

Overrepresentation of a Gene Family Encoding Extracytoplasmic Solute Receptors in *Bordetella*

Rudy Antoine,¹ Françoise Jacob-Dubuisson,^{1*} Hervé Drobecq,² Eve Willery,¹
Sarah Lesjean,¹ and Camille Locht¹

INSERM U447¹ and CNRS UMR 8525,² Institut de Biologie de Lille,
Institut Pasteur de Lille, 59019 Lille Cedex, France

Received 16 September 2002/Accepted 12 November 2002

A family of genes that are likely to encode extracytoplasmic solute receptors is strongly overrepresented in several β -proteobacteria, including *Bordetella pertussis*. This gene family, of which members have been called *bug* genes, contains some examples that are contained within polycistronic operons coding for tripartite uptake transporters of the TTT family, while the vast majority are “orphan” genes. Proteomic and functional analyses demonstrated that several of these genes are expressed in *B. pertussis*, and one is involved in citrate uptake. The *bug* genes probably form an ancient family that has been subjected to a large expansion in a restricted phylogenetic group.

A number of bacterial genome sequences contain large families of paralogs, genes that encode proteins of related, though not necessarily identical, functions (6, 13, 19). We have recently described a large gene family of unknown function in *Bordetella pertussis*, the whooping cough agent (3). These genes, named *bug* genes (*Bordetella* uptake genes; see below), code for predominantly hydrophilic proteins with predicted signal peptides. The first *bug* gene was identified while sequencing the regions flanking the pertussis toxin (PTX) locus and was thus called *bugT* (3). *B. pertussis* expresses its pathogenicity through the action of virulence factors coordinately regulated at the transcriptional level by the two-component regulatory system BvgAS (14, 21). Genes positively regulated by BvgAS are called *vag* genes (virulence-activated genes), while genes negatively regulated by BvgAS are called *vrg* genes (virulence-repressed genes) (8). In spite of its genomic position, *bugT* is not Bvg regulated, and its deletion did not affect the production or secretion of PTX (3). In contrast, another *bug* gene was found to correspond to the previously identified *vrg24* (4).

A homology search with TBLASTN using the predicted BugT protein sequence as the query sequence (3) was performed on the genome of *B. pertussis* TohamaI (www.sanger.ac.uk/Projects/B_pertussis). The genes for 90 other highly similar putative proteins were found, making the *bug* family one of the most populated in *B. pertussis* (http://www.ibl.fr/articles/jbact_antoine.htm lists the sequences of the Bug proteins). The G+C percentage (67.7%) and codon usage of the Bug proteins do not significantly differ from those of other *B. pertussis* genes. Among the 91 copies, 12 are not functional based on the currently available genomic data, carrying large internal deletions, frameshift mutations, or the *B. pertussis*-specific insertion sequence IS481. The *bug* genes are scattered throughout the

chromosome and are not systematically linked to virulence genes or insertion sequences (not shown). All 79 intact *bug* genes were numbered according to their position in the genome, with *bugT* being numbered 1 (3).

Bug genes in other bacterial species. The genomic sequences of more than 200 bacterial genomes whose sequencing is completed or in progress were scanned using BLASTP or TBLASTN to identify *bug* homologs (Table 1). *Bordetella bronchiseptica*, *Bordetella parapertussis*, and a β -proteobacterium relative, *Ralstonia metallidurans* (formerly *Alcaligenes eutrophus*), are so far the only species with as many *bug* paralogs as *B. pertussis*. Several other eubacteria have between 1 and 11 *bug* genes, with the larger numbers found in soil-dwelling bacteria. One *bug* was found so far in an archaeobacterium, *Methanosarcina barkeri*.

Salmonella enterica serovar Typhimurium has a gene called *tctC* that is homologous to *bug* genes (25, 27). *tctC* codes for a periplasmic citrate-binding protein (22) and is part of a polycistronic operon containing two other genes, *tctB* and *tctA*, that encode putative cytoplasmic membrane proteins (28) (Fig. 1). Recently, it has appeared that TctABC is the prototype of a new family of uptake transporters called tripartite tricarboxylate transporters (TTT) (www-biology.ucsd.edu/~msaier/transport/classf.html, TC no. 2.A0.80). The TTT systems are thus the third type of transporters, in addition to the ATP-binding cassette (ABC) and the tripartite ATP-independent periplasmic (TRAP) transporters, to make use of extracytoplasmic solute receptors (ESR) (12, 23).

