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NOTES

Identification of a *Bordetella pertussis bvg*-Regulated Porin-Like Protein

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***Bordetella pertussis* 18323 produces a *bvg*-regulated 39.1-kDa porin-like protein, OmpQ. OmpQ had 61% similarity to the major porin of *B. pertussis* and contains conserved regions common to both the neisserial and enteric porin families. The results of Southern blot analysis indicate that strains of *Bordetella parapertussis* and *Bordetella bronchiseptica* but not *Bordetella avium* contain this gene.**

In *Bordetella pertussis*, the *vir*/*bvg* locus coordinately regulates the expression of many of the known virulence-associated determinants of the organism (1, 21–23). Tn*phoA* and Tn*5lac* mutagenesis studies have indicated that other *bvg*-regulated proteins are coordinately expressed with the more thoroughly studied *B. pertussis* virulence-associated proteins such as pertussis toxin, filamentous hemagglutinin, and pertactin (5, 24). The genes encoding these proteins have been named *vag* for *vir*-activated genes (14). Expression of these genes is affected by environmental signals in a phenomenon called antigenic modulation, such that expression is maximal in the absence of $MgSO_4$ or nicotinic acid (9, 15, 20).

One of these Tn*phoA* insertions is in a gene originally designated *vag-49* and renamed *ompQ* in this report (5). This gene has been cloned and sequenced, and it encodes a porin-like protein, OmpQ. Thus, *B. pertussis* may express at least two porins: a major porin (monomers of which we designate OmpP, encoded by the *ompP* gene, which appears to be expressed under both modulating and nonmodulating conditions [2, 11, 16, 19]) and OmpQ (encoded by the *ompQ* gene and expressed only under nonmodulating conditions).

Bacteria, plasmids, and cloning of *OmpQ*. Bacterial strains and plasmids used are listed in Table 1. *Bordetella* species were grown on Bordet Gengou (BG) medium supplemented with 12% sheep blood or in modified Stainer-Scholte (SS) broth cultures (13). *Escherichia coli* strains were grown on Luria-Bertani medium. For modulation studies, BG agar was supplemented with either $MgSO_4$ (20 mM) or both $MgSO_4$ (20 mM) and nicotinic acid (5 mM). Antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; kanamycin, 50 μg/ml; streptomycin, 100 μg/ml; tetracycline, 12.5 μg/ml; and cephalexin, 50 μg/ml.

By standard DNA manipulation methods (17), the fusion junction between the Tn*phoA* insertion and *B. pertussis* DNA was cloned from SK49 as a 7-kb *Bam*HI fragment in pUC18 to generate pTF101. Ligation of this 7-kb *Bam*HI fragment into *Bam*HI-digested pLAFR2 generated pTF003. This plasmid was transferred into *Bordetella bronchiseptica* IT-2 by using pRK2013 to provide transfer facilities. An exconjugate from this mating expressed modulated alkaline phosphatase activity. In a *B. pertussis* 347 background, pTF003 did not produce

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant feature(s)	Source or reference
Bordetella strains		
B. pertussis 18323	Wild type	ATCC 9797
B. pertussis SK49	18323 *vag-49*::Tn*phoA*	5
B. pertussis Tohama I		Laboratory of Pertussis
B. pertussis 347	338 *bvgS*::Tn*5*	A. A. Weiss
B. bronchiseptica IT-2		C. Lee
B. bronchiseptica 058		Laboratory of Pertussis
B. bronchiseptica 1104		Laboratory of Pertussis
B. parapertussis 23054		Laboratory of Pertussis
B. parapertussis 482		Laboratory of Pertussis
B. avium Gobl124		C. Gentry-Weeks
E. coli K-12 derivative, DH5α		Gibco BRL, Gaithersburg, Md.
Plasmids		
pUC18		26
pLAFR2	IncP1 *cos* Tc^r	6
pRK2013	IncP1 *tra oriE1* Km^r	6
pTF101	pUC18 with *vag-49*::Tn*phoA′* insert, Km^r	This study
pTF003	pLAFR2 with *vag-49*::Tn*phoA′* insert	This study
pZM101	4-kb *Sal*I-*Pst*I fragment containing *vag-49* in pBluescript	This study

