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The autotransporter protein from *Bordetella avium*, Baa1, is involved in host cell attachment

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ABSTRACT

Bordetella avium is a Gram negative upper respiratory tract pathogen of birds. *B. avium* infection of commercially raised turkeys is an agriculturally significant problem. Here we describe the functional analysis of the first characterized *B. avium* autotransporter protein, Baa1. Autotransporters comprise a large family of proteins found in all groups of Gram negative bacteria. Although not unique to pathogenic bacteria, autotransporters have been shown to perform a variety of functions implicated in virulence. To test the hypothesis that Baa1 is a *B. avium* virulence factor, unmarked *baa1* deletion mutants (Δ*baa*1) were created and tested phenotypically. It was found that *baa1* mutants have wild-type levels of serum sensitivity and infectivity, yet significantly lower levels of turkey tracheal cell attachment *in vitro*. Likewise, semi-purified recombinant His-tagged Baa1, expressed in *Escherichia coli*, was shown to bind specifically to turkey tracheal cells via western blot analysis. Taken together, we conclude that Baa1 acts as a host cell attachment factor and thus plays a role *B. avium* virulence.

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Introduction

Autotransporters comprise a large family of secreted proteins that use the Type V secretion system which is conserved among all major groups of Gram negative bacteria (reviewed in Henderson et al. 2004; Hayes et al. 2010; Dautin and Bernstein 2007). Autotransporters are modular proteins consisting of an N-terminal signal sequence for targeting to the Sec apparatus, a C-terminal beta-barrel domain for targeting to the outer membrane, and a passenger domain that is exposed to the bacterial surface (reviewed in Dautin and Bernstein 2007; Henderson et al. 2004). The beta-barrel domain facilitates the translocation of the N-terminal passenger domain through the outer membrane, although the mechanism of this translocation is under debate (reviewed in Dautin and Bernstein 2007). In some cases, the passenger domain is cleaved via autoproteolysis and thus released from the cell surface (Dautin et al. 2007; Yen et al. 2008). While the transport domains of autotransporters are structurally and functionally conserved, differences within surface exposed or released N-terminal passenger domains allow for the diversity of autotransporter protein function.

Although not unique to pathogenic bacteria, autotransporters have been found to perform a variety of virulence-related functions in Gram negative pathogens. Functions include those such as protein/lipid degradation (*e.g. Moraxella catarrhalis* McaP (Timpe et al. 2003)), serum resistance (*e.g. Bordetella pertussis* BrkA (Fernandez and Weiss 1994), *Yersinia pestis* YadA (El Tahir and Skurnik 2001)), host actin polymerization (*e.g. Shigella flexneri* IcsA (Sansonetti 1991)), as well as attachment to host tissues (*e.g. Escherichia coli* AIDA-1 (Sherlock et al. 2004), *Y. pestis* YadA (El Tahir and Skurnik 2001), and *B. pertussis* Pertactin (Sansonetti 1991) and BrkA (Weingart and Weiss 2000)). Surface localization and high antigenicity of these virulence factor proteins make them ideal candidates for inclusion in subunit vaccines. For instance, *B. pertussis* Pertactin is an important component of the current whooping cough subunit vaccine.

Bordetella avium causes a disease of birds called bordetellosis (*i.e.* turkey coryza; Jackwood and Saif 2008), with similarities to whooping cough in humans. Current antibiotic treatments and live-attenuated vaccines have proven ineffective in controlling this pathogen within commercial poultry flocks. Upon respiratory introduction, *B. avium* specifically attaches to and colonizes ciliated tracheal cells of infected birds. In doing so, ciliary cells are effaced and poults demonstrate upper respiratory tract symptoms including profuse nasal and ocular discharge in addition to signature sneezing/coughing called "snicking." Although usually non-fatal, *B. avium* infection of commercial flocks leads to significant economic

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Table 1
Strains used in this study.

Strain or plasmid	Relevant genotype/description	Source/Reference
B. avium		
197 N	Wild type parental *B. avium* strain	Temple et al. (1998)
Δ*baa*1	Unmarked *baa1* deletion mutant	This work
E. coli		
DH5α	Cloning strain	Invitrogen (Carlsbad, CA)
Top10F′	Cloning strain	
MC4100λpir	Conjugation donor strain	Hoffmann et al. (1998)
BL21(DE3)	Expression strain	Invitrogen (Carlsbad, CA)
Plasmids		
pBluescript (pSK-)	3.0 kb cloning plasmid, Amp^R	Stratagene (La Jolla, CA)
pET30b	5.4 kb expression plasmid, Kan^R, T7/*lac* inducible promoter, N-terminal 6× HIS	Stratagene (La Jolla, CA)
pKAS46	*B. avium* suicide plasmid. *oriR6K oriT rpsL* Amp^R Kan^R	Skorupski and Taylor (1996)
pRK2103	*E. coli* conjugation helper plasmid, Kan^R	
pSK(−)(*baa1*)	Full-length *baa1* ORF cloned into the *Bam*HI/*Eco*RI sites of pBluescript(−)	This work
pSK(−)(Δ*baa*1)	*baa1* deletion allele at the *Bam*HI/*Eco*RI sites of pBluescript(−)	This work
pKAS46(Δ*baa1*)	*baa1* deletion allele at the *Kpn*I/*Sac*Isites of pKAS46	This work
pET30b(*baa1*-pass)	pET30b containing *baa1*-pass (1777 bp) Downstream of the T7/*lac* inducible promoter, Kan^R	This work
pΩ-$hagA_{his}$	Qiagen His-tag expression plasmid, pQE30, containing *B. avium hagA*, Amp^R	Temple et al. (2010)

losses due to increased secondary infection prevalence and slow growth rates.