All genomes containing *bug/tctC* homologs have between one and five operons homologous to *tctBA*, at least one of which includes a *bug/tctC* homolog (Table 1; Fig. 1). Two operons homologous to *tctBA* are present in *B. pertussis*. Only one of them forms a polycistronic operon with *bug4* (Fig. 1), while the other is bicistronic and does not comprise any *bug* gene. In several genomes, *bug/tctC* homologs are more abundant than *tctBA* homologs, indicating the existence of “orphans” (Table 1). In *B. pertussis*, these orphan *bug* genes are

* Corresponding author. Mailing address: INSERM U447, IBL, Institut Pasteur de Lille, 1 rue Calmette, 59019 Lille Cedex, France. Phone: 33 320 87 11 55. Fax: 33 320 87 11 58. E-mail: francoise.jacob@pasteur-lille.fr.

TABLE 1. Predicted *bug* and *tctBA* homologs in bacterial genomes

Bacterial species	No. of <i>bug</i> genes	No. of <i>tctBA</i> homologs ^a (no. of putative TTT-coding operons)
<i>Agrobacterium tumefaciens</i>	4	3 (3)
<i>Azotobacter vinelandii</i>	5	2 (1)
<i>Bacillus halodurans</i>	1	1 (1)
<i>Bordetella bronchiseptica</i>	181	4 (1)
<i>Bordetella parapertussis</i>	143	4 (1)
<i>Bordetella pertussis</i>	79	2 (1)
<i>Burkholderia fungorum</i>	11	4 (2)
<i>Corynebacterium glutamicum</i>	1	1 (1)
<i>Desulfotobacterium hafniense</i>	5	5 (3)
<i>Erwinia chrysanthemi</i>	4	1 (1)
<i>Escherichia coli</i> K1	1	1 (1)
<i>Escherichia coli</i> CFT073	1	1 (1)
<i>Methanosarcina barkeri</i>	1	1 (1)
<i>Mycobacterium smegmatis</i>	2	2 (2)
<i>Pasteurella multocida</i>	1	1 (1)
<i>Pseudomonas aeruginosa</i>	2	2 (2)
<i>Pseudomonas fluorescens</i>	1	1 (1)
<i>Pseudomonas putida</i> KT2440	1	1 (1)
<i>Pseudomonas syringae</i>	1	1 (1)
<i>Ralstonia metallidurans</i>	102	5 (2)
<i>Ralstonia solanacearum</i>	2	1 (1)
<i>Rhodobacter sphaeroides</i>	3	3 (3)
<i>Rhodospseudomonas palustris</i>	7	2 (2)
<i>Salmonella enterica</i> serovar Typhimurium	1	1 (1)
<i>Silicibacter pomeroyi</i>	5	5 (1)
<i>Sinorhizobium meliloti</i>	5	4 (4)
<i>Streptomyces coelicolor</i>	1	1 (1)
<i>Thermobifida fusca</i>	1	1 (1)
<i>Vibrio cholerae</i>	1	1 (1)

^a *tctA* and *tctB* homologs are always found within operons, usually in the order *BA*.

either in monocistronic operons or in polycistronic operons involved in various metabolic pathways.