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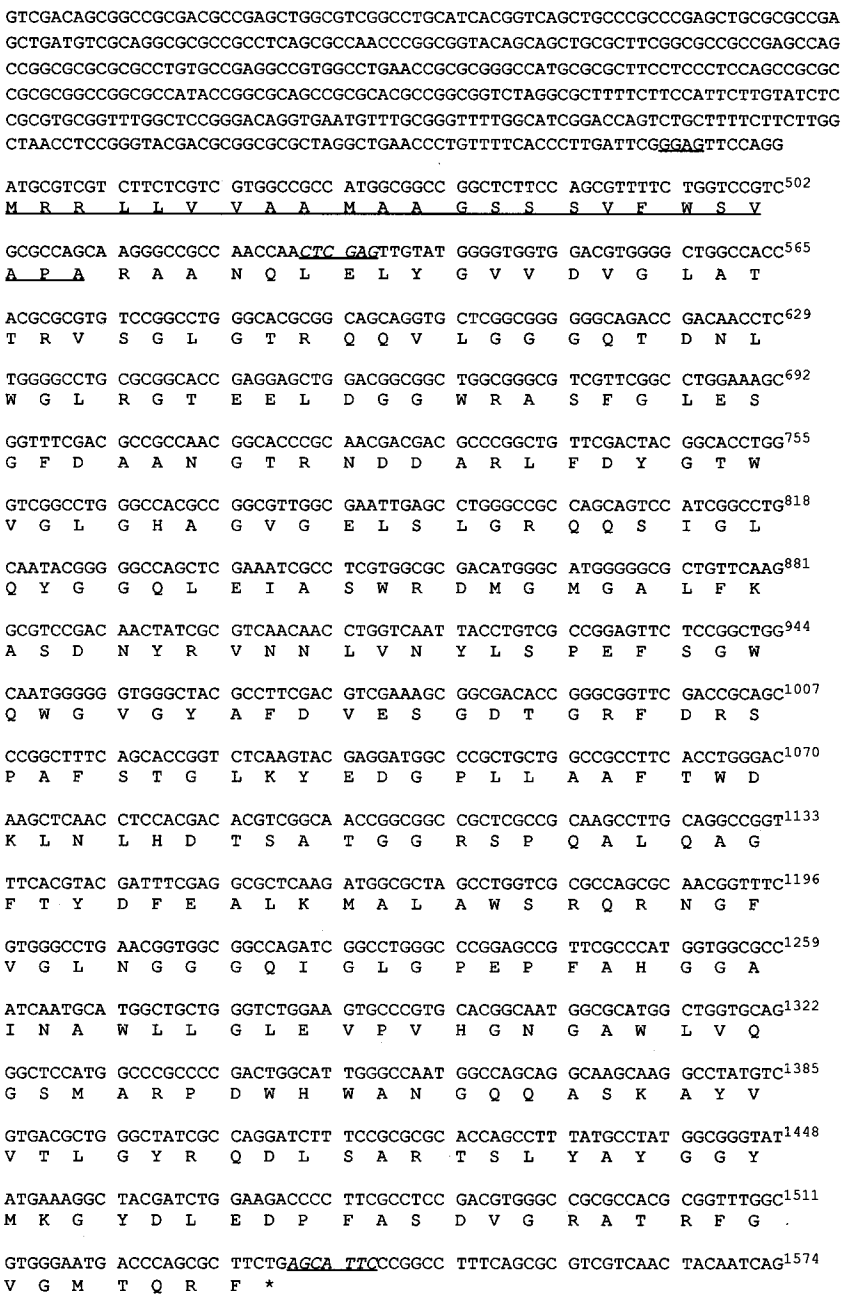

FIG. 1. Nucleotide sequence and derived amino acid sequence of *ompQ* (*vag-49*). The signal sequence is underlined. A possible ribosome binding site near the methionine start site is also underlined. The *Xho*I and *Bsm*I sites are underlined and in italic type.

regulated alkaline phosphatase activity (data not shown). Thus, the fusion was *bvg* regulated and contained all sequences required for regulation. The wild-type gene was isolated from a 18323 cosmid library (14) by using the 7-kb *Bam*HI fragment from pTF101 as a probe. A cosmid which reacted with this probe was subcloned as a 4-kb *Pst*I-*Sal*I fragment into pBluescript (Stratagene, La Jolla, Calif.) to generate pZM101. *ompQ* was sequenced from ExoIII subclones of pZM101 by the dideoxy termination method by Lark Sequencing Technologies (Austin, Tex.). The reading frame was deduced by sequencing M13 subclones of pTF101.

