The Ornithine Decarboxylase Gene odc Is Required for Alcaligin Siderophore Biosynthesis in *Bordetella* spp.: Putrescine Is a Precursor of Alcaligin

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Chromosomal insertions defining *Bordetella bronchiseptica* siderophore phenotypic complementation group III mutants BRM3 and BRM5 were found to reside approximately 200 to 300 bp apart by restriction mapping of cloned genomic regions associated with the insertion markers. DNA hybridization analysis using *B. bronchiseptica* genomic DNA sequences flanking the cloned BRM3 insertion marker identified homologous *Bordetella pertussis* UT25 cosmids that complemented the siderophore biosynthesis defect of the group III *B. bronchiseptica* mutants. Subcloning and complementation analysis localized the complementing activity to a 2.8-kb *B. pertussis* genomic DNA region. Nucleotide sequencing identified an open reading frame predicted to encode a polypeptide exhibiting strong similarity at the primary amino acid level with several pyridoxal phosphate-dependent amino acid decarboxylases. Alcaligin production was fully restored to group III mutants by supplementation of iron-depleted culture media with putrescine (1,4-diaminobutane), consistent with defects in an ornithine decarboxylase activity required for alcaligin siderophore biosynthesis. Concordantly, the alcaligin biosynthesis defect of BRM3 was functionally complemented by the heterologous *Escherichia coli* odc gene encoding an ornithine decarboxylase activity. Enzyme assays confirmed that group III *B. bronchiseptica* siderophore-deficient mutants lack an ornithine decarboxylase activity required for the biosynthesis of alcaligin. Siderophore production by an analogous mutant of *B. pertussis* constructed by allelic exchange was undetectable. We propose the designation *odc* for the gene defined by these mutations that abrogate alcaligin siderophore production. Putrescine is an essential precursor of alcaligin in *Bordetella* spp.

Nutritional iron limitation, mediated primarily by specific host iron-binding glycoproteins, is a front-line host defense against disease-causing infectious agents. Strategies aimed at defeating host iron restriction may involve the action of low-molecular-mass, high-affinity, ferric iron-specific chelators of microbial origin, termed siderophores, that are synthesized coordinately with their cognate surface receptors and transport machinery in response to iron starvation (28). The role of siderophores in microbial pathogenesis is well established (29, 53, 54).

*Bordetella pertussis*, the causative agent of human whooping cough or pertussis, and *Bordetella bronchiseptica*, the agent of swine atrophic rhinitis and kennel cough in dogs, are mucosal pathogens that colonize the upper respiratory tracts of their mammalian hosts. In the first reported molecular genetic studies of *Bordetella* iron acquisition systems, Armstrong and Clements (3) described the isolation of *B. bronchiseptica* mutants deficient in siderophore activity following transposon mutagenesis. DNA hybridization analysis using DNA probe sequences flanking the transposon insertions established the existence of homologs of *B. bronchiseptica* siderophore genes in *B. pertussis*. Reciprocal cross-feeding experiments provided additional support for the hypothesis that the hydroxamate siderophores produced and utilized by *B. pertussis* and *B. bronchiseptica* were structurally similar or identical. In a subsequent report, complementation of deregulated iron transport system mutants of *B. bronchiseptica* by the fur gene of *B. pertussis* (9) confirmed the involvement of common regulatory determinants governing iron transport in the two related species.

Recent purification and spectroscopic analysis of *Bordetella* siderophores (10, 33) found that the iron chelators produced by both *B. pertussis* and *B. bronchiseptica* were identical to the potent macrocyclic dihydroxamate siderophore alcaligin, 1,8(S), 11,18(S)-tetrahydroxy-1,6,11,16-tetraazacycloeicosane-2,5,12,15-tetraeno (molecular formula, C_{16}H_{28}N_{4}O_{8}; molecular weight, 404), previously isolated from the taxonomically related bacterial species *Alcaligenes denitrificans* subsp. *xylosoxydans* (35, 36). Evidence for biological activity of the purified siderophores was gathered with growth stimulation and ^{55}Fe-alcaligin transport assays of *Bordetella* spp. (10). Uptake rates and saturability of iron uptake observed in ^{55}Fe transport assays were indicative of a high-affinity ferric alcaligin transport system and provided the first direct evidence, beyond simple growth stimulation assays, of alcaligin-mediated *Bordetella* iron transport.