A functional TTT system in *B. pertussis*. Since *bug4* is the only *B. pertussis* *bug* gene in a TTT-coding operon, we investigated whether this operon is involved in citrate uptake, similar to its *tctCBA* relative. The following three isogenic strains were used to monitor the in vivo incorporation of radiolabeled citrate: the Tohama I derivative BPSM (16) and two mutants, one with a nonpolar in-frame deletion of *bug4* and the other carrying a knockout mutation of the *tctA* homolog coding for the large putative membrane component of the transporter and generated by the targeted insertion of the suicide vector pFUS2 (2). The bacteria were grown in liquid modified Steiner-Scholte (MSS) medium as described previously (15). When the cultures reached optical densities of 1.5 at 600 nm, 1 mM sodium citrate (pH 7) was added, because transcriptional fusion analyses have indicated that the operon is induced by citrate (our unpublished observations). The cells were harvested 1 h later by centrifugation and resuspended in fresh MSS medium without citrate. Sixty micromolar [1,5-¹⁴C]citric acid was added (Amersham Pharmacia Biotech), and aliquots were taken after increasing labeling times. Cells were harvested by centrifugation and washed twice in MSS medium. Cell pellets were analyzed by scintillation counting. The wild-type strain incorporated citrate at a very fast rate (Fig. 2), in contrast to the mutant strains, demonstrating that both the

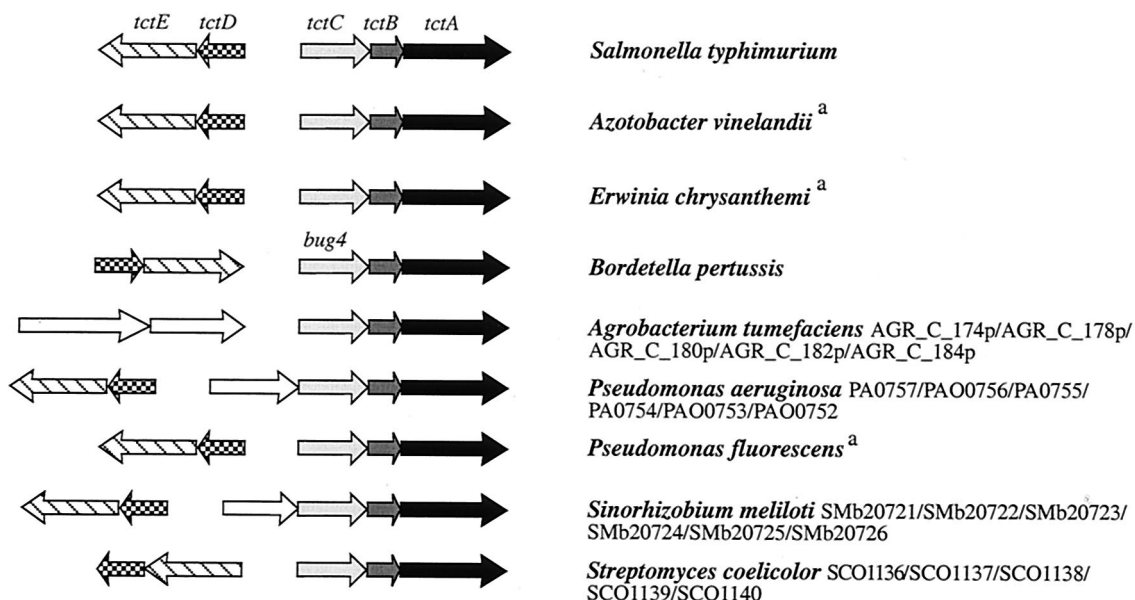
Bug4 protein and the TctA ortholog of *B. pertussis* are involved in citrate uptake. Whether the low residual citrate uptake activity was mediated by another transporter has not been investigated further.

Phylogenetic analyses. Multiple alignments of 253 Bug proteins without their predicted signal peptides were performed with ClustalW and used to construct a phylogenetic tree using the PAUP software (<http://www.lms.si.edu/PAUP/>). This analysis revealed one small cluster of closely related Bug proteins whose genes are part of polycistronic operons involved in catechol utilization and carried on mobile genetic elements, suggesting that it has arisen from recent horizontal transfer (<http://www.ibl.fr/articles/jbact.antoine.htm> [Fig. A1 and Table A2]). Intriguingly, these operons did not appear to comprise genes coding for membrane components of transporters. Another cluster comprises Bug proteins whose genes are found in TTT-coding operons, including TctC of *S. enterica* serovar Typhimurium and a single Bug protein of *B. pertussis*, Bug4. The proteins of this latter cluster belong to evolutionarily divergent species, including both gram-positive and gram-negative bacteria, which suggests that they represent ancestral members. The other Bug proteins have diverged more widely. In particular, the levels of divergence between paralogs of *B. pertussis* or of *R. metallidurans* are similar to those between Bug proteins of the two species, suggesting ancient events of duplication and divergence in a common ancestor (19). Nevertheless, Bug proteins clearly form a distinct family, as evidenced by phylogenetic analyses incorporating several bona fide members of other ESR families (23; also data not shown).