Nucleotide sequence analysis. Sequence analysis was performed with the Genetics Computer Group (GCG) package (version 7.0, April 1991) (4). Using FASTA to do a Pearson Lipman search within the GenBank database revealed that *vag-49* had 55% identity with the nucleotide sequence of the major porin of *B. pertussis* (16). This identity starts at the 5′ ends of both sequences, extending from the nucleotides encoding the signal sequences over 559 bp of the *vag-49* sequence (data not shown).

Amino acid sequence analysis. The derived amino acid sequence predicts the *vag-49* gene product, OmpQ, to be a protein of 364 amino acids with a molecular mass of 39,133 Da, (Fig. 1). The signal sequence terminates in the sequence Ala-Pro-Ala-Arg-Ala which contains two potential signal peptidase processing sites. We have designated the first of these Ala-X-

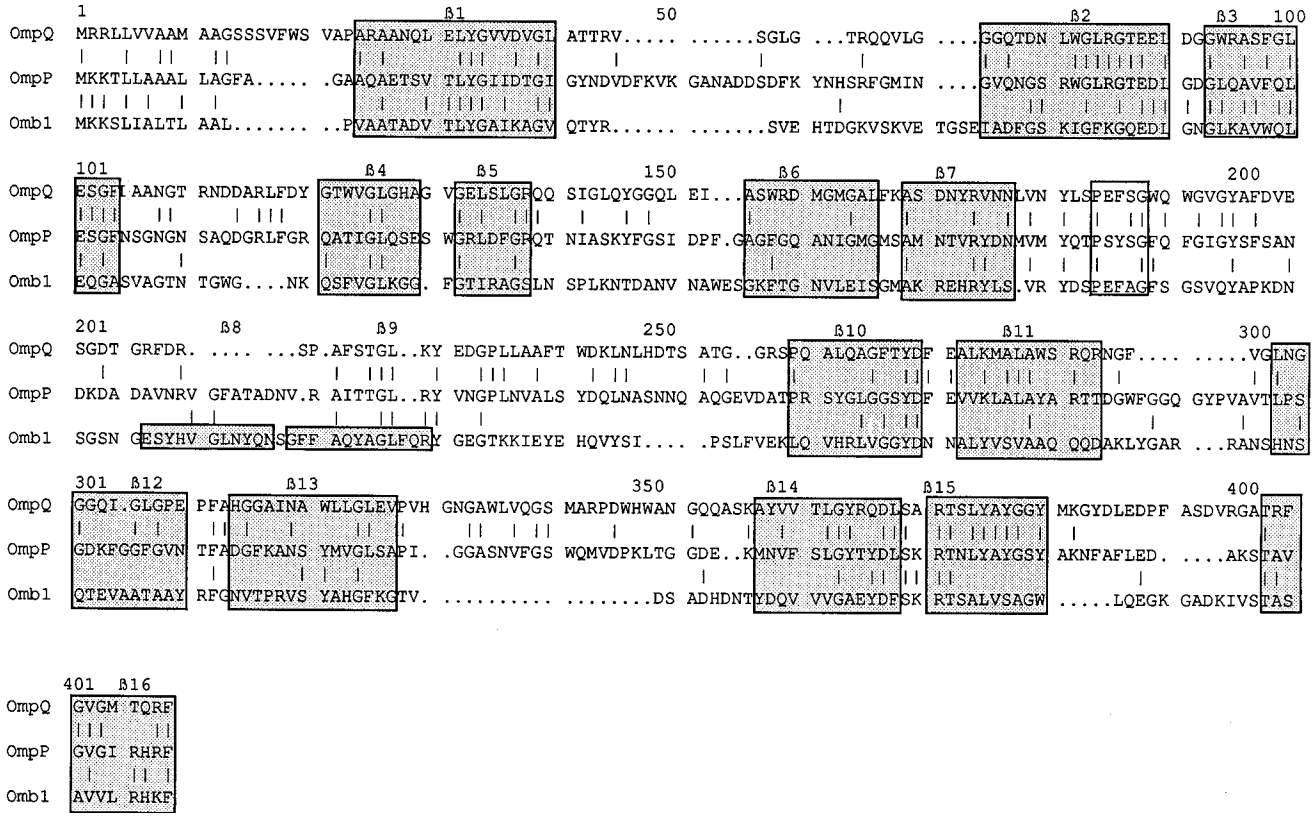

FIG. 2. Comparison of the amino acid sequences of the *B. pertussis* OmpQ, OmpP, and *N. gonorrhoeae* Omb1. Sequences were aligned with PILEUP within the GCG package. Identical residues are designated with bars. The beta sheets of *N. gonorrhoeae* Omb1 were predicted on the basis of the determined beta sheets of OmpF (3) and the porin superfamily alignment (10). The predicted beta sheets of *N. gonorrhoeae* Omb1 and aligned sequences of *B. pertussis* OmpP and OmpQ, which may represent the beta sheets of the *B. pertussis* proteins, are boxed and shaded. The PXXXG region is boxed.

