

Polymorphism of Repeated Regions of Pertactin in *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica*

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Pertactin is an outer membrane protein expressed by *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* that induces protective immunity to *Bordetella* infections. The immunodominant and immunoprotective epitopes of pertactin include two repeated regions, I and II. Comparison of these two repeated regions showed that *B. parapertussis* pertactin is invariant, whereas *B. pertussis* pertactin varies mostly in region I and *B. bronchiseptica* pertactin varies in both repeated regions I and II, but mostly in region II. These differences may result from specific characteristics of these *Bordetella* species.

The genus *Bordetella* includes seven species; the most studied species are *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*. *B. pertussis* is responsible for respiratory infections only in humans. *B. parapertussis* causes infections in humans and sheep, and *B. bronchiseptica* infects many animal species, including humans. These pathogens produce an array of virulence factors, the synthesis of which is regulated by the two-component Bvg AS (2, 22) system. These factors include toxins such as pertussis toxin, which is the only toxin specific to *B. pertussis*; tracheal cytotoxin; adenylate cyclase-hemolysin; and adhesins such as filamentous hemagglutinin, fimbriae, and pertactin (PRN). PRN is an outer membrane protein with an apparent molecular mass of 69 kDa in *B. pertussis*, 70 kDa in *B. parapertussis*, and 68 kDa in *B. bronchiseptica* (5, 15, 16). The precursors of PRN are 91.5, 93, and 92.5 kDa in size, respectively. In *B. pertussis*, PRN has been demonstrated to be an agglutinin (4), promoting attachment to certain eukaryotic cells via an Arg-Gly-Asp (RGD) motif (14).

Antibodies specific for the *B. bronchiseptica*-PRN are detected at high titer in immunized piglets, whereas few if any of these antibodies are detected in unprotected animals (20). Synthesis of PRN by *B. bronchiseptica* correlates with protection (17). The immunization of mice or piglets with preparations of PRN induces protective immunity against *B. bronchiseptica* infection (13, 20), and passively administered monoclonal antibodies prevent the death of animals challenged with *B. bronchiseptica* (17). *B. pertussis* PRN has also been shown to induce protective immunity to intracerebral, aerosol, and intranasal challenge with *B. pertussis* in mice (12, 19, 21). PRN is therefore now included in some acellular pertussis vaccines (i.e., vaccines composed of purified bacterial proteins) (10). However, the PRNs of these three species, although clearly related, have different immunogenic properties. For example, preparations of *B. pertussis* PRN protect mice against intranasal *B. pertussis* challenge but not against intranasal *B. parapertussis* challenge (12). They also protect mice against intracerebral *B. pertussis* challenge, whereas the *B. bronchiseptica* PRN protein does not (19).

Comparison of the deduced amino acid sequences of the three PRN proteins reveals a high degree of similarity, with the *B. bronchiseptica* and *B. parapertussis* proteins being more sim-

ilar to each other than to the *B. pertussis* PRN protein (5, 15, 16). The sequences of the three proteins differ mostly in the number of repeats in regions I and II (Fig. 1A). Using monoclonal antibodies, Charles et al. identified and characterized a protective immunodominant epitope of the *B. pertussis* PRN (6). This epitope spans the (Pro-Gln-Pro)₅ repeat sequences located in region II. Differences in this region may account for the observation that sera from piglets that recognize *B. bronchiseptica* PRN do not react with *B. pertussis* PRN despite the high degree of similarity between these proteins (13) and the lack of cross-protection provided by the three proteins (12, 19, 21). It has recently been shown that the PRN produced by clinical isolates of *B. pertussis* varies (3, 18).

The sequences of the *prn* gene of various clinical isolates revealed three major types of PRN variant. It has been suggested that epidemics result from changes in the sequences of the genes encoding PRN and pertussis toxin because the proteins present in the clinical isolates currently in circulation differ in sequence from those expressed by the vaccine strains used in the Netherlands (18). For PRN, all the observed deduced amino acid differences are located in region I. The deduced amino acid sequences of *B. pertussis prn* types A=1 and C=3 are very similar, differing by only two amino acids, whereas type B=2 is quite different, having a five-amino-acid insertion in the same region (18). Only one type was found to differ in region II. This type, A*=6, is produced by the *B. pertussis* World Health Organization reference strain 18323 and one French clinical isolate (3). It does not, however, seem to be common because it has been detected in only one clinical isolate (3). The production by this *B. pertussis* strain of this unusual PRN reflects the many common properties shared with the *B. parapertussis* and *B. bronchiseptica* species. No differences were found in the phenotype and behavior in the animal model of *B. pertussis* clinical isolates expressing different PRNs (3).