We report the identification of a gene encoding an alcaligin siderophore biosynthetic activity defined by previously reported transposon mutations abrogating alcaligin production in *B. bronchiseptica* (3) as an ornithine decarboxylase (Odc) gene, indicating that putrescine is an essential precursor for alcaligin siderophore biosynthesis in *Bordetella* spp.

**MATERIALS AND METHODS**

Bacterial strains and plasmids. *B. bronchiseptica* B013N, a nalidixic acid-resistant derivative of strain B013, and the isolation of siderophore biosynthetic mutants BRM3 and BRM5 by mini-Tn5lacZ1(16) insertional inactivation have been described previously (3). Virulent-phase *B. pertussis* UT25 has also been described previously (18), *Escherichia coli* DH5α [*F′ F’λlacZΔM15 Δ(lacZYA-argF)]U169 endA1 recA1 hsdR17(rK- mK−) deoR thi-1 supE44 λ− gyrA96 relA1].

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(Bethesda Research Laboratories, Gaithersburg, Md.) was used as the host strain for routine plasmid construction and propagation and as the donor strain in conjugal transfer of plasmids to Bordetella strains. Plasmid pRK3/2.2 carrying the speC constitutive ornithine decarboxylase gene of E. coli, was constructed by subcloning the 3.2-kb E. coli PstI insert DNA fragment of plasmid pODCI (8) into the unique SalI site of plasmid vector pBRM3 (8). DH5α was used as the source of plasmid-encoded mobilization functions (19) in triparental matings to transfer the cosmid pCP13-based gene bank of B. pertussis UT25 (11) as well as plasmid vector pRK415 derivatives to Bordetella strains. E. coli K38 (pCP1-2) (46) was used as the host strain in Bordetella protein expression studies. 

Growth conditions. B. pertussis was maintained on Bordet-Gengou agar plates (7), and B. bronchiseptica was maintained on blood agar plates or standard Luria-Bertani agar plates. Modified Stainer-Scholte medium (39, 41) (SS) was used to grow each of Bordetella spp. in liquid culture. Medium conditions were achieved by the methods of Armstrong and Clements (3); media and supplements were purchased from Chrome-Lex 100 resin (Bio-Rad Laboratories, Richmond, Calif.). Tetracycline was used at 15 μg/ml to select for pCP13-based cosmids and pRK415 plasmid derivatives. Kanamycin was used at 50 μg/ml for maintenance of pRK3/2.2 and pCP1-2 and for selection of kanamycin cassette insertion markers in allelic exchange procedures, while ampicillin was used at 100 μg/ml for maintenance of other plasmids in E. coli. Culture media were supplemented as required with cadaverine, arginine, putrescine, ornithine, arginine, or spermidine at a 50-μg/ml final concentration, or with 1,4-diamino-2-butane dihydrochloride (DAB) (Sigma Chemical Co., St. Louis, Mo.) at concentrations ranging from 0 to 2 mM. All glassware was acid cleaned and rinsed with distilled deionized water prior to use. Optical densities of SS cultures were measured with a Klett-Summerson colorimeter fitted with a no. 54 filter (Klett Manufacturing Co., Long Island City, N.Y.).

Conjugation. Conjugal transfer of pCP13 and pRK415 derivatives to Bordetella strains was accomplished by triparental mating of E. coli DH5α as the E. coli donor strain and DH5α (pRK2013) as the source of mobilization functions. Susensions of donor, helper, and recipient strains in SS plus 10 mM MgSO4 were combined at an estimated 1:1:3 cell ratio, spotted onto Luria-Bertani or blood agar plates (B. bronchiseptica recipients) or Bordet-Gengou agar plates (for B. pertussis recipients), and incubated at 37°C for 3 to 5 h. Bacteria were streaked from mating spots directly onto agar plates containing the appropriate selective antibiotics and incubated at 37°C for 18 to 24 h. Plates were scored for resistance to chloramphenicol and folic acid.

