Plasmid pBP136 from *Bordetella pertussis* represents an ancestral form of IncP-1 β plasmids without accessory mobile elements

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The complete 41 268 bp nucleotide sequence of the IncP-1 β plasmid pBP136 from the human pathogen *Bordetella pertussis*, the primary aetiological agent of whooping cough, was determined and analysed. This plasmid carried a total of 46 ORFs: 44 ORFs corresponding to the genes in the conserved IncP-1 β backbone, and 2 ORFs similar to the *XF1596* and *XF1597* genes with unknown function of the plant pathogen *Xylella fastidiosa*. Interestingly, pBP136 had no accessory genes carrying genetic traits such as antibiotic or mercury resistance and/or xenobiotic degradation. Moreover, pBP136 had only two of the *kle* genes (*kleAE*) that have been reported to be important for the stability of IncP-1 plasmid in *Pseudomonas aeruginosa*. Phylogenetic analysis of the Kle proteins revealed that the KleA and KleE of pBP136 were phylogenetically distant from those of the present IncP-1 plasmids. In contrast, IncC1 and KorC, encoded upstream and downstream of the *kle* genes respectively, and the replication-initiation protein, TrfA, were closely related to those of the IncP-1 β 'R751 group'. These results suggest that (i) pBP136 without any apparent accessory genes diverged early from an ancestor of the present IncP-1 β plasmids, especially those of the R751 group, and (ii) the *kle* genes might be incorporated independently into the backbone region of the IncP-1 plasmids for their stable maintenance in various host cells.

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INTRODUCTION

The bacterial incompatibility group P-1 (IncP-1) plasmids can replicate and be stably maintained in almost all Gram-negative bacteria, and these plasmids are very promiscuous as either antibiotic-resistance or xenobiotic-degradative plasmids (Adamczyk & Jagura-Burdzy, 2003; Dennis, 2005; Thomas, 2000; Thomas & Smith, 1987). From the 1970s, many new members of the IncP-1 group have been discovered in bacteria from aquatic and soil environments (pB3, Heuer *et al.*, 2004; pADP-1, Martinez *et al.*, 2001; pB10, Schlüter *et al.*, 2003; pB8, Schlüter *et al.*, 2005; pUO1, Sota *et al.*, 2003; pB4, Tauch *et al.*, 2003; pTB11, Tennstedt *et al.*, 2005; pJP4, Trefault *et al.*, 2004; pEST4011, Vedler

et al., 2004). The complete genome sequences of these newly identified IncP-1 plasmids have been determined, and the sequence data revealed that these plasmids have IncP-1-specific backbone modules for their replication, stable inheritance and conjugative transfer which show high similarity to the corresponding modules of two well-characterized IncP-1 plasmids, the IncP-1 α subgroup plasmid RP4 (Pansegrau *et al.*, 1994) and the IncP-1 β subgroup plasmid R751 (Thorsted *et al.*, 1998). Their genomes have various mobile genetic elements (transposons and their remnants) that carry genes encoding antibiotic resistance or degradation of xenobiotic compounds. Most of these accessory genes are inserted in one or both of two specific regions of the backbone, e.g. the IncP-1 β plasmid pUO1 contains two haloacetate-catabolic transposons in the *oriV-trfA* region and two mercury-resistance transposons in the *trb-tra* region (Sota *et al.*, 2003). In general, IncP-1 plasmids have at least one accessory gene (encoding degradation of xenobiotic compounds or resistance to antibiotics or heavy metals) that has been postulated to be acquired during adaptive evolution of this group of

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A supplementary table showing the localization and predicted functions of the ORFs of pBP136 is available with the online version of this paper.

plasmids (Heuer *et al.*, 2004). However, recently, an IncP-1 β plasmid without any of these typical accessory genes, pA1, was identified in *Sphingomonas* sp. A1 (Harada *et al.*, 2006).