The presence of a large number of orphan *bug* genes not included in TTT-coding operons in *Bordetella* and *R. metallidurans* remains to be explained. It is possible that several Bug proteins interact with the same set of TctAB homologs, which could represent a thrifty way to diversify transport capabilities with a minimal number of low-specificity membrane components. Alternatively, some orphan Bug proteins may have been recruited by ABC or TRAP transporters (12, 23). In addition, some Bug proteins may be involved in signal transduction, as described for other ESRs (12, 23). We have obtained preliminary evidence that Bug4 might play a role in signaling the presence of citrate (our unpublished observations).

Expression of *bug* genes. To determine whether orphan *bug* genes are expressed in *B. pertussis*, a representative set was chosen for transcriptional analysis by use of *lacZ* fusions (2). Most genes appeared to be expressed, albeit at various levels (Table 2). No *bug* gene whose expression is positively regulated by BvgAS was identified. In contrast, after the addition of nicotinic acid or magnesium sulfate, which are both negative modulators of the virulence regulon, to the medium, the expression of the *bug71* gene was increased approximately 2.5-fold. This indicates that the *bug71* gene is a *vrg*, similar to *bug73/vrg24* (2). The *bug71* gene is found in the vicinity of the *B. pertussis* dermonecrotic toxin locus (3), and its ortholog in *B. bronchiseptica* has recently been reported to be a *vrg* as well (20). The biological functions of the *vrg*-encoded proteins in *B. pertussis* remain unknown, while in *B. bronchiseptica* several are involved in metabolic pathways or in survival under unfavorable conditions (5, 8, 20). As suggested recently, these proteins might have been relevant to nonpathogenic, free-living ancestors of present-day pathogenic *Bordetella* (11, 26).

A



B

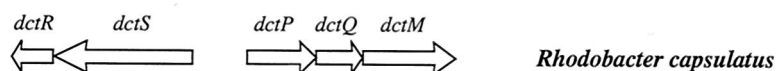


FIG. 1. Organization of selected operons encoding predicted TTTs and comparison with the *dctPQM* operon that encodes the archetype of TRAP transporters. (A) *tct* operon of *S. enterica* serovar Typhimurium is shown as the paradigm for TTT systems. Homologs of *tctC*, *tctB*, and *tctA* are shown in light gray, dark gray, and black, respectively. Homologs of *tctE* and *tctD*, coding for the sensor kinase and response regulator of a two-component system involved in the regulation of *tctCBA* (29), are depicted as hatched and checkered arrows, respectively. Among the three predicted TTT-coding operons of *Agrobacterium tumefaciens*, only the one depicted here is preceded by an operon encoding a two-component system. It is not homologous to TctDE but to DctRS of *Rhodobacter capsulatus*, involved in the regulation of the dicarboxylate transporter operon *dctPQM* (see panel B). Additional genes (in white) are found in a few operons; these include genes presumably coding for a porin in *Pseudomonas aeruginosa* and for an ESR associated with ABC uptake transporters in *Sinorhizobium meliloti*.^a, no accession numbers are yet available for *Azotobacter vinelandii*, *Erwinia chrysanthemi*, and *Pseudomonas fluorescens*. (B) The *dctPQM* operon that encodes the dicarboxylate TRAP transporter of *Rhodobacter capsulatus* (10) and the adjacent *dctRS* operon that encodes a two-component system are shown for comparison purposes. Although not homologous, TTT and TRAP transporters have similar molecular organizations.