Routine DNA procedures. DNA cloning and hybridization analysis were performed as described previously (38), and transformation of E. coli was by the CaCl2 method of Cohen and coworkers (14).

Radiochemicals. Tran 35S-label used for radiolabeling plasmid-encoded B. pertussis proteins in E. coli, [3H]dATP for nucleotide sequencing, and [32P]dCTP for DNA hybridization analysis were purchased from ICN Radiochemicals (Irvine, Calif.). Tr-1-14C]ornithine used in Odc assays and for DNA hybridization analysis were purchased from ICN Radiochemicals (Irvine, Calif.).

Conditional expression of plasmid-encoded Bordetella proteins in E. coli. Exclusive expression of Bordetella proteins in E. coli used the temperature-sensitive T7 RNA polymerase-promoter system of Tabor and Richardson (46). Recombinant DNA plasmids containing cloned 2.8-kb odc DNA fragment of B. pertussis UT25 in the T7 promoter plasmid vector pGEM4Z (Promega Corporation, Madison, Wis.). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of radiolabeled bacterial cell proteins was performed essentially as described previously (27).

Nucleotide sequencing. Nucleotide sequencing was performed by the dideoxy chain termination method with a Sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, Ohio). Double-stranded plasmid templates were isolated by the alkaline lysis procedure (6) and further purified by precipitation in the presence of 500 mM sodium chloride and 6.5% polyethylene glycol 8000. Nucleotide sequence data were derived from plasmid subclones of odc of B. pertussis cosms, as well as from a collection of deletion derivatives generated by treatment of odc plasmid subclones with exonuclease HI (21) by using a commercially available kit (Erase-a-Base; Promega Corporation) or from deletion derivatives resulting from in vivo intramolecular transposition of transposon γ (52) with the Deletion Factory System version 2.0 (Life Technologies, Inc., Gaithersburg, Md.). Nucleotide sequence data management and analysis used DNA Strider 1.2 software (Service de Biochimie et de Génétique Moléculaire, Gil sur Yvette, France) for the Macintosh computer. Database searches and data retrieval and database management and electronic mail server functions were operated by the National Center for Biotechnology Information at the National Library of Medicine. For protein database BLASTP searches, Bordetella DNA sequences were translated in all six possible reading frames, and the resulting amino acid sequences were transmitted to the NCBI Center for Biotechnological Information for analysis. Multiple amino acid sequence alignments were performed with the MegAlign module of a demonstration version of the Lasergene sequence analysis software system for the Power Macintosh computer (DNA STAR). A nucleotide sequence accession number is U34895.

Transposition inserts defining group III siderophore-deficient mutants of B. bronchiseptica interrupt a genetic region predicted to encode an amino acid decarboxylase. Molecular cloning of mini-Tn5lacZ1 transposon insertion markers associated with group III B. bronchiseptica siderophore-deficient mutants BRM3 and BRM5, resulting in recombinant plasmids pBRM3 and pBRM5, was accomplished in a previous study (3). B. bronchiseptica genomic sequences flanking the inser-
otide sequencing of pCP3.3 plasmid subclones and deletion insertions were identical to corresponding sequences determined from 222 bp. Nucleotide sequences flanking the cloned transposon Tn 

synthetic oligonucleotide primers complementary to the mini-terminus generated by exonuclease III treatment; the genetic fragment extending from a derivative mapped the genetic region encoding the complementing activity (Fig. 1). Cosmids shared an 8.5-kb fragment and complementation analysis established that the cosmids extended their complementing activity (Fig. 1). Cosmids shared an 8.5-kb fragment and complementation analysis established that the cosmids extended their complementing activity (Fig. 1).

Restriction mapping and Southern hybridization analysis of pBRM3 and pBRM5 with the probes BRM3 and BRM5 displayed no significant growth defect other than the inability to cope with iron starvation.