The genus *Bordetella* includes small, aerobic, Gram-negative coccobacilli associated with respiratory infections in human and other animals. *Bordetella pertussis* infects only humans. It adheres to the ciliate epithelium of the trachea and bronchi, and causes the disease whooping cough (pertussis), a highly contagious disease with severe clinical manifestations in infants. *Bordetella bronchiseptica* is a respiratory tract pathogen for dogs, pigs and laboratory animals, and *Bordetella avium* causes turkey coryza. In virulent *B. bronchiseptica* and *B. avium*, naturally occurring plasmids have been observed (Antoine & Loch, 1992; Graham & Abruzzo, 1982; Hedges *et al.*, 1974; Lax & Walker, 1986; Shimizu *et al.*, 1981; Speakman *et al.*, 1997; Terakado *et al.*, 1973; Terakado & Mitsuhashi, 1974). Most of the plasmids identified in *B. bronchiseptica* and *B. avium* belong to the IncP-1 or IncQ groups, and their presence is associated with resistance to various antibiotics and heavy metals. IncP-1 plasmids have been shown to be transferred to and stably maintained in *B. pertussis* strains under laboratory conditions (Smith *et al.*, 1986; Weiss & Falkow, 1982); however, naturally occurring plasmids have not been reported in *B. pertussis* to date.

In 2002, we identified a *B. pertussis* isolate from a pertussis case that harboured an IncP-1 β plasmid. This is believed to be the first reported case of *B. pertussis* with a naturally occurring plasmid. To understand the basic properties of the IncP-1 β plasmid, designated pBP136, we determined its complete nucleotide sequence. Here we report that pBP136, which lacks any apparent accessory genes in the typical insertion sites, could represent an ancestral form of the IncP-1 β plasmids of the 'R751 group'.

METHODS

Bacterial strains. In 2002, a pertussis-death case occurred in a general hospital in Oita prefecture, Japan. The dead body was submitted for an autopsy to confirm *B. pertussis* infection. Permission for the autopsy was obtained from the infant's parents and grandfather. In the autopsy, small specimens were excised from the pharynx, bronchial tube and lower lobe of the right lung. The specimens were immersed in Casamino acid solution (1% Difco-Casamino acid, 0.6% NaCl, pH 7.1), and vortexed. Small portions (50–100 μ l) of the supernatant were inoculated on Bordet–Gengou agar (Difco) supplemented with 1% (v/v) glycerol, 15% (v/v) defibrinated horse blood and 20 μ g cephalixin ml⁻¹. A total of 77 *B. pertussis* isolates were collected from the infant's respiratory tract: 11 isolates from the pharynx, 33 from the bronchial tube and 33 from the lower lobe of the right lung. All of the isolates were confirmed as *B. pertussis* using PCR identification (Houard *et al.*, 1989). Among the isolates from the pharynx, a *B. pertussis* strain harbouring the IncP-1 β plasmid pBP136 (named strain BP136) was identified.

DNA sequence analysis and annotation. *B. pertussis* strain BP136 was grown at 36 °C on Bordet–Gengou agar plates. The plasmid DNA from the strain was purified using two cycles of CsCl/ethidium bromide gradient as described elsewhere (Ausubel *et al.*,

1987). The nucleotide sequence of plasmid pBP136 was determined by the shotgun approach. Purified pBP136 DNA was randomly fragmented by using a sonicator equipped with a microtip probe (UP50H, dr. hielsher, Germany). The >0.4 kb size fractions were cloned into plasmid pUC18 and transferred to *Escherichia coli* DH5 α . Clones containing inserts were picked randomly and sequenced. Sequence reactions were carried out with BigDye terminator v.1 cycle sequencing kit (Applied Biosystems), and the products were sequenced on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems). Sequencing reads were assembled by using the SEQUENCHER DNA sequencing software (v.4.1.2, Gene Codes). Gap closure of the sequence was achieved by primer walking with custom-made primers (Invitrogen). The analysis of the ORFs present in the pBP136 sequence was completed using the web-based versions of the GeneHacker (<http://www.btls.jst.go.jp/GeneHacker/>) and GeneMark.hmm (<http://opal.biology.gatech.edu/GeneMark/hmmchoice.html>) programs. Those predicted ORFs and their products were analysed further using the BLAST and BLASTP programs (Altschul *et al.*, 1997) implemented at the DNA Database of Japan (DDBJ) in order to predict their functions.