In this study, we sequenced and compared the deduced amino acid sequences of the repeated regions of the *prn* genes of 10 *B. parapertussis* isolates of human origin and 40 *B. bronchiseptica* isolates of animal and human origin (Table 1). The aim of this study was to analyze whether the PRN polymorphism observed in *B. pertussis* species also occurs in *B. parapertussis* and *B. bronchiseptica*. Sequencing was limited to regions I and II mostly because variation in the *B. pertussis prn* gene was observed in these regions and because these regions are immunodominant (Fig. 1A).

DNA was extracted, amplified by PCR, and sequenced as

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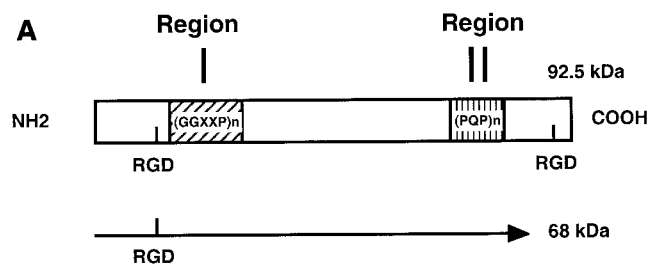


FIG. 1. (A) Structure of the *B. bronchiseptica* PRN, which harbors two repeated regions (I and II) sequenced in this study. (B and C) Multiple alignments of region I (B) and region II (C).

B

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I-1   QRATIRRGDAPAGGAVPGGAVPGGAVPG-----GFGPLLDGWYGVDVSDSTVDLAQ
I-2   QRATIRRGDAPAGGAVPG-----GAVPG-----GFGPLLDGWYGVDVSDSTVDLAQ
I-3   QRATIRRGDAPAGGGVPG-----GAVPG-----GFDPGGFGPGGFGPVLGDWYGVDVSGSTVELAQ
prn1  QRATIRRGDAPAGGAVPG-----GAVPG-----GAVPGGFGPGGFGPVLGDWYGVDVSGSSVELAQ
prn2  QRATIRRGDAPAGGAVPG-----GAVPGGFGPGGFGPGGFGPVLGDWYGVDVSGSSVELAQ
prn3  QRATIRRGDAPAGGAVPG-----GAVPG-----GFGPGGFGPGGFGPVLGDWYGVDVSGSSVELAQ
prn4  QRATIRRGDAPAGGAVPG-----GAVPG-----GFGPGGFGPVLGDWYGVDVSGSSVELAQ
*****.***.....*****.*****:*****.***:***
    
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C

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II-1   GAKAPPAPKPAPQPGPQPGP-----QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-2   GAKAPPAPKPAPQPGPQPGPQPP-----QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-3   GAKAPPAPKPAPQPGPQPGPQPGPQPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-4   GAKAPPAPKPAPQPGPQPGPQPGP-----QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-5   GAKAPPAPKPAPQPGPQPGPQPGPQPP-----PQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-6   GAKAPPAPKPAPQPGPQPGPQPPQPP-----QPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-7   GAKAPPAPKPAPQPGPQP-P-----QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA
II-8   GAKVPPAPKPAPQPGPQP-PQPP-----QPPQPPQPQPPQPP--EAPAPQPPAGRELSAA
II-9   GAKVPPAPKPAPQPGPQP-PQPP-----QPPQPPQPQPPQPPQPEAPAPQPPAGRELSAA
prn1  GAKAPPAPKPAPQPGPQP-----PQPPQP----QP--EAPAPQPPAGRELSAA
prn6  GAKAPPAPKPAPQPGPQP-----PQP----QP--EAPAPQPPAGRELSAA
*****.*****.....*****.***.....*****.*****
    
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previously described (3). Amplified PCR products were purified and sequenced by ESGS (Cybergene Group, Evry, France). Deduced amino acid sequences were analyzed with GCG software (Wisconsin package version 9.1; Genetics Computer Group, Madison, Wis.) (11). The deduced amino acid sequences of regions I and II were compared, and multiple alignments of the amino acid sequences were created with the CLUSTAL W program of GCG for each region (Fig. 1B and C).