Isolated the B. pertussis homolog of the mutated group III siderophore gene(s), an approximately 700-bp NotI-SalI fragment of pBRM3 was used to probe a B. pertussis UT25 cosmid-based genomic library by in situ DNA hybridization. Four distinct recombinant cosmids that hybridized strongly with the B. bronchiseptica DNA probe at high stringency were identified. Restriction mapping and Southern hybridization analysis of the four cosmids, pCP3.1, pCP3.2, pCP3.3, and pCP3.4, determined that they shared B. pertussis DNA sequences homologous to the B. bronchiseptica DNA region interrupted by the insertions defining the group III siderophore-deficient mutants (data not shown). All four B. pertussis cosmids restored wild-type levels of iron-regulated siderophore production to mutants BRM3 and BRM5 when introduced by conjugal transfer, as determined by the CAS siderophore assay and absorption spectroscopy of iron-depleted culture supernatants. Subcloning and complementation analysis established that the cosmids shared an 8.5-kb XhoI B. pertussis UT25 genomic DNA fragment that encoded the complementing activity (Fig. 1). Cosmid pCP3.3 was selected for further examination. Complementation analysis of pCP3.3 plasmid subclones and deletion derivatives mapped the genetic region encoding the complementing activity to an approximately 2.8-kb B. pertussis DNA fragment extending from a B. pertussis NotI restriction site to a terminus generated by exonuclease III treatment; the genetic limits of this fragment are depicted in Fig. 1. The minimal complementing plasmid pRK/143.1 carries the 2.8-kb fragment in the broad-host-range plasmid vector pRK415.

Nucleotide sequencing analysis of pBRM3 and pBRM5 with synthetic oligonucleotide primers complementary to the mini-Tn5lacZ1 transposon “I” and “O” ends confirmed that the BRM3 and BRM5 chromosomal insertions were separated by 222 bp. Nucleotide sequences flanking the cloned transposon insertions were identical to corresponding sequences determined later for the complementing B. pertussis DNA. Nucleotide sequencing of pCP3.3 plasmid subclones and deletion derivatives and subsequent BLAST database searches of the high-scoring B. pertussis DNA sequences identified high-scoring segment pairs with a family of pyridoxal phosphate-dependent amino acid decarboxylase protein sequences. These decarboxylases included LDC, the lysine decarboxylase of Hafnia alvei (17); CadA, the inducible lysine decarboxylase of E. coli (32); Adi, the biodegradative arginine decarboxylase of E. coli (43); SpeC, the constitutive biosynthetic Odc of E. coli (4); and SpeF, the inducible Odc of E. coli (25). The putative B. pertussis decarboxylase homolog was encoded by a DNA region corresponding to the B. bronchiseptica genomic region disrupted by the transposon insertions defining BRM3 and BRM5 (Fig. 1).

Over the highest-scoring 63-amino-acid segment of similarity with the decarboxylases, multiple sequence alignments found 54% identity of the deduced partial Bordetella amino acid sequence with LDC, 52% identity with CadA, 46% with Adi, and 38% with both SpeC and SpeF. A multiple amino acid sequence alignment of this region is shown in Fig. 2. Percent identity between the deduced B. pertussis polypeptide and each of the same known decarboxylases over a 20-amino-acid region spanning the highly conserved pyridoxal phosphate cofactor-binding site ranged from 50 to 80.

Expression of a putative Bordetella amino acid decarboxylase protein in E. coli. The 2.8-kb B. pertussis DNA fragment including the region encoding the putative pyridoxal phosphate-dependent amino acid decarboxylase homolog was subcloned into plasmid vector pGEM4Z to produce pT7/odc, allowing bacteriophage T7 promoter-directed expression of Bordetella proteins in E. coli K38 (pGPI-1). Plasmid pT7/odc-encoded proteins were exclusively 35S radiolabeled and analyzed by SDS-polyacylamide gel electrophoresis and autoradiography. E. coli B. pertussis DNA-encoded product of approximately 86 kDa in apparent molecular mass was consistently observed in autoradiographs of SDS-polyacylamide gels (Fig. 3), as was the T7-transcribed vector β-lactamase product(s) also seen in pGEM4Z vector control samples. The observed 86-kDa molecular-mass product is within the calculated limits of the coding capacity of the 2.8-kb DNA fragment and approximates the 82- to 84-kDa mass of the homologous decarboxylases identified in database searches.