Phylogenetic analysis. Multiple-alignment and phylogenetic analyses were performed by using the CLUSTALW program with default parameters provided by DDBJ (<http://www.ddbj.nig.ac.jp/search/clustalw-j.html>). Tree topology and evolutionary distance were analysed by the neighbour-joining method, and were displayed with the TreeView program (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>).

Bacterial mating. Plasmid pBP136Km^R was constructed by inserting a kanamycin-resistance gene in the *Xba*I site of pBP136, and was

Fig. 1. Genetic map of the 41 268 bp IncP-1 β plasmid pBP136. Coding regions are shown by arrows indicating the direction of transcription. ORF1 and ORF2 are similar to XF1597 and XF1596 of *Xylella fastidiosa*. The positions of the origins of vegetative replication (*oriV*) and plasmid transfer (*oriT*) are marked by black boxes. Arrows indicate positions of insertion (mobile genetic elements and phenotypic markers) in other IncP-1 β plasmids.

Fig. 2. Comparisons of the *oriV-trfA* (a) and *trb-tra* (b) regions of pBP136 with other IncP-1 β plasmids. Plasmids pB3 and pJP4 have no interrupting mobile genetic elements in their *oriV-trfA* and *trb-tra* regions, respectively. Plasmid pA1 contains no interrupting mobile genetic elements in its backbone. Conserved 20 bp inverted repeat sequences, black box; binding site of TrfA, open box; binding site of DnaA, hatched box; A/T- and G/C-rich regions, white ellipses.

transformed into *E. coli* EC100. Conjugative transfer of pBP136Km^R from *E. coli* EC100 donor to *B. pertussis* recipients was performed by the plate mating method (Weiss & Falkow, 1982). Donor and recipient were inoculated on a Bordet-Gengou agar plate, incubated at 36 °C for 3 h, harvested, and then plated on a Bordet-Gengou agar plate containing 25 μ g kanamycin ml⁻¹ and 20 μ g cephalixin ml⁻¹. Transfer frequencies were calculated per recipient titre.

PFGE and Southern blotting. Pulsed-field gel electrophoresis (PFGE) was performed according to standardized recommendations for typing of *B. pertussis* (Mooi *et al.*, 2000), with minor modifications (Kodama *et al.*, 2004). DNA from *B. pertussis* isolates was digested with restriction enzyme *Xba*I, and the digested fragments were separated using a CHEF DR II apparatus (Bio-Rad). For Southern blotting analysis, DNA fragments separated by PFGE were cleaved by UV irradiation and transferred to a nylon membrane (Zeta-probe blotting membranes, Bio-Rad) according to the manufacturer's instructions. Southern blots were hybridized with HRP-labelled *traL* gene as a probe using an ECL direct nucleic acid labelling and detection system (Amersham Pharmacia Biotech). The *traL*-specific probe (500 bp) was PCR-amplified using the forward primer 5'-ATGGCAAAATTCACATGGT-3' and the reverse primer 5'-TCGAACCCCTCCCTCGTG-3' with purified pBP136 as a template.

Plasmid stability test. *B. pertussis* strain pBP136 was grown at 36 °C in cyclodextrin liquid medium (Imaizumi *et al.*, 1983). A sample of the culture was collected every 72 h, and transferred into fresh medium at a dilution of 1/100. After 10 subcultures, a sample of the culture was diluted and plated onto a Bordet-Gengou agar plate. Thirty-two colonies were picked up, and tested for the presence of pBP136 by PCR amplification using the *traL*-specific primers.