Proteomic analyses of Bug proteins. To identify Bug proteins, total protein extracts of the BPSM derivative BPDR (FHA⁻ PTX⁻) (1) were analyzed by two-dimensional (2-D) electrophoresis using immobilized pH 4 to 7 or 6 to 9 gradient strips (Pharmacia). BPDR was grown in MSS medium to an optical density at 600 nm of 2. The cells were then harvested by centrifugation, and the samples for 2-D electrophoresis were prepared as described previously (24), except that the cells were broken by three passages through a French pressure cell. Proteins in the 20- to 45-kDa mass range were identified by mass fingerprinting (9) (Fig. 3). Six Bug proteins, Bug2, Bug4, Bug9, Bug20, Bug71, and Bug73, were identified. In particular, the Bug9 protein was one of the most intense protein spots in the gels. Several other abundant proteins in that region of the gel were found to correspond to predicted ESRs that belong to ABC and TRAP transporters (Fig. 3) (<http://www.ibl.fr/articles/jbact.antoine.htm> [Table A3] shows the sequences of these proteins).

B. pertussis has large coding capabilities for transport systems, including approximately 100 ABC transporters and a dozen TRAP transporters (our unpublished observations), and these proteomic analyses demonstrate that it produces a number of such proteins. Generally, the global transport and met-

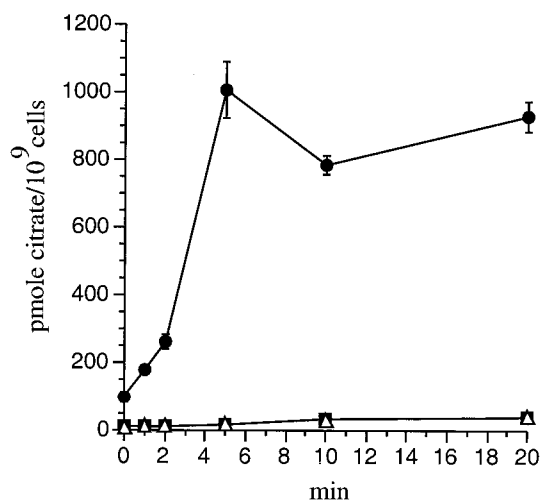


FIG. 2. Citrate uptake by *B. pertussis* strains. BPSM carrying an intact copy of the operon (black circles) and two isogenic mutants, one with an in-frame deletion of the *bug4* gene (black squares) and the other carrying a knockout mutation of the *B. pertussis tctA* ortholog (open triangles), were used to monitor the uptake of radiolabeled citrate over time. The results are expressed as picomoles of radioactive citrate incorporated by 10^9 cells.

TABLE 2. β -Galactosidase activities of *B. pertussis* *bug-lacZ* recombinants

Target gene	β -Galactosidase activity (Miller units) ^a in:		
	BG ^b	BG + MgSO ₄	BG + NA
<i>bug1</i>	40 ± 1	33 ± 7	36 ± 9
<i>bug4</i>	77 ± 9	107 ± 22	132 ± 27
<i>bug9</i>	2,863 ± 997	2,639 ± 603	1,998 ± 240
<i>bug20</i>	205 ± 39	292 ± 97	274 ± 61
<i>bug24</i>	<5 ^c	<5	<5
<i>bug43</i>	411 ± 109	343 ± 118	346 ± 53
<i>bug60</i>	22 ± 4	49 ± 5	31 ± 5
<i>bug64</i>	85 ± 40	107 ± 37	93 ± 26
<i>bug71</i>	1,193 ± 61	2,178 ± 292	2,964 ± 399
<i>bug73</i>	207 ± 15	490 ± 50	496 ± 60
<i>bug79</i>	323 ± 161	267 ± 171	209 ± 52

^a Data are means ± standard deviations from 3 to 5 independent experiments.

^b BG, Bordet Gengou medium; NA, 20 mM nicotinic acid.

^c Below the limit of detection.

abolic capacities of an organism correlate with its ecological niche(s), and the expansion of some gene families is linked to their ability to generate physiological diversification (18, 19). The large numbers of *bug* genes in *Bordetella* and *R. metallidurans* indicate that they probably fulfill important functions or have done so during the evolutionary history of these bacteria. In this respect, large transport capabilities are most likely an asset to soil-dwelling *R. metallidurans*. In contrast, *B. pertussis* is a strict pathogen with more-restricted metabolic capabilities, but it is believed to have originated from free-living ancestors (11, 26). That 12 of the *B. pertussis bug* genes appear to be nonfunctional may indicate some genome decay, as has been observed for many genes in other pathogens (7, 17). If *bug* genes have evolved to bind and transport a large range of substrates for the complex metabolism of soil-dwelling microorganisms, it is plausible that some have become obsolete in the ecologically specialized, human-adapted *B. pertussis*.