Restoration of alcaligin siderophore production to mutant BRM3 by culture medium supplementation with amino acid decarboxylase products. In an attempt to circumvent the siderophore biosynthesis defect associated with the loss of a putative amino acid decarboxylase activity in group III mutant BRM3, iron-depleted SS culture medium was supplemented with cadaverine, agmatine, or putrescine, which are the products of the highest-scoring 63-amino-acid segment of similarity with the decarboxylases, multiple sequence alignments found 54% identity of the deduced partial Bordetella amino acid sequence with LDC, 52% identity with CadA, 46% with Adi, and 38% with both SpeC and SpeF. A multiple amino acid sequence alignment of this region is shown in Fig. 2. Percent identity between the deduced B. pertussis polypeptide and each of the same known decarboxylases over a 20-amino-acid region spanning the highly conserved pyridoxal phosphate cofactor-binding site ranged from 50 to 80.

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FIG. 1. Restriction map of the B. pertussis UT25 genomic region complementing alcaligin siderophore-deficient group III B. bronchiseptica mutants BRM3 and BRM5. The upper map depicts the 8.5-kb XhoI DNA fragment shared by four distinct group III-complementing B. pertussis cosmids. The relative positions of opposing mini-Tn5lacZ1 transposon insertions defining BRM3 and BRM5 are indicated by the triangles (3. BRM3 insertion, and 5. BRM5 insertion). The lower map shows the limits of the minimal complementing 2.8-kb B. pertussis DNA fragment identified. The Δ symbol indicates that one terminus of the fragment was generated by exonuclease III treatment. The relative position of an open reading frame resembling pyridoxal phosphate-dependent amino acid decarboxylases is indicated by the arrow labeled odc.

FIG. 2. Multiple primary amino acid sequence alignment of translated B. pertussis DNA sequences with amino acid decarboxylases identified in BLAST database searches. The partial decarboxylase sequences shown represent the highest-scoring 63-amino-acid segment of similarity with a deduced B. pertussis polypeptide sequence. Residue positions of translated decarboxylase amino acid sequences are numbered as they appear in the GenBank database submissions (GenBank accession numbers: B. pertussis Odc, U34898; H. alvei LDC, P05033; E. coli CadA, P23892; E. coli Adi, P28629; E. coli SpeC, P21169; E. coli SpeF, P21469).
products of lysine decarboxylase, arginine decarboxylase, or Odc activities, respectively. Iron-regulated alcaligin production by BRM3 was restored to wild-type levels by medium supplementation with putrescine (1,4-diaminobutane) and only partially restored by supplementation with agmatine (1-aminoo-4-guanidobutane) (Fig. 4). Cadaverine supplementation had no effect on siderophore production by BRM3, nor did supplementation with ornithine, arginine, or spermidine in other experiments. All supplements had no effect on alcaligin production by wild-type B013N, except putrescine, which resulted in a slight enhancement of siderophore production.

In E. coli, putrescine can be produced by two alternative biochemical pathways (34): the first and most efficient route is direct decarboxylation of ornithine, and the second is a two-step process involving decarboxylation of arginine to form agmatine, followed by the conversion of agmatine to putrescine by the action of agmatine ureohydrolase (Fig. 5). Full restoration of siderophore production to BRM3 by putrescine and partial restoration by agmatine were consistent with the predicted involvement of an amino acid decarboxylase activity, possibly either an Odc or an arginine decarboxylase, in the production of putrescine, the proposed precursor of the Bordetella siderophore alcaligin on the basis of structural analysis.