RESULTS AND DISCUSSION

Nucleotide sequence and ORFs of pBP136

Among the 77 *B. pertussis* isolates from the pertussis infant, we identified 24 isolates that harboured a plasmid of

similar size; we characterized the plasmid from isolate BP136, designated plasmid pBP136. Since this is the first finding of a naturally occurring plasmid in *B. pertussis*, the complete sequence of pBP136 was determined by a shotgun approach. As shown in Fig. 1, sequence determination of pBP136 resulted in a circularly closed nucleotide sequence of 41 268 bp with a mean G + C content of 65.1 mol% (*ORF1-ORF2*, 65.8 mol%; *kleE-kleA*, 62.9 mol%). Annotation of the sequence data revealed that pBP136 contains 46 ORFs. The localization and predicted functions of the ORFs are presented in supplementary Table S1 (available with the online version of this paper). Forty-three ORFs correspond to a well-conserved IncP-1 β backbone, and one corresponds to *parA*, encoding a resolvase, ParA. This partitioning gene *parA* is present as an intact gene, as is the case for the IncP-1 β plasmids pB2, pB3, pJP4 and pA1 (Heuer *et al.*, 2004; Trefault *et al.*, 2004; Harada *et al.*, 2006). Plasmid pBP136 possesses a classical IncP-1 β backbone: two regions involved in plasmid conjugation (the *tra* and *trb* operons), and a region carrying the genes for plasmid replication, central control, stable inheritance and partitioning (*trfA*, *klc*, *kor*, *kle*, *inc*, *kfrA* and *parA*). The origins of vegetative replication (*oriV*) and plasmid transfer (*oriT*) are also conserved. In the present study, *traO* and *traN* are shown as *kfrB* (*traO*) and *kfrC* (*traN*), respectively (Adamczyk *et al.*, 2006).

Almost all the gene products of the pBP136 backbone showed high amino acid identities (87–100%) to the corresponding gene products of other IncP-1 β plasmids, R751, pJP4, pUO1, pB3, pB8, pTSA and pADP-1 (supplementary Table S1). Exceptions were the products of *traC* (76%), *kleE* (70%) and *kleA* (61%). The remaining two ORFs, ORF1 and ORF2, which had not been found in other

IncP-1 β plasmids, showed 80 and 90 % amino acid sequence identity to the hypothetical proteins (XF1597 and XF1596) of the plant pathogen *Xylella fastidiosa* (Shimpson *et al.*, 2000; accession no. AE003987), respectively. XF1596 belongs to the helix–turn–helix XRE-family-like proteins and xenobiotic response element family of transcriptional regulators, and XF1597 is a member of the DUF891 family (bacterial protein of unknown function, pfam05973, COG3657). However, no function has yet been assigned to the proteins. *X. fastidiosa* is a fastidious, xylem-limited bacterium, and causes citrus variegated chlorosis through colonization of the plant xylem (Chang *et al.*, 1993; Li *et al.*, 1999). The role of these proteins in *B. pertussis* is unknown.

Plasmid pBP136 dose not carry any accessory mobile elements

No accessory mobile elements were identified in the pBP136 sequence (Fig. 1). Moreover, no remnants of insertion sequences and transposons similar to Tn21- or Tn402, which have been found on other IncP-1 β plasmids, were identified. Typically, mobile elements with accessory genes are found on IncP-1 β plasmids between *oriV* and *trfA* and between the *trb* and *tra* operons. These regions all have the conserved 20 bp inverted repeat sequence CATCGCCA-NNTCYGRCGATG, which has been proposed to be a target site for insertion of foreign DNA segments (Tauch *et al.*, 2003; Thorsted *et al.*, 1998). Interestingly, pBP136 contains two copies of the repeat sequence in each region, but it is the first IncP-1 β plasmid reported not to contain any accessory genes in these two regions. The only two potential accessory genes found on the plasmid, *ORF1* and *ORF2*, were inserted between *klaA* and *oriV* (Figs 1 and 2). Other examples of