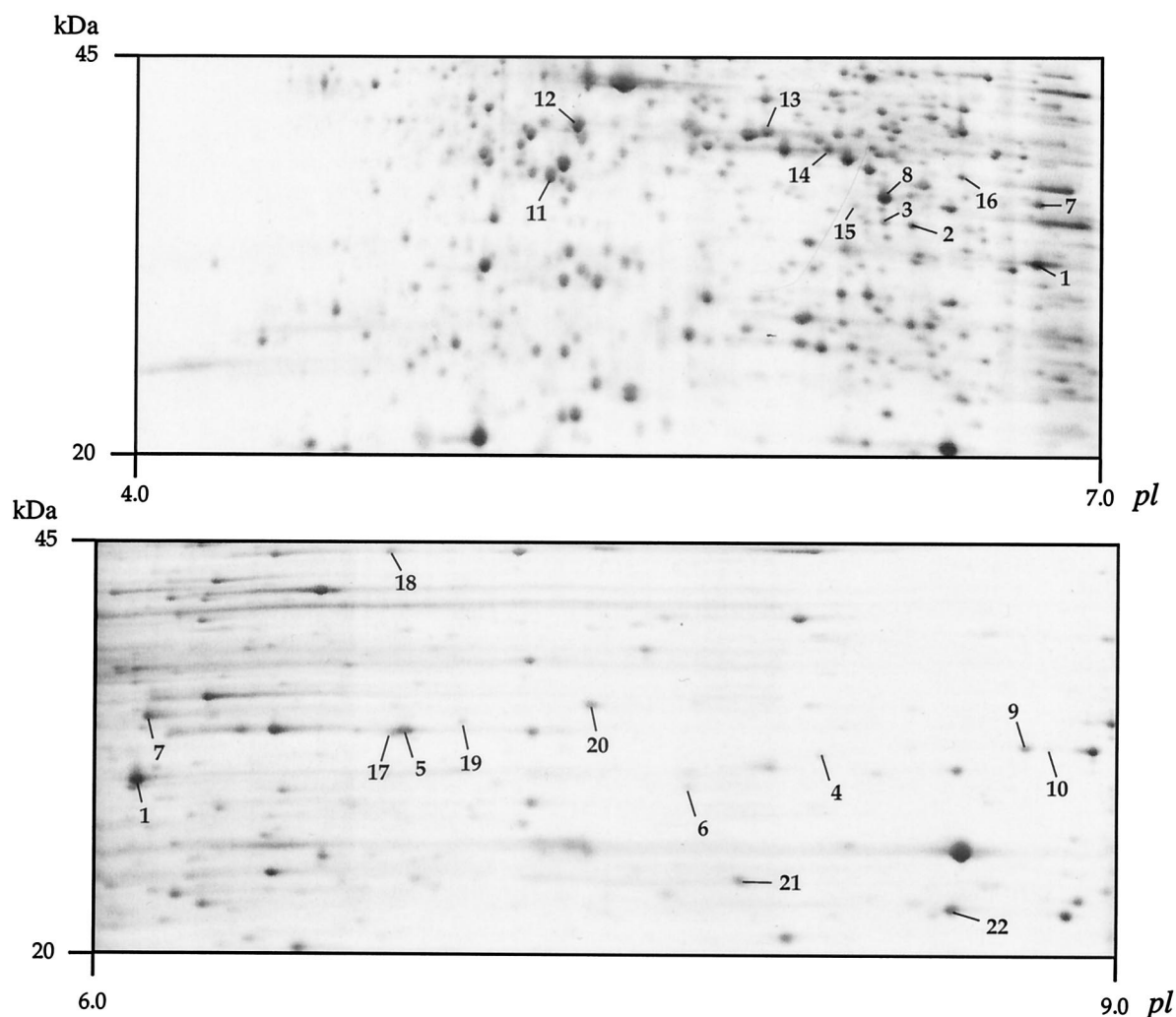


FIG. 3. Analyses of *B. pertussis* BPCR proteins by 2-D electrophoresis. The portions of pH 4 to 7 (top) and pH 6 to 9 (bottom) silver-stained gels corresponding to the 20- to 45-kDa range are shown. Only the putative periplasmic proteins involved in solute binding as identified by mass fingerprinting have been numbered (1 to 6, Bug9, -2, -73, -20, -71, and -4, respectively; 7 to 10, predicted TRAP transporter-associated ESRs; 11 to 22, predicted ABC transporter-associated ESRs). See <http://www.ibl.fr/articles/jbact-antoine.htm> (Table A3) for the sequences of these 22 proteins.

R.A. and F.J.-D. contributed equally to this work.

We thank Julian Parkhill of the Sanger Center for his authorization to use the *Bordetella* genomic data prior to publication. We are grateful to P. Supply, A. Baulard, and J. Dubuisson for critical reading of the manuscript and to P. Supply for his help with phylogenetic analyses. The sequence data used in this work were obtained from ftp://ftp.sanger.ac.uk/pub/pathogens/bp/.

H.D. is supported by the University des Sciences et Technologies de Lille I. F.J.-D. is a researcher of the CNRS. This work was supported in part by INSERM, the Institut Pasteur de Lille, and the Région Nord-Pas-de Calais.