No difference was found between the sequences of regions I and II of the PRNs produced by the 10 *B. parapertussis* isolates and the published sequence (16). However, three different types were found among the 40 *B. bronchiseptica prn* genes analyzed, with differences in the number of repeats in region I (Fig. 1B). The largest group corresponded to sequences with three copies of the repeated sequence, identical to the sequence reported previously (15). A higher degree of variability was observed in the second repeated region of the *B. bronchiseptica* PRN (Fig. 1C). Nine variants were observed. Again, the differences concerned the number of repeats (6 to 9). Some *B. bronchiseptica* variants are of the *B. parapertussis* type, but no *B. bronchiseptica* variant presented the same pattern as the *B. pertussis* isolates. Furthermore, no unique association between one type of region I and one type of region II was observed.

We did not observe in any of the three species a pattern similar to those of the 18323 strain and the CZ isolate (3), which are considered intermediate between *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis*. These data are consistent with the *B. parapertussis* and *B. bronchiseptica prn* genes' being more similar to each other than to the *B. pertussis prn* gene (1). The lack of variation in the PRN expressed by the 10 human *B. parapertussis* isolates is consistent with other studies which showed that these isolates constitute a highly clonal group (23, 25).

No host specificity was observed with respect to PRN type. It was thought that *B. pertussis* allelic *prn* types 2 and 3 emerged after 30 years of high-coverage vaccination with the same vaccine strains producing allelic *prn* type 1. If this is the case, the lack of variation in the PRN of the 10 *B. parapertussis* PRN isolates, 6 of which were collected recently (1990 to 1997) and 4 of which were collected some years ago (1963 to 1964), suggests that selection pressure due to 30 years of vaccination with a *B. pertussis* whole-cell vaccine may have affected *B. pertussis* PRN but not *B. parapertussis* PRN. However, more *B. parapertussis* isolates must be analyzed before any firm conclusion can be drawn.

It has been shown that region II plays a role in the induction of protective immunity (6). The lack of cross-protection between *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* PRN

TABLE 1. Isolates used in this study and corresponding PRN types

<i>Bordetella</i> species ^a	Isolate	PRN types (regions I and II)/no. of isolates	Accession no. (region I, region II)
BB	CN7531	I-2, II-4	Q03035
BB	9.73H+	I-1, II-3/3	AJ250076, AJ250077
BB	LAPR	I-2, II-3/8	AJ250078, AJ250079
BB	5	I-2, II-4/8	AJ250080, AJ250081
BB	335	I-2, II-1/3	AJ250082, AJ250083
BB	CVGEO	I-2, II-5/6	AJ250084, AJ250085
BB	BBCH	I-2, II-6/4	AJ250086, AJ250087
BB	DEL	I-1, II-2/5	AJ250088, AJ250089
BB	CAT1	I-1, II-7/1	AJ250090, AJ250091
BB	286	I-3, II-8/1	AJ250093, AJ250092
BB	SEI	I-3, II-9/1	AJ250094, AJ250095
BPP	CN2591	I-1, II-2	P24328
BPP	63.2	I-1, II-2/10	Identical to P24328
BP	Tohama	<i>prn1</i>	AJ006158
BP	18323	<i>prn6</i>	AJ006152
BP	Hav	<i>prn2</i>	AJ007361
BP	Fr287	<i>prn3</i>	AJ006156

^a BB, *B. bronchiseptica*; BP, *B. pertussis*; BPP, *B. parapertussis*.

is consistent with this because the major differences between these proteins occur in this region. No variation in this region was observed for the PRN produced by *B. pertussis* isolates. These data suggest that 30 years of vaccination may have induced variation in one immunodominant repeat region (I) but not in the other region (II). One can make the hypothesis that region II, which was already known to be implicated in the induction of protective immunity (6), is the most involved region. In contrast, analysis of the PRN of *B. bronchiseptica* shows polymorphism in both regions. This may account for the inability of *B. bronchiseptica* vaccines to induce long-lasting protection. This polymorphism may also be linked to the ability of *B. bronchiseptica* to induce chronic infections (7, 9, 24). In fact, we have previously shown that *B. bronchiseptica* may persist in the host (9) by varying the expression of one of its toxins (adenylate cyclase hemolysin) or the structure of its lipopolysaccharide (8, 9). Variation in PRN region II may also provide a mean for this bacterium to escape host immune responses. Our observations, as well as those published previously (18), indicate that a continuous surveillance of antigens expressed by *B. pertussis* isolates is necessary to verify whether vaccine formulation should be adapted.

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