IncP-1 β plasmids that have one or two ORFs between *klaA* and *oriV* are pADP-1, pB4, pB8, pB10 and pJP4, whereas such ORFs are absent in plasmids pB3 and pA1 (Heuer *et al.*, 2004; Harada *et al.*, 2006). Interestingly, some of these ORFs also show similarity with genes found in *X. fastidiosa* and/or might encode similar functions (pB4, Tauch *et al.*, 2003; pJP4, Trefault *et al.*, 2004; pB10, Schlüter *et al.*, 2003). Heuer *et al.* (2004) considered these genes to be accessory since they were absent in the potentially ancestral *oriV* region on plasmid pB3 (see below). However, their presence on many of the IncP-1 β plasmids may also suggest that they are part of the plasmid backbone. Future work will have to unravel whether *ORF1* and *ORF2* confer a selective advantage to some hosts under specific conditions and thus would qualify as true accessory genes, or are essential for stable plasmid replication or horizontal transfer, and thus would be considered backbone genes. While three IncP-1 β plasmids have recently been described that lack accessory genes in one of the two typical insertion regions, none have been reported that completely lack accessory genes in both regions. The IncP-1 β plasmids pB3 and pJP4 have no interrupting mobile genetic elements in one region (*oriV*–*trfA* and *trb*–*tra* regions, respectively) but carry mobile elements in the other. Similar to pB136, these non-interrupted regions contain respectively two and three copies of the repeat sequence. Recently an IncP-1 β plasmid, pA1, was identified that does not contain any of the typical accessory genes, but it contains two cryptic ORFs in the *trb*–*tra* region between *para* and *traC* (Harada *et al.*, 2006). Since these ORFs in pA1 clearly do not belong to the conserved IncP-1 β backbone, they should probably be considered as potential accessory genes. Unlike other IncP-1 β plasmids, pA1 contains only one copy of the inverted repeat sequence in the *oriV*–*trfA* region, and none in the *trb*–*tra* region. In conclusion,

Fig. 3. Comparison of the genetic organization of the central control/stable-inheritance regions located on the IncP-1 β plasmids pBP136, pB10, pUO1, R751 and pADP-1. Accession numbers for pB10, pUO1, R751 and pADP-1 are AJ564903, AB063332, U67194 and U66917, respectively.

Fig. 4. Phylogenetic trees of KleE, KleA, IncC1, KorC and TrfA2 proteins of different IncP-1 plasmids. Multiple sequence alignments were conducted using CLUSTALW provided by DDBJ. Accession numbers other than those given in the legend of Fig. 3 are as follows: IncP-1 α plasmids (RP4, L27758; pTB11, AJ744860); IncP-1 β plasmids (pJP4, AY365053; pB3, AJ639924; pB4, AJ431260; pB8, AJ863570; pA1, AB231906); and IncP-1 δ plasmid pEST4011, AY540995. The IncC1 gene was not identified in pB8. The result of TrfA2 of pA1 is not shown.

pBP136 is the first IncP-1 β plasmid reported to have no accessory genes in the typical insertion sites of its IncP-1 β backbone, and only the second IncP-1 β plasmid without any mobile elements.

Genetic organization of the central control/stable-inheritance region of pBP136

Fig. 3 shows the genetic organization of the central control/stable-inheritance region of the five IncP-1 β plasmids pB136, pB10, pUO1, R751 and pADP-1. In pBP136, the operon composed of *incC* (plasmid partitioning), *kor* (transcriptional regulation), *klc* (stable plasmid inheritance) and *kfrA* (transcriptional regulation) was similar to those found in other IncP-1 β plasmids. Plasmids R751, pUO1 and pADP-1 have the *kleABEF* genes (stable plasmid maintenance) between *korA* and *korC*, while plasmids pB10 and pB4 only have *kleAEF* (Tauch *et al.*, 2003). Interestingly, we identified only the *kleAE* genes (G+C