REFERENCES

- Alonso, S., K. Pethe, N. Mielcarek, D. Raze, and C. Locht. 2001. Role of ADP-ribosyltransferase activity of pertussis toxin in toxin-adhesin redundancy with filamentous hemagglutinin during *Bordetella pertussis* infection. *Infect. Immun.* **69**:6038–6043.
- Antoine, R., S. Alonzo, D. Raze, L. Coutte, S. Lesjean, E. Willery, C. Locht, and F. Jacob-Dubuisson. 2000. New virulence-activated and virulence-repressed genes identified by systematic gene inactivation and generation of transcriptional fusions in *Bordetella pertussis*. *J. Bacteriol.* **182**:5902–5905.
- Antoine, R., D. Raze, and C. Locht. 2000. Genomics of *Bordetella pertussis* toxins. *Int. J. Med. Microbiol.* **290**:301–305.
- Beattie, D. T., S. Knapp, and J. J. Mekalanos. 1990. Evidence that modulation requires sequences downstream of the promoters of two *vir*-repressed genes of *Bordetella pertussis*. *J. Bacteriol.* **172**:6997–7004.
- Beattie, D. T., M. J. Mahan, and J. J. Mekalanos. 1993. Repressor binding to a regulatory site in the DNA coding sequence is sufficient to confer transcriptional regulation of the *vir*-repressed genes (*vrg* genes) in *Bordetella pertussis*. *J. Bacteriol.* **175**:519–527.
- Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry, III, et al. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**:537–544.
- Cole, S. T., K. Eiglmeier, J. Parkhill, K. D. James, N. R. Thomson, P. R. Wheeler, N. Honore, T. Garnier, C. Churcher, D. Harris, et al. 2001. Massive gene decay in the leprosy bacillus. *Nature* **409**:1007–1011.
- Cotter, P. A., and V. J. DiRita. 2000. Bacterial virulence gene regulation: an evolutionary perspective. *Annu. Rev. Microbiol.* **54**:519–565.
- Coutte, L., R. Antoine, H. Drobecq, C. Locht, and F. Jacob-Dubuisson. 2001. Subtilisin-like autotransporter serves as maturation protease in a bacterial secretion pathway. *EMBO J.* **18**:5040–5048.
- Forward, J. A., M. C. Behrendt, N. R. Wyborn, R. Cross, and D. J. Kelly. 1997. TRAP transporters: a new family of periplasmic solute transport systems encoded by the *dctPQM* genes of *Rhodobacter capsulatus* and by homologs in diverse gram-negative bacteria. *J. Bacteriol.* **179**:5482–5493.
- Gerlach, G., F. von Wintzingerode, B. Middendorf, and R. Gross. 2001. Evolutionary trends in the genus *Bordetella*. *Microbes Infect.* **3**:61–72.
- Kelly, D. J., and G. H. Thomas. 2001. The tripartite ATP-independent periplasmic (TRAP) transporters of bacteria and archaea. *FEMS Microbiol. Rev.* **25**:405–424.
- Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, et al. 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**:249–256.
- Locht, C., R. Antoine, and F. Jacob-Dubuisson. 2001. *Bordetella pertussis*, molecular pathogenesis under multiple aspects. *Curr. Opin. Microbiol.* **4**:82–89.