Ala sequences as the termination of the signal sequence, which would give a 24-amino-acid signal sequence. Comparison of the amino acid sequence of the 39,103-Da constitutive porin, OmpP, with OmpQ, showed 61% similarity and 39% identity over the entire length of the two proteins when the GAP program was used. With FASTA in the GCG software package, 27% identity was seen over a 250-amino-acid overlap to the *Comomonas acidovorans* porin Omp32 (7). OmpQ also contains areas of conserved amino acids in common with enteric and neisserial porins. Using the GAP program in the GCG package, we found that OmpQ showed similarities of 50% to *E. coli* OmpF (SwissPro accession number P02931), 55% to *E. coli* OmpC (P06996), 46% to *Salmonella typhimurium* OmpC (P029878), 52% to the *Neisseria meningitidis* porin Oma2 (P18194), and 48% to the *Neisseria gonorrhoeae* Omb1 (P18195) (data not shown). The highest identity among this group was seen to Omb1 at 24.3%. Many of the sequence similarities are common to the trimeric, enteric, and neisserial porin superfamily (10). With the multiple sequence alignment program PILEUP within the GCG package, the sequences of the above porins were aligned with OmpP and OmpQ. The data were analyzed using the GCG DISTANCES program which indicated that the *B. pertussis* sequences were more similar to the neisserial porin family than to the enteric porin family (data not shown). Thus, PILEUP was run to align OmpQ, OmpP, and the *N. gonorrhoeae* Omb1 to predict where the membrane-spanning regions of the pertussis proteins might be (Fig. 2). A greater similarity to the neisserial porins than the enteric porins would imply that the longest loop of the porin monomer would be predicted to occur after the seventh beta sheet (10). This prediction is based on the positions of the PEFSG and PSYSG motifs of OmpQ and OmpP, respectively. In the largest loop of *E. coli* OmpF, between the fifth and sixth beta strands, there is a PEFGG motif (10). The crystallographic data indicates that this loop folds into the barrel constricting the size and shape of the pore at about half of the height of the barrel, defining the exclusion limit for diffusing particles (3, 25).

The identities between the *B. pertussis* proteins and Omb1 are mainly at the amino and carboxy termini of the proteins. The central area between the sixth and tenth beta sheets aligns less well. The sixth and seventh beta sheets predicted for neisserial porins (10) align with OmpP and OmpQ, but the predicted eighth and ninth beta sheets do not align. It is possible that the area of identity, between OmpQ and OmpP, downstream of the nonaligned sequence of OmpQ corresponds to the eighth and ninth beta sheets of the pertussis proteins. This is the area which aligns in both OmpQ and OmpP to the eighth and ninth beta sheets of *E. coli* OmpF when either GCG GAP is run on sequence pairs or a multiple sequence alignment is run (data not shown). Obviously a definitive alignment of the beta sheets must await X-ray crystallographic analysis of the pertussis porins.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel identification of OmpQ. OmpQ was seen to be a minor band migrating at 36 kDa, similar to the predicted size of 36.4 kDa after cleavage of the signal sequence (Fig. 3). This protein is slightly smaller than OmpP, the mature form of which is 37.2 kDa.

