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Short communication

Phenotypic and genotypic characterization of *Paenibacillus larvae* isolates

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Abstract

Paenibacillus larvae is the causative agent of American Foulbrood (AFB), a severe disease of honeybees (*Apis melifera*). The aim of this work was to develop a strategy for the subtyping and the epidemiological analysis of *P. larvae*. Phenotypic characterisation, susceptibility to several antibiotics, electrophoresis of whole bacterial proteins, *rep*-PCR, ribotyping and DGGE were assessed using a collection of *P. larvae* isolates from different Uruguayan and Argentinean locations. Results indicated that there are two *P. larvae* genotypes circulating in Uruguay ERIC I-BOX A (worldwide distributed) and ERIC I-BOX C (exclusively detected in Argentina until this study). These results suggest that *P. larvae* isolates had moved between Argentina and Uruguay, probably through the Uruguay River. Patterns of whole bacterial proteins, DGGE and ribotyping did not improve the *P. larvae* intraspecific discrimination. Antibiotic susceptibility assays showed that 100% isolates were OTC-sensitive and 22% (belonging to ERIC I-BOX A group) were sulfisoxazole-resistant. This work may contribute to the elucidation of basic aspects related to the epidemiology of AFB in Uruguay and in the region.

Keywords: American Foulbrood; *Paenibacillus larvae*; Honeybee disease; Epidemiology

1. Introduction

American Foulbrood (AFB) is a severe disease-affecting larvae of the honeybee *Apis mellifera*, caused by the spore-forming bacteria *Paenibacillus larvae* (Hansen and Brodsgaard, 1999; Genersch et al., 2006).

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Serious effects are associated to AFB, such as decrease of bee population and honey production. *P. larvae* was first isolated in the South Cone of South America in Argentina, in 1989 (Alippi, 1992). In Uruguay, it was first isolated in 1999 in provinces adjacent to Argentina (Piccini and Zunino, 2000). Currently, it is widely spread throughout the country (Antúnez et al., 2004, Fig. 1).

Several approaches have been carried out to evaluate the genetic diversity of *P. larvae*. Alippi and Aguilar (1998) reported the use of BOX-PCR for the subtyping of former *P. larvae* subsp. *larvae*. Using a worldwide isolates collection, these authors identified three genotypes (A–C). According to their results, the genotype C included exclusively Argentinean isolates, while genotypes B and C were widely distributed. Later, Genersch and Otten (2003) reported the use of BOX-PCR with MBO REP1 primers to identify four genotypes on German isolates. After the reclassification of *P. larvae* subsp. *larvae* and *P. larvae* subsp. *pulvifaciens* as *P. larvae*, a new subtyping method based on ERIC-PCR was proposed, allowing the differentiation of four genotypes, I and II for former *P. larvae* subsp. *larvae* and III and IV for former *P. larvae* subsp. *pulvifaciens* (Genersch et al., 2006).

Apiculture is becoming a very important economic activity in Uruguay and worldwide. However, AFB appears as a growing problem that may affect honey production. The aim of this study was to develop a subtyping strategy for *P. larvae* and assess genotypes prevalence and geographic distribution in Uruguay.

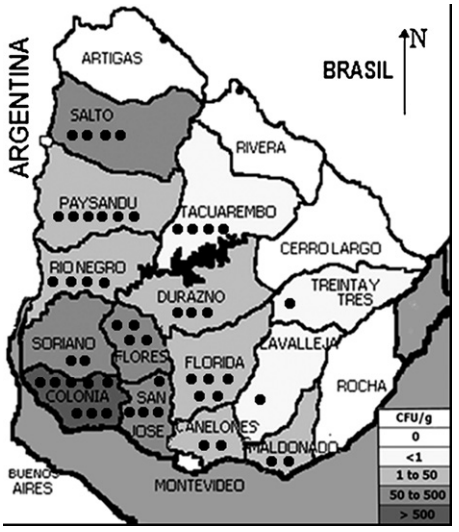

Fig. 1. *Paenibacillus larvae* spores in honey samples from different geographic locations of Uruguay and origins from isolates used in this study (Antúnez et al., 2004). Each black point indicates one isolate.

2. Methods

2.1. P. larvae isolates

Fifty isolates presumptively identified as *P. larvae*, obtained from bees, larvae and honey from Uruguayan provinces between 1999 and 2002 were used for this study (Fig. 1). Four Argentinean isolates corresponding to genotypes A, B and C (PL8 and PL99 corresponding to genotype A, PL35 corresponding to genotype C and PL63 corresponding to genotype B) (Alippi and Aguilar, 1998) and one additional isolate, PLP (former *P. larvae* subsp. *pulvifaciens*) were also used. Isolates were grown on J medium (Hornitzky and Nicholls, 1993) and standard biochemical tests were performed for initial identification (Alippi, 1992).

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility tests were assessed by the disk diffusion method (NCCLS, 1986). Disks with oxytetracycline (OTC, 5, 10, 15, 20 and 30 μg), gentamicin (10 μg), ampicillin (10 μg), sulfisoxazole (300 μg) and nitrofurantoin (300 μg) were used (Bauer et al., 1966).

2.3. SDS-PAGE of whole bacterial proteins

Crude bacterial proteins extracts were obtained by sonication of cell suspensions in phosphate-buffered saline. SDS-PAGE was carried out as described by Laemmli (1970).

2.4. DNA extraction, PCR and DNA sequencing

For PCR experiments, DNA was extracted by bacterial lysis (Govan et al., 1999). Isolates identification was carried out by amplification of a specific *P. larvae* 16S rRNA gene fragment using primers PL5 and PL4 (Piccini et al., 2002). Universal primers 27F and 1494R were used to amplify the complete 16S rRNA gene for sequencing analysis (Lane, 1991). PCR