- Locht, C., M.-C. Geoffroy, and G. Renaud. 1992. Common accessory genes for the *Bordetella pertussis* filamentous hemagglutinin and fimbriae share sequence similarities with the *papC* and *papD* gene families. *EMBO J.* **11**:3175–3183.
- Menozi, F. D., R. Mutombo, G. Renaud, C. Gantiez, J. H. Hannah, E. Leininger, M. J. Brennan, and C. Locht. 1994. Heparin-inhibitable lectin activity of the filamentous hemagglutinin adhesin of *Bordetella pertussis*. *Infect. Immun.* **62**:769–778.
- Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. Holden, M. B. Prentice, M. Sebahia, K. D. James, C. Churcher, K. L. Mungall, et al. 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature* **413**:523–527.
- Paulsen, I. T., L. Nguyen, M. K. Sliwinski, R. Rabus, and M. H. Saier, Jr. 2000. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. *J. Mol. Biol.* **301**:75–100.
- Saier, M. H., Jr., and I. T. Paulsen. 1999. Paralogous genes encoding transport proteins in microbial genomes. *Res. Microbiol.* **150**:689–699.
- Schneider, B., D. Stubs, and R. Gross. 2002. Identification and genomic organization of gene loci negatively controlled by the virulence regulatory BvgAS two-component system in *Bordetella bronchiseptica*. *Mol. Genet. Genomics* **267**:526–535.
- Smith, A. M., C. A. Guzman, and M. J. Walker. 2001. The virulence factors of *Bordetella pertussis*: a matter of control. *FEMS Microbiol. Rev.* **25**:309–333.
- Sweet, G. D., C. M. Kay, and W. W. Kay. 1984. Tricarboxylate-binding proteins of *Salmonella typhimurium*. Purification, crystallization, and physical properties. *J. Biol. Chem.* **259**:1586–1592.
- Tam, R., and M. H. Saier, Jr. 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. *Microbiol. Rev.* **57**:320–346.
- Tonella, L., B. J. Walsh, J. C. Sanchez, K. Ou, M. R. Wilkins, M. Tyler, S. Frutiger, A. A. Gooley, I. Pescaru, R. D. Appel, et al. 1998. '98 *Escherichia coli* SWISS-2DPAGE database update. *Electrophoresis* **19**:1960–1971.
- Valdivia, R. H., D. M. Cirillo, A. K. Lee, D. M. Bouley, and S. Falkow. 2000. mig-14 is a horizontally acquired, host-induced gene required for *Salmonella enteritica* lethal infection in the murine model of typhoid fever. *Infect. Immun.* **68**:7126–7131.
- von Wintzingerode, F., A. Schattke, R. A. Siddiqui, U. Rosick, U. B. Gobel, and R. Gross. 2001. *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *Int. J. Syst. Evol. Microbiol.* **51**:1257–1265.
- Widenhorn, K. A., W. Boos, J. M. Somers, and W. W. Kay. 1988. Cloning and properties of the *Salmonella typhimurium* tricarboxylate transport operon in *Escherichia coli*. *J. Bacteriol.* **170**:883–888.
- Widenhorn, K. A., J. M. Somers, and W. W. Kay. 1988. Expression of the divergent tricarboxylate transport operon (*tctI*) of *Salmonella typhimurium*. *J. Bacteriol.* **170**:3223–3227.
- Widenhorn, K. A., J. M. Somers, and W. W. Kay. 1989. Genetic regulation of the tricarboxylate transport operon (*tctI*) of *Salmonella typhimurium*. *J. Bacteriol.* **171**:4436–4441.