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Characterization of IS*1001*, an Insertion Sequence Element of *Bordetella parapertussis*

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By analysis of repetitive DNA in *Bordetella parapertussis*, an insertion sequence element, designated IS*1001*, was identified. Sequence analysis revealed that IS*1001* comprised 1,306 bp and contained inverted repeats at its termini. Furthermore, several open reading frames that may code for transposition functions were identified. The largest open reading frame coded for a protein comprising 406 amino acid residues and showed homology to TnpA, which is encoded by an insertion sequence element (IS*1096*) found in *Mycobacterium smegmatis*. Examination of flanking sequences revealed that insertion of IS*1001* occurs preferentially in stretches of T's or A's and results in a duplication of target sequences of 6 to 8 bases. IS*1001* was found in about 20 copies in 10 *B. parapertussis* strains analyzed. No restriction fragment length polymorphism was observed in *B. parapertussis* when IS*1001* was used as a probe. An insertion sequence element similar or identical to IS*1001* was found in *B. bronchiseptica* strains isolated from pigs and a rabbit. In these strains, about five copies of the IS*1001*-like element were present at different positions in the bacterial chromosome. Neither *B. pertussis* nor *B. bronchiseptica* strains isolated from humans and dogs contained an IS*1001*-like element. Therefore, IS*1001* may be used as a specific probe for the detection of *B. parapertussis* in human clinical samples.

Bacterial insertion sequence (IS) elements are DNA segments capable of inserting themselves into different positions and orientations into the genome (8). Many IS elements are characterized by the presence of inverted repeats at their termini, which are essential for transposition, and duplication of target DNA, as a consequence of staggered cleavage of target sequences during transposition. The proteins involved in transposition are encoded by the IS sequence, and often overlapping reading frames are found. Many IS elements seem to have coevolved with their hosts and are therefore species specific. This quality makes IS elements very useful targets for the detection of pathogens in clinical specimen by means of the polymerase chain reaction (PCR). Furthermore, because many IS elements occur in multiple copies per genome, a high sensitivity of detection can be achieved (11).

An IS element designated IS*481*, which is found in *Bordetella pertussis*, the causative agent of whooping cough, has been used to detect this pathogen in clinical specimen by means of PCR (10, 12). IS*481* is found only in *B. pertussis*, not in the two closely related species *B. parapertussis* and *B. bronchiseptica* (16, 17). It is present in about 80 copies in the chromosome, comprises approximately 1,050 bp, and has inverted repeats of 28 bp at its termini. For two of the three copies of IS*481* analyzed, duplication of 3 and 5 bp in the target sequence has been observed (17). Open reading frames (ORFs) of IS*481*, coding for putative proteins involved in transposition, showed no homology to other IS-encoded sequences (16), and IS*481* could not be assigned to known families of IS elements by sequence similarities.

A considerable proportion (3 to 35%) of whooping cough cases is caused by *B. parapertussis* (13, 18), and a PCR-based diagnostic test should also include this pathogen. Although other repetitive DNA has been found in the *Bordetella* species (1), including a DNA fragment adjacent to one copy of IS*481* (16), which is present in nine copies in *B. parapertussis*, no sequences specific for *B. parapertussis* that would enable the discrimination between the two causative agents of whooping cough have been identified.

To find sequences specific for *B. parapertussis*, we set out to identify IS elements in this species by using a positive selection system (27). However, this method was not successful, and here we describe another approach to search for sequences specific for *B. parapertussis*. The method used identifies repeated elements by denaturation and renaturation of chromosomal DNA (22) and allowed us to identify and isolate a repeated IS element that appeared to be specific for *B. parapertussis*. This element may be used for the detection of *B. parapertussis* in clinical samples.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Bordetella* strains used in this study are listed in Table 1. The strains were stored in milk containing 30% glycerol at −70°C and were recovered by growth on Bordet-Gengou agar plates (4). Cloning procedures were carried out by using pEMBL18 (7) and *Escherichia coli* DH5α (Bethesda Research Laboratories, Gaithersburg, Md.).

Heteroduplex formation. Enrichment of repetitive heteroduplex DNA by self-renaturation was performed essentially as described by Ohtsubo and Ohtsubo (22). Briefly, 100 μg of chromosomal DNA in 400 μl of Tris-EDTA (TE) buffer was sheared by 20 passages through a 26-gauge needle. The sheared DNA was ethanol precipitated and resuspended in 50 μl of TE buffer containing 50% formamide. The DNA was denaturated by incubation at 100°C for 10 min. Subsequently, heteroduplex formation was allowed for 4 h at 20 to 25°C, after which the DNA was precipitated and dissolved in

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TABLE 1. *Bordetella* strains used