**Inhibition of Bordetella alcaligin production by an inhibitor of Odc.** Supplementation of iron-depleted SS culture medium with the Odc inhibitor DAB resulted in strong reduction in siderophore levels achieved by wild-type B. bronchiseptica B013N (Fig. 6). Alcaligin production was highly sensitive to DAB inhibition; the lowest inhibitor concentration tested, 0.001 mM, decreased siderophore production by approximately 20%. At 0.080 mM DAB, inhibition was near maximal at approximately 50% inhibition of siderophore production compared with untreated cultures. DAB concentrations used...
were not growth inhibitory over the culture period examined, and control assays established that DAB did not interfere with the CAS siderophore assay.

Restoration of siderophore production to mutant BRM3 by the speC constitutive Odc-encoding gene of E. coli. Concordant with the decarboxylase product feeding results, the siderophore biosynthesis defect of BRM3 was functionally complemented in *trans* by plasmid pRK/3.2P, encoding the heterologous Odc activity SpeC of *E. coli* (8) (Table 1). Alcaligin levels produced by BRM3 (pRK/3.2P) approximated those of wild-type *B. bronchiseptica* B013N and BRM3 carrying the group III-complementing *B. pertussis* plasmid pRK/143.1.

Restoration of Odc activity to BRM3 by plasmids complementing its siderophore biosynthesis defect. In Odc assays, group III siderophore-deficient mutant BRM3 was found to lack significant levels of Odc activity compared with the wild-type parent strain B013N (Table 1). Counts determined were in close agreement with integrated values resulting from computer analysis of digitized autoradiographic images. Activities for mutant BRM3 and BRM3 carrying the plasmid vector pRK415 were essentially the same as nonenzymatic background activity of boiled B013N suspensions. Levels of Odc activity comparable to those expressed by wild-type B013N were restored to BRM3 by the *B. pertussis* plasmid, pRK/143.1, that complemented its siderophore biosynthesis defect, indicating that the amino acid decarboxylase activity required for alcaligin biosynthesis is an Odc. *Bordetella* Odc enzymatic activities appeared to be largely independent of iron concentration in the culture medium, suggesting that the reported Fur-mediated iron regulation of *Bordetella* siderophore biosynthesis (9) is exerted over other alcaligin biosynthetic activities.

Constitution and analysis of a group III-analogous mutant of *B. pertussis*. Siderophore-deficient *B. pertussis* mutant P12 was constructed by allelic exchange by using a deletion-replacement pRTP1 derivative to delete approximately 1 kb of N-terminal coding sequences of the *B. pertussis* Odc-encoding gene required for alcaligin biosynthesis. Deletion of the 3.8-kb *B. pertussis* genomic DNA fragment depicted in Fig. 1 and replacement by the 2.1-kb Tn15-derived kanamycin resistance cassette were confirmed by Southern hybridization analysis of genomic DNA isolated from a dozen independent kanamycin-resistant derivatives. As with *B. bronchiseptica* mutant BRM3, *B. pertussis* P12 was incapable of alcaligin siderophore production unless supplied with the wild-type DNA sequences in *trans* or unless the iron-depleted growth medium was supplemented with putrescine (Fig. 7). These data establish that the Odc, for which we propose the genetic designation *odc*, is required for biosynthesis of the siderophore alcaligin (Fig. 8) in *B. pertussis* as well as in *B. bronchiseptica*.

**TABLE 1. Alcaligin siderophore production and Odc activities of *B. bronchiseptica***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Alcaligin siderophore activity</th>
<th>Odc activity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+ Iron</td>
<td>− Iron</td>
</tr>
<tr>
<td>B013N (wt)</td>
<td>0.019</td>
<td>0.574</td>
</tr>
<tr>
<td>BRM3</td>
<td>0.014</td>
<td>0.052</td>
</tr>
<tr>
<td>BRM3 (pRK415 vector)</td>
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<td>0.085</td>
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<tr>
<td>BRM3 (pRK143.1) odc</td>
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<td>0.570</td>
</tr>
<tr>
<td>BRM3 (pRK3.2P) speC</td>
<td>0.013</td>
<td>0.543</td>
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*Mean relative CAS siderophore activities (1−A/A<sub>ref</sub>, n = 3) of 24-h SS cultures.

