SUMMARY

Paenibacillus popilliae and *P. lentimorbus* are pathogens of Japanese beetle larvae and related scarab larvae. Differentiation between the two organisms has been based on phenotypic characteristics and, more recently, on DNA similarities. *Paenibacillus popilliae* was characterized by the formation of a parasporal body during sporulation, the ability to grow on medium supplemented with 2% sodium chloride, and resistance to the antibiotic vancomycin, whereas *P. lentimorbus* was believed to lack these traits. Phenotypic characterization, DNA similarity studies, and random amplified polymorphic DNA (RAPD) studies have been reported on strains of *P. popilliae* and *P. lentimorbus* from North America and Europe. The studies on the North American and European strains revealed that the presence of a parasporal body and the ability to grow on medium supplemented with 2% sodium chloride were not ideal phenotypic traits. In this study, 31 strains of bacteria isolated in Mexico and throughout Central and South America were examined. The strains were received as *P. popilliae* based on the appearance of milky disease in infected larvae. The species of these strains were determined using DNA similarity studies and the strains were characterized phenotypically.

Twenty-one of the Mexican and Central and South American strains were determined to be *P. popilliae* based on DNA similarity to the *P. popilliae* type strain ATCC 14706. The remaining strains were more closely related to the *P. lentimorbus* type strain ATCC 14707. Two European strains (NRRL B-4081 and H1) included in this study appear as a subgroup within the *P. popilliae* group of strains. Only one of the *P. popilliae* strains (strain 508) was resistant to the antibiotic vancomycin. The polymerase chain reaction (PCR) was used to amplify a portion of a ligase gene, *vanE*, necessary for vancomycin resistance. The PCR product was sequenced and

the sequence was identical to a North American *P. popilliae* strain (Bp17) previously described. Eighteen of the twenty-one *P. popilliae* strains and four of the ten *P. lentimorbus* strains were able to grow on medium supplemented with 2% sodium chloride. No correlation between species and host insect or geographic or environmental source could be determined. Also, no correlation between species and phenotypic traits could be made because there were exceptions to each phenotypic characteristic typically associated with the species.

All of the *P. popilliae* and *P. lentimorbus* strains from Mexico and Central and South America produced a parasporal body. These results support a report that described a subgroup of North American *P. lentimorbus* strains that produce a paraspore. The Mexican and Central and South American strains of *P. popilliae* and *P. lentimorbus* were screened for the presence of a parasporal gene resembling the published *cry18Aa1* parasporal gene. Two regions of the *cry* operon were amplified using PCR. One set of primers, CryBP2, amplified a region beginning in the 3' end of the *orf1* gene and extending into the 5' end of the *cry* gene. CryBP2 primers detected the parasporal gene in 17 of the *P. popilliae* strains and in all of the *P. lentimorbus* strains. The PCR reactions produced amplicons of three sizes: eleven of the *P. popilliae* strains produced amplicons of approximately 660 bp; six of the *P. popilliae* strains produced amplicons of approximately 1100 bp; all of the *P. lentimorbus* strains produced amplicons of approximately 750 bp. The CryBP2 primers did not detect the parasporal gene in four of the *P. popilliae* strains. The second set of primers, CryBP4, amplified a region within the *cry* gene. CryBP4 primers detected the parasporal gene in all of the *P. lentimorbus* strains and in twenty of the twenty-one *P. popilliae* strains. All of the CryBP4 amplicons were approximately 616 bp in size. I was also able to detect the parasporal gene in the *P. lentimorbus* type strain ATCC 14707, a strain characterized by its inability to produce a paraspore.

The PCR products for CryBP2 amplicons for *P. popilliae* strain 381 (660 bp), *P. popilliae* strain 492 (1100 bp), and *P. lentimorbus* strain 266 (750 bp). The CryBP2 amplicon for the *P. lentimorbus* type strain (ATCC 14707) was also sequenced. Strains 266 and 391 differed by 67 and 47 bp, respectively, from the published *cry18Aa1* sequence and these same strains differed by 30 bp from each other. The *P. lentimorbus* type strain showed no similarity to the published *cry18Aa1* sequence. Strain 492 has a 453 bp insert that appears to begin in the intergenic region between the *orf1* and *cry* genes. Approximately 114 bp at the 3' end of the insert are a duplication of the region between bp 132 and bp 214 at the 5' end of the *orf1* gene of the published sequence, which includes the ribosome binding site (RBS) for the *orf1* gene. Since the alignment of strain 492 doesn't show a RBS similar to the *cry18Aa1* gene, it seems likely that the insert provides a RBS for the *cry* gene in strain 492.