Strain	Designation	Source or reference
B. parapertussis	B24	Clinical isolate (human)
	841805	Clinical isolate (human)
	840750	Clinical isolate (human)
	840994	Clinical isolate (human)
	841637	Clinical isolate (human)
	850038	Clinical isolate (human)
	841892	Clinical isolate (human)
	850170	Clinical isolate (human)
	851754	Clinical isolate (human)
	871470	Clinical isolate (human)
	873318	Clinical isolate (human)
B. pertussis	Tohama	24
	Wellcome 28	23
B. bronchiseptica	851705	Clinical isolate (human)
	841434	Clinical isolate (human)
	B14	Clinical isolate (pig)
	B15	Clinical isolate (pig)
	B16	Clinical isolate (pig)
	B171	Clinical isolate (rabbit)
	B19	Clinical isolate (dog)
	B20	Clinical isolate (dog)
	B81	Clinical isolate (dog)

50 μl of S1 buffer (0.25 M NaCl, 1 mM $ZnSO_4$, 30 mM sodium acetate [pH 4.5]). S1 nuclease (100 U) was added, and the solution was incubated for 30 min at 37°C, after which it was extracted with phenol. Finally, the DNA was precipitated and dissolved in 50 μl of TE, and 10 μl was used for analysis by means of agarose gel electrophoresis or Southern blotting. The heteroduplex product was labeled after purification by gel electrophoresis.

DNA techniques. Chromosomal DNA was prepared as described previously (2). Restriction enzymes, T4 ligase, and S1 nuclease were used according to the instructions of the manufacturer. DNA fragments were labeled with digoxigenin according to the instructions provided with the AMPPD kit (Boehringer, Mannheim, Germany). Probes used in this study are depicted in Fig. 1. Plasmid DNA was isolated by the method of Birnboim and Doly (3) and further purified on Qiagen columns (Diagen, Düsseldorf, Germany). DNA fragments were isolated from agarose gels by using Geneclean (Bio 101, La Jolla, Calif.). Southern blotting was carried out as described by Maniatis et al. (15).

PCR. PCRs were performed in a final volume of 25 μl containing 67 mM Tris-HCl (pH 8.8), 16.6 mM $NH_4(SO_4)_2$, 6.7 mM $MgCl_2$, 10 mM β-mercaptoethanol, 5% dimethyl sulfoxide, 200 μM each of the four deoxynucleotides, 125 ng of each primer, 0.6 U of AmpliTaq polymerase (Perkin-Elmer Cetus), and approximately 200 ng of DNA. The reaction mixtures were preheated at 94°C for 3 min. Thirty-three amplification cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C were performed in a DNA thermocycler (Perkin-Elmer Cetus), followed by 7 min at 72°C to complete the final polymerase reaction.

Synthetic oligonucleotides. On the basis of the partial sequence of IS*1001*, harbored by pRPP3 (IS*1001*b; Fig. 1), two oligonucleotides were synthesized, using a DNA synthesizer (Applied Biosystems Inc., Foster City, Calif.). In primers V (5′-GCAaGcttAACTCGTCCATCG-3′) and T (5′-TATCAAGcTtATCAAGCGGCG-3′), which correspond to nucleotides 555 to 576 and 1210 to 1231 of IS*1001*, respectively (see Fig. 4), a *Hin*dIII site (underlined) was created by base substitutions (lowercase letters) to facilitate cloning of the PCR products. These primers were used to obtain DNA fragments harboring the termini and flanking regions of IS*1001* by means of inverse PCR (21). Inverse PCR allows the amplification of unknown sequences that flank an IS element of known sequence. DNA containing IS*1001* was digested with *Eco*RI, diluted to a concentration of 2 μg/ml, circularized with ligase, and subjected to PCR. The primers used, V and T (Fig. 1), were homologous to the ends of IS*1001* included within the circle formed by ligation but oriented such that chain elongation proceeded across the uncharacterized region rather than across the IS*1001* region separating the primers. DNA fragments obtained by amplification were cleaved with *Hin*dIII and inserted into the

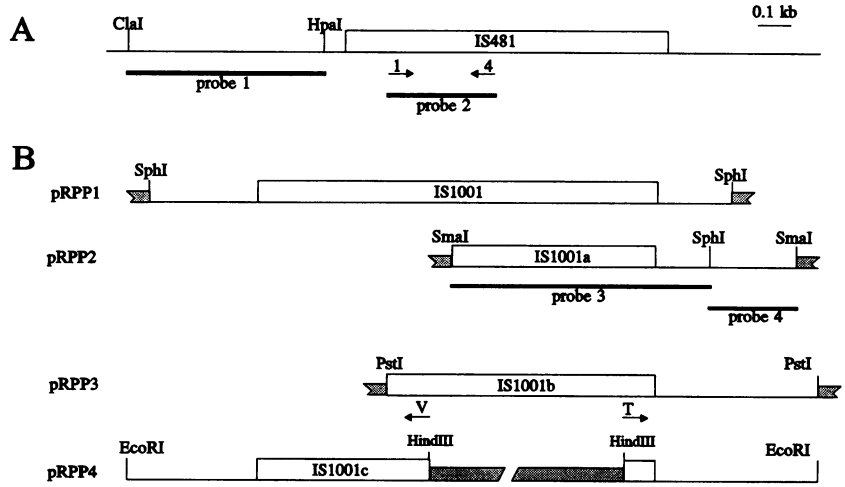

FIG. 1. Overview of cloned *B. pertussis* and *B. parapertussis* chromosