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Short communication

Characterization of *Bordetella bronchiseptica* strains using phenotypic and genotypic markers

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Abstract

Thirty-five strains of *Bordetella bronchiseptica*, recovered primarily from pigs, rabbits, dogs, cats and humans, were characterized by phenotypic and genotypic markers. Biochemical typing only showed variation in the ability to reduce nitrate to nitrite. OMP profiles from virulent strains showed variations in the region of 85–95 kDa, which lead us to describe five OMP-types α, β, γ, δ and ε. Genotypic markers included the presence of *IS*1001, and polymorphisms in the flagellin gene (*flaA*) and pertussis toxin (PT) promoter region. The *IS*1001 was detected in 16 isolates (2 from humans and 10 from pigs) but was absent in rabbit isolates. The restriction profiles of the *flaA* gene allowed us to differentiate the strains into types A–C. The PT types were characterized by an RFLP assay and could be typed through patterns III–V. There was no apparent association between the *flaA* or PT types and the origin of the isolates. Eleven groups of isolates were identified on the basis of specific combinations of the analyzed markers. The combination of phenotypic and genotypic tests used could be useful in characterizing isolates and differentiating between certain clonal types of *B. bronchiseptica*.

Keywords: *Bordetella bronchiseptica*; Flagellin; PT promoter; OMP profiles; Polymorphisms; PCR-RFLP; Diversity

1. Introduction

Bordetella bronchiseptica causes acute and chronic respiratory infections in diverse animal species and occasionally in humans (Goodnow, 1980; Stefanelli et al., 1997). The virulent strains (Bvg$^+$) of *B. bronchiseptica* express several characterized virulence factors, such as filamentous hemagglutinin, pili, pertactin, adenylate cyclase-haemolysin toxin and dermonecrotic toxin, and outer membrane proteins (OMPs) of approximately 200, 90–95 and 30–32 kDa encoded by *vag* genes, which are positively regulated by the *bvgAS* locus. The avirulent variants (Bvg$^-$), originating by spontaneous mutations in *bvgAS*,

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express *vrg* genes that encode the production of flagellin and urease, but do not express the *vag* genes (Cotter and Miller, 2001).

In certain bacterial species, the OMP profiles have been shown to be closely associated with types and clones identified by multilocus enzyme electrophoresis (MLEE) (Davies et al., 1997). Moreover, OMPs exhibit different degrees of inter-strains heterogeneity, which could be used to assay intra-species diversity and determine epidemiological relationships. In a previous study, we evaluated the potential usefulness of OMP profiles for typing *B. bronchiseptica* isolates (Friedman et al., 2003).

To study the epidemiology of *B. bronchiseptica*, several molecular methods have been proposed, such as randomly amplified polymorphic DNA, ribotyping, pulse-field gel electrophoresis, the presence of *IS*1001 and polymorphism in the flagellin gene (*flaA*) (Stefanelli et al., 1997; van der Zee et al., 1997; Keil and Fenwick, 1999; Winstanley et al., 2001). A *flaA* PCR-RFLP assay was reported for the discrimination among *B. bronchiseptica* strains in three sequence types A–C (Winstanley et al., 2001). However, none of these methods showed a high correlation with the origin of the isolates. We have recently demonstrated that veterinary *B. bronchiseptica* isolates can be classified into three groups based on pertussis toxin (PT) promoter region polymorphisms (Messina et al., 2004).

There are few studies on the epidemiology of *B. bronchiseptica* and characterization of isolates from Argentina. The aim of this study was to analyze several phenotypic and genotypic markers for the characterization of *B. bronchiseptica* strains isolated from different hosts potentially useful for epidemiologic analysis.

2. Materials and methods

2.1. Bacterial strains

Thirty-five strains of *B. bronchiseptica* were included in the study. Of them, 32 were recovered from pigs ($n = 11$), rabbits ($n = 11$), dogs ($n = 4$), cats ($n = 2$), human ($n = 3$) and guinea pig ($n = 1$), while 3 were of unknown origin (Table 1). Twenty-four isolates were recovered from different geographical areas within Argentina between 1967 and 2004. Six isolates from the UK [BB SB660, BB SB998, BB SB55, BB SB521; Winstanley et al., 2001; BB A48, and BB D78] and five collection strains [CCUG1326, CCUG4878, CCUG7865, ATCC4617 and 8101 (University of Laval, PQ, Canada)] were also included. The strains were stored in 15% (v/v) glycerol at −20 °C. Local isolates were previously identified as *B. bronchiseptica* by conventional bacteriological tests (Pittman, 1984). The 33 Bvg^+ isolates were characterized by colonial morphology and haemolytic activity on Bordet–Gengou agar (Difco) containing 5% horse blood (BG), the hemagglutination assay and SDS-PAGE analysis of OMP profiles, as described previously (Friedman et al., 2001).

2.2. Biochemical typing

Biochemical testing was performed with the API20NE phenotypic identification system (Biomerieux) following the manufacturer's instructions.

2.3. Preparation of OMP-enriched fractions

Stock cultures stored at −20 °C were streaked onto BG and incubated overnight at 37 °C. For preparation of OMPs, the overnight culture was inoculated on BG plates and incubated for 48 h at 37 °C. Then, β-haemolytic colonies of Bvg^+ strains were transferred to 50 ml SS broth (Stainer and Scholte, 1971) and incubated for 30 h at 37 °C at 150 rpm. After subsequent subculturing on BG, 97–100% haemolytic colonies were observed. OMPs were prepared by Sarkosyl extraction, as described previously (Leyh and Griffith, 1992; Friedman et al., 2001).

2.4. SDS-PAGE and Western blot

OMPs were separated by SDS-PAGE on 7.5% polyacrylamide gels according to Laemmli (1970) with a Mini-Protean II system (Bio-Rad). Ten micrograms of protein was loaded per lane and visualized with Coomassie brilliant blue or electrophoretically transferred to nitrocellulose membranes by Towbin's method (1979). Blots were blocked with 3% bovine serum albumin and incubated with a mouse polyclonal antibody raised against *Bordetella*