**DISCUSSION**

We report the identification of the *odc* gene encoding an enzymatic activity essential for alcaligin siderophore biosynthesis in *Bordetella* spp. This Odc supplies the alcaligin precursor 1,4-diaminobutane, commonly known as putrescine. The involvement of an amino acid decarboxylase activity in the biosynthesis of alcaligin was predicted on the basis of database searches of translated DNA sequences corresponding to the genomic region interrupted by transposon insertions defining previously reported *B. bronchiseptica* alcaligin-deficient mu-

**FIG. 6. Inhibition of alcaligin siderophore production in *B. bronchiseptica* by an inhibitor of Odc. Mean relative CAS siderophore activities (1 − A/A<sub>ref</sub>, n = 3) of 18-h B013N cultures are plotted as a function of concentration (millimolars) of the Odc inhibitor DAB added to the culture medium.**

**FIG. 7. Alcaligin siderophore production by *B. pertussis* odc mutant P12 constructed by allelic exchange. Mean relative CAS siderophore activities (1−A/A<sub>ref</sub>, n = 3) of 24-h cultures of odc mutant P12 carrying the designated plasmids or supplemented with putrescine are shown versus that of the wild-type parent strain UT25, wt, wild type.**
tants BRM3 and BRM5. Evidence supporting the involvement of an Odc activity was gained in mutant feeding experiments in which culture medium supplementation with decarboxylated ornithine (putrescine) fully restored alcaligin biosynthetic capacity to BRM3. Accordingly, the alcaligin biosynthesis defect of BRM3 was functionally complemented by expression of a heterologous Odc activity, SpecE of E. coli. Ultimately, BRM3 was shown to be defective in Odc activity compared with its precursor putrescine. Dashed arrows indicate unknown biosynthetic activities.

Certain catechol-type siderophores, such as agroboactin (37), vibriobactin (20), and parabactin (47) also contain polyamines, and in Paracoccus denitrificans, along with parabactin siderophore production, spermine production increases by an order of magnitude in response to iron starvation, although spermidine and not spermine donates its triamine backbone to the siderophore (5).

Remarkable parallels exist between the Bordetella odc gene and the recently described hdc gene of Vibrio anguillarum required for biosynthesis of the siderophore anguibactin (49), a trait necessary for virulence. As with Bordetella odc, the V. anguillarum hdc gene was identified after transposon mutagenesis on the basis of a siderophore-deficient phenotype. Nucleotide sequence analysis of the mutated region of plasmid pJMI identified deduced amino acid sequence similarities with known histidine decarboxylases. Anguibactin siderophore production by V. anguillarum hdc mutants was restored by culture medium supplementation with histamine, the product of histidine decarboxylation, confirming the predicted role of histamine as an essential precursor of that siderophore. Further, neither odc nor hdc is repressible by iron, although alcaligin and anguibac-
tin siderophore production have been shown to be negatively regulated by the action of a Fur repressor (9, 50), indicating that iron concentration controls other siderophore biosynthetic activities.

On the basis of the elucidation of the alcaligin biosynthesis role of odc in Bordetella spp. and the known structure of alcaligin, we hypothesize the subsequent action of an oxygenase catalyzing the hydroxylation of putrescine. This putative Bordetella oxygenase would supply an activity analogous to the IucD l-lysine N\(^6\)-hydroxylase of E. coli (22), involved in the biosynthesis of the hydroxamate siderophore aerobactin, and to the l-ornithine N\(^2\)-oxygenases PvdA of Pseudomonas aerugi-
nosa (51) and SidI of Ustilago maydis (31), required for pyoverdin and ferrichrome siderophore biosynthesis, respectively. Preliminary nucleotide sequencing of the B. pertussis DNA corresponding to the B. bronchiseptica genomic region interrupted by transposons defining the group I alcaligin siderophore-deficient mutants BRM1 and BRM9 (GenBank accession number U34894) identified an open reading frame predicted to encode such a Bordetella homolog of the IucD, PvdA, and SidI oxygenases (24). Confirmation of the role of this putative Bordetella oxygenase in the biosynthesis of alcaligin awaits further biochemical analysis.

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