Using a bioinformatics approach to search for novel *B. avium* virulence factors, four ORFs with significant sequence similarity to known autotransporter proteins have been identified (Sebaihia et al. 2006). The passenger domain of one of these putative *B. avium* autotransporter proteins, Baa1 (*B. avium* autotransporter 1), shows sequence similarity (28% ID, 48% sim. at the amino acid level) to the known *E. coli* tissue adhesin autotransporter, AIDA-I (Sherlock et al. 2004). Based on this finding, we hypothesized that Baa1 is involved in *B. avium* host attachment. To test this hypothesis, a *baa1* deletion mutant (Δ*baa*1) and cloned recombinant Baa1 passenger domain (Baa1-Pass) were assayed for their ability to specifically bind to turkey tracheal cells.

Materials and methods

Bacterial strains, plasmids, and growth conditions

The strains used in this study are described in Table 1. *E. coli* and *B. avium* strains were routinely grown in rich media (*i.e.* Luria-Bertani (LB) or brain-heart infusion (BHI)) at 37 °C. Prior to turkey tracheal attachment assays, *B. avium* cells were grown on Bordet Gengou (BG) agar supplemented with 15% defimbrinated sheep's blood. Antibiotics were added where appropriate, at the following concentrations: ampicillin (100 μg/ml); chloramphenicol (30 μg/ml); kanamycin (30 μg/ml for *E. coli*, 100 μg/ml for *B. avium*).

Restriction enzymes were purchased from New England Biolabs (Ipswich, MA). Primer synthesis and DNA sequencing were performed by Elim Biopharmaceuticals (Hayward, CA). Advantage GC cDNA Polymerase (Clonetech, Mountain View, CA) was used in all PCR amplifications.

Construction of the Δbaa1 mutant

Full-length *baa1* ORF was PCR amplified from *B. avium* 197 N chromosomal DNA with restriction enzyme site engineered primers (Forward P1, Reverse P2; see Table 2) using the following reaction conditions: 94 °C for 6 min, followed by 25 cycles of 94 °C for 30 s, 65.5 °C for 30 s, and 68 °C for 3.5 min, finishing with 68 °C for 7 min. The resulting ~2800 bp product was digested with *Bam*HI and *Eco*RI and ligated into the similarly cut pBluescript(−) plasmid (Stratagene, La Jolla, CA). The ligation mixture was introduced into Top10F' *E. coli* cells (Invitrogen, Carlsbad, CA) via heat shock, and transformed clones were selected and screened. To create an ~2000 bp deletion within the *baa1* coding region, long range PCR was performed on pSK(*baa1*). Specifically, primers were designed to amplify out of the *baa1* ORF and around the plasmid backbone (Forward P3, Reverse P4; see Table 2). In doing so, only 400 bp on each end of the *baa1* ORF was maintained. Long range PCR was performed as follows: 94 °C for 2 min, followed by 25 cycles of 94 °C for 30 s, 63 °C for 30 s, and 68 °C for 4 min, finishing with 68 °C for 7 min. The ~3.8 kb amplicon was blunted, self-ligated, and transformed into Top10F' *E. coli* cells. Recombinant plasmids containing the *baa1* deletion allele were confirmed by restriction enzyme digestion and sequence analysis. The *baa1* mutant allele was released from pBluescript with *Kpn*I and *Sac*I digestion and ligated into similarly cut allelic exchange vector, pKAS46. The recombinant pKAS46(Δ*baa1*) plasmids were selected for in MC4100λpir *E. coli* cells.

The Δ*baa1* allele (Fig. 1) was introduced into *B. avium* via homologous recombination. A tri-parental mating was performed between MC4100λpir/pKAS(Δ*baa1*), DH5α/pRK2103, and parental *B. avium* 197 N cells, as previously described (Temple et al. 2010). Two Δ*baa1* isolates were obtained independently and confirmed by PCR and sequencing. These strains were used in all further studies.

Construction and induction of the Baa1-Pass-HIS construct

A 1777 bp *baa1* fragment encoding the putative Baa1 passenger domain (Fig. 1) was PCR amplified from *B. avium* 197 N chromosomal DNA with restriction enzyme site engineered primers (Forward P5, Reverse P6; see Table 2) using the following reaction conditions: 94 °C for 6 min, followed by 25 cycles of 94 °C for 2 min, 62 °C for 30 s, and 68 °C for 2 min, finishing with 68 °C for 7 min. The resulting *baa1-pass* amplicon, flanked with 5′ *Eco*RI and 3′ *Xho*I sites was directionally cloned into similarly cut pBluescript (pSK-, Stratagene, La Jolla, CA) and introduced into DH5α *E. coli* cells

Table 2
Primers used in this study.

Primer	Sequence (5′→3′)[a]
P1	TGGATCCATCAACCCGCGAGAAAG
P2	AGAATTCATGCCTGTGCTCGATCTTC
P3	TACCAGGCCCACGGTTGG
P4	CCGGCCATTGGTGAGGGT
P5	TTGAATTCCTGATCTTGGCCTTGACCCTG
P6	ATCTCGAGGAGGGGCTCATCCGGCTGGTTATTG

[a] Underlined sequences indicate engineered restriction enzyme sites.