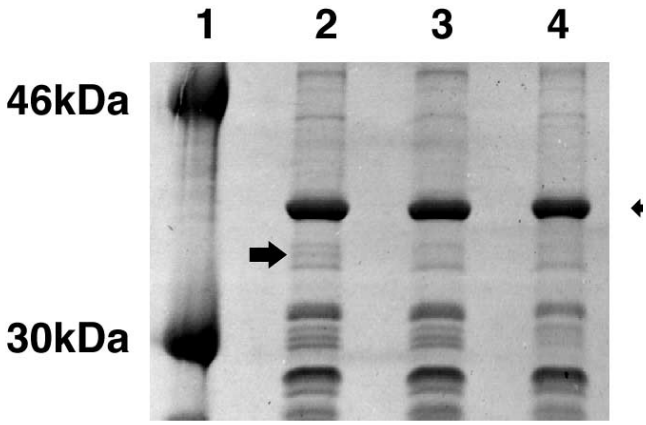

FIG. 3. SDS-polyacrylamide gel of outer membrane protein-enriched preparations from 18323 and SK49. Triton-insoluble outer membrane protein fractions were prepared from 2-day SS cultures by the method of Hantke (8). Samples were separated on SDS–12.5% polyacrylamide gels (ISS, Natick, Mass). Gels were stained by the Integrated Separations Systems Problue Staining System (Enprotech, Natick, Mass). Lane 1, prestained molecular mass markers (Amersham Corp., Arlington Hgts., Ill.); lane 2, 18323; lane 3, SK49; lane 4, 18323 grown in the presence of magnesium sulfate and nicotinic acid. The large arrow indicates the presence of OmpQ in unmodulated 18323. The major band at 40 kDa, indicated by the small arrowhead on the right, is OmpP. This picture was submitted as an Adobe Photoshop 2.5 file.

This band was confirmed to be a *vag* gene product by demonstrating that it is no longer present in outer membrane samples of 18323 grown in media containing nicotinic acid and magnesium sulfate.

Aerosol model. To determine whether OmpQ affects the ability of *B. pertussis* to persist in vivo, groups of 10 mice were challenged with an aerosol of SK49 or wild-type 18323 (12, 18). Lungs and tracheas were harvested separately 14 days after challenge, homogenized, and plated on BG agar to determine CFU. The recovery of 18323 from the lungs of mice was $\log_{10}$ 8.2712 (standard deviation [SD], $\pm$0.18), that of SK49 was 8.163 (SD, $\pm$0.23). Recovery of 18323 from the trachea was $\log_{10}$ 6.15 (SD, $\pm$0.163) and recovery of SK49 was 5.87 (SD, $\pm$0.51). These levels were not significantly different from each other at the $P < 0.05$ level of probability. Thus, the mutation did not affect the ability of the strain to colonize and persist in the mouse model nor did it affect the ability of the strain to cause lymphocytosis or death (results not shown).

Presence of *vag-49* in other *Bordetella* species. Southern blot analysis of chromosomal DNA from the other *Bordetella* species using a probe which extends from 528 to 1535 bp of the *vag-49* sequence (Fig. 1) indicated that the *vag-49* gene is present in *B. pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* (Fig. 4). There was no hybridization to DNA from *Bordetella avium.*

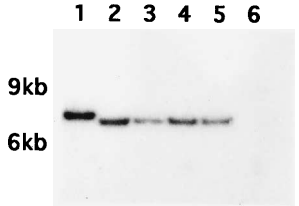

FIG. 4. *Sal*I-digested chromosomal DNAs from strains of *B. pertussis*, *B. bronchiseptica*, *B. parapertussis*, and *B. avium* were probed with a 1.1-kbp *Xho*I-*Bsm*I ^{32}P-labelled fragment of the *vag-49* structural gene. Lane 1, *B. pertussis* Tohama I; lane 2, *B. parapertussis* 23054; lane 3, *B. parapertussis* 482; lane 4, *B. bronchiseptica* 058; lane 5, *B. bronchiseptica* 110H; lane 6, *B. avium* GOBL124.

Summary. The *bvg*-regulated gene product OmpQ, encoded by *ompQ*, appears to be a porin-like protein. The predictions from the sequence analyses are that both OmpQ and the non-*bvg*-regulated OmpP are more similar to the neisserial than enteric porin family in overall structure. Lack of OmpQ did not have an adverse effect on the ability of the 18323 derivative, SK49, to survive in the mouse aerosol challenge model, nor did it appear to affect the ability of the strain to survive in vitro. The lack of effect in vivo was somewhat surprising, since we had postulated that this protein may have an important role in allowing access to an essential nutrient. In this model system, either the other outer membrane proteins may be able to substitute for OmpQ, or the protein may not be involved in a critical infectious process in the mouse model. However, OmpQ is expressed with the other virulence-associated determinants, and it is tempting to speculate that OmpQ plays a role during the infectious process, perhaps in the colonization of the human host or in the establishment of a carrier state.

Nucleotide sequence accession number. The nucleotide sequence of *ompQ* (*vag-49*) and the derived amino acid sequence have been deposited in the GenBank database and assigned accession number U16266.

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