content 62.9 mol%) in this region of pB136. The non-coding regions, *korA*–*kleE* and *kleE*–*kleA*, were highly similar to the corresponding regions of other IncP-1 β plasmids, pB10, pUO1, R751 and pADP-1 (data not shown). Therefore, the difference in the pBP136 *kle* region could not be explained by a deletion event. Wilson *et al.* (1997) demonstrated that several of the *kleABCDEF* genes are required for stable maintenance of IncP-1 α plasmid RK2 in *Pseudomonas aeruginosa*, but not in *E. coli*. In order to examine if pBP136 was stably maintained, we investigated the stability of pBP136 in *B. pertussis* strain BP136. Interestingly, no loss of the plasmid from the host cell was observed after 10 subcultures in liquid culture medium (approx. 67 generations; data not shown). This observation indicates that pBP136 is quite stably maintained in the *B. pertussis* cells, and suggests that (i) the two *kle* genes (*kleAE*) are sufficient for the stable maintenance of the IncP-1 β plasmids in general, and/or (ii) the *kle* genes (*kleBGF*) are not required for the stability of pBP136 in *B. pertussis*.

Fig. 5. Characterization of *B. pertussis* harbouring pBP136 by PFGE with restriction enzyme *Xba*I (a) and hybridization with *traL*-specific probe on PFGE-Southern blot (b). Lane 1, purified IncP-1 β plasmid pBP136 (undigested); lane 2, *Xba*I-digested pBP136; lane 3, *B. pertussis* harbouring pBP136 (A1 strain); lane 4, *B. pertussis* not harbouring plasmid pBP136 (A2 strain); lane 5, *B. pertussis* strain Tohama (Japanese vaccine strain); lane M, lambda DNA ladder size marker. The Southern blot was hybridized with HRP-labelled *traL* gene as a probe. The lambda DNA ladder size marker was also visualized using *Hind*III-digested lambda DNA as a probe.

The IncC, KorA, KorC and Klc proteins of pBP136 showed high identities (93–97%) to those of the other IncP-1 β plasmids, whereas the KleE and KleA proteins showed much lower identities (70 and 61%, respectively). To understand the phylogenetic relationship of the Kle proteins, we constructed neighbour-joining trees derived from multiple alignments of KleE, KleA, IncC1, KorC and TrfA of different

IncP-1 plasmids, IncP-1 α (RP4 and pTB11), IncP-1 β (R751, pUO1, pADP-1, pJP4, pB3, pB4, pB8, pB10 and pA1) and IncP-1 δ (pEST4011). For the alignment of TrfA proteins, TrfA2 regions (approx. 280 amino acids) were used. As shown in Fig. 4, the phylogenetic trees for KleE and KleA clearly showed that these proteins of pBP136 are phylogenetically distant from those of the IncP-1 β plasmids. In contrast, the trees for IncC1 and KorC showed that these proteins of pBP136 are closely related to those of the IncP-1 β 'R751 group' (R751, pUO1, pADP-1, pB3 and pB8). This is interesting, given that the *incC1* and *korC* genes are located upstream and downstream of the *kle* genes, respectively (Fig. 3). Similarly, the tree for the replication initiation protein, TrfA2, also showed that this protein of pBP136 is closely related to those of the R751-group plasmids. These observations strongly suggest that pBP136 is an ancestral form of the present IncP-1 β plasmids, especially the R751-group plasmids. In addition, we postulate that the *kle* genes of the present IncP-1 β plasmids may have been acquired independently in the establishment of the different current plasmid backbones, resulting in high plasmid stability in various host cells. This hypothesis is consistent with a proposal that plasmids have evolved by acquiring, clustering and assembling the component parts that encode their survival and dissemination functions (Thomas, 2000).

