

INTRODUCTION

Paenibacillus popilliae and *P. lentimorbus* (formerly genus *Bacillus*) are the causative agents of milky disease in larvae of Japanese beetles and related scarabs. In the field, these organisms are identified by the appearance of a milky white color in scarab larvae infected with the bacteria. *Paenibacillus popilliae* spores have been used as a microbial insecticide since the 1940's, but the inability to achieve mass *in vivo* sporulation has limited its widespread use and production as a control agent. Differentiation between the two organisms has typically relied on three phenotypic characteristics: the presence of a parasporal body, resistance to the antibiotic vancomycin, and growth in a medium supplemented with 2% sodium chloride. Until the 1960's, classification of organisms into taxonomic groups was based largely on morphological, biochemical, and serological traits. Since the elucidation of the molecular basis of inheritance, determining relationships at the molecular level (DNA, RNA, and protein) has become increasingly popular. One such example is a technique known as DNA-DNA hybridization. Whole genomes of organisms are compared and data from these studies are used to determine the relatedness of species. DNA similarity studies have shown that the phenotypic traits used to differentiate between *P. popilliae* and *P. lentimorbus* are not reliable. Increased geographic distribution of strains used in DNA similarity studies have revealed that species are often misidentified when based solely on the traditional phenotypic characteristics. Prior to this study, phenotypic characterizations and DNA similarities have been performed mainly on strains of *P. popilliae* and *P. lentimorbus* from North America and Europe. In this study, *P. popilliae* and *P. lentimorbus* strains from Mexico and throughout Central and South America were characterized phenotypically and speciation was determined based on data from DNA similarity

studies. The formation of a parasporal body by *P. popilliae* and the absence of this structure in *P. lentimorbus* was one of the first traits described to distinguish the species, but North American and Central and South American strains of *P. lentimorbus* that produce a paraspore have been identified. Resistance to the antibiotic vancomycin was believed to be restricted to *P. popilliae*. No strains of *P. lentimorbus* have been identified that are resistant to vancomycin, however all but one of the Mexican and Central and South American strains of *P. popilliae* are sensitive to vancomycin. Growth in 2% sodium chloride was believed to be a trait unique to *P. popilliae*. This trait may be of some use in identifying *P. popilliae* from all the geographic areas, especially those from Central and South America, but it is not completely reliable. Lack of a phenotypic test for differentiation of these genetically distinct species does not imply that speciation should be based entirely at the molecular level. A classification system that includes both phenotypic and molecular data will provide a better insight into the relationship of *P. popilliae* and *P. lentimorbus* as well as provide a simple means of identification of strains within these species. Determining the genetic relationships between *P. popilliae*, *P. lentimorbus*, and related species could also help clarify bacteria-host specificity and develop a more effective biopesticide.