

Bacillus thuringiensis Berliner (*Bt*) has been applied for gypsy moth (*Lymantria dispar* L.) control in forests in the northeastern U.S. for many years. The subspecies of *Bt* that is used (*kurstaki*) is not common in U.S. soil. We attempted to recover *Bt* from soil that had been sprayed 2 years prior with *Bt* for gypsy moth control. By amplifying the bacteria found in the soil on bacterial agar and feeding this diverse microbial population to tobacco hornworm larvae, an insect that is very sensitive to *Bt*, we were able to further improve on traditional recovery methods. Out of 20 soil samples, 15 were found to have spore-forming bacteria that killed these larvae. These strains were identified to species by their 16S rDNA sequences. Most of the bacteria belonged to the *Bacillus cereus* Franklin and Franklin group (14/15) including 3 *Bt*.

All the *Bt* strains and most of the *B. cereus* strains were closely related (<0.001 substitutions/site in their 16S rDNA) to the strain that was sprayed. The other two spore formers were identified as *B. weihenstephanensis* Lechner et al. and *Lysinibacillus fusiformis* (Meyer and Gottheil). All 15 strains were phenotypically distinguishable from one another, as well as from the *Bt* strain that was applied, by substrate utilization and antibiotic resistance, although seven strains differed from the *Bt* strain applied by 5 or less of the 24 traits tested. The toxicity to gypsy moth larvae of the three *Bt* strains isolated was similar to that of the applied *Bt* strain. Thus, amplification of bacteria present in soil, in combination with a sensitive insect, can recover insect toxic strains that are related to an applied strain.