Distribution of *B. pertussis* harbouring pBP136 in the pertussis infant's respiratory tract

In this pertussis case, we isolated not only *B. pertussis* strains harbouring plasmid pBP136 (referred to as A1 strains), but also *B. pertussis* strains that did not harbour any plasmid (named A2 strains). As shown in Fig. 5, PFGE analysis revealed that the A1 strains had the same PFGE profile as the A2 strains except for one band corresponding to plasmid pBP136, and thus probably represent the same or very similar genotypes. All 77 *B. pertussis* isolates from the infant's respiratory tract could be classified as A1 or A2 strains by PFGE analysis. Interestingly, the fraction of A1 strains isolated differed between the different parts of the infant's respiratory tract: pharynx, 55%; bronchial tube, 39%; lung, 15% (Table 1). This observation indicates that the localization of A1 strains was different from that of A2 strains in the patient's respiratory tract. Further analysis is

Table 1. Isolation of *B. pertussis* harbouring pBP136 (A1 strain) and not harbouring plasmid (A2 strain) from the pertussis infant's respiratory tract

Part of respiratory tract	No. of isolates	No. (%) of isolates:	
		A1 strain (pBP136)	A2 strain (no plasmid)
Pharynx	11	6 (55)	5 (45)
Bronchial tube	33	13 (39)	20 (61)
Lung*	33	5 (15)	28 (85)
Total	77	24 (31)	53 (69)

*Lower lobe of right lung.

needed to understand the ability of A1 and A2 strains to adhere to host cells, especially lung ciliated cells.

Role of pBP136 in *B. pertussis*

We investigated the ability of pBP136Km^R, a marked derivative of pBP136, to transfer by conjugation from *E. coli* EC100 to several *B. pertussis* strains, the vaccine strain Tohama and three clinical isolates. The transfer frequencies were estimated to be $1.8\text{--}4.1 \times 10^{-2}$, indicating that pBP136 is a highly transferable plasmid. Interestingly, pBP136 could also be transferred to *B. pertussis* A2, collected in this study (transfer frequency $1.5\text{--}2.0 \times 10^{-2}$). Although pBP136 is a transferable plasmid, pBP136-like plasmids without accessory mobile elements are rarely found in microbial communities (pA1 is the only other example so far; Harada *et al.*, 2006). Previously, Heuer *et al.* (2004) suggested that IncP-1 plasmids without any accessory genes must exist in microbial communities, but are difficult to detect due to the lack of genes encoding selectable phenotypes. Plasmid pBP136 supports this hypothesis, but its presence may be inconsistent with the hypothesis that plasmids are maintained in bacterial communities because they confer one or several advantageous traits to their host, which are intrinsically unnecessary for usual growth and survival (Bergstrom *et al.*, 2000). Since the cost of maintaining a plasmid that does not encode beneficial traits can be very small, but not zero, it is not clear how pBP136 persists in *B. pertussis*. A first possible explanation is that the two cryptic gene products, ORF1 and ORF2, provide as yet unknown benefits to *B. pertussis*. Secondly, it is possible that some of the plasmid backbone genes confer some advantage to *B. pertussis*, such as promotion of biofilm formation (Ghigo, 2001). Under these two assumptions, the plasmid provides a benefit to its host and therefore is maintained in the population. Alternatively, it could also be envisaged that pBP136 provides a benefit to *B. pertussis* as a vehicle that can capture mobile elements with beneficial phenotypic markers from other conjugative plasmids or even chromosomes of surrounding bacteria. A last hypothesis is that IncP-1 plasmids such as pBP136 can be maintained in microbial communities as parasitic genetic elements without any benefit for the host: since their transfer efficiency on surfaces is very high, it may be high enough to overcome their cost and occasional segregational loss and allow them to spread through and persist in microbial populations.

Conclusions

The IncP-1 β plasmid pBP136, described here, is the first reported case of a naturally occurring plasmid in *B. pertussis*. It seems to have diverged early from an ancestor of the IncP-1 β 'R751 group', and is the first IncP-1 β plasmid with uninterrupted *oriV-trfA* and *trb-tra* regions, and only the second, after pA1, shown not to contain any accessory mobile elements. The IncP-1 group plasmids are thought to have evolved by the acquisition of accessory genes encoding various phenotypic markers, which are being selected by the various compounds introduced in environmental and

clinical habitats, such as pesticides and antimicrobial agents (Top & Springael, 2003). Since pBP136 is an IncP-1 plasmid that has no such accessory genes in the expected insertion regions, it is an interesting model system for future studies on the adaptive evolution of IncP-1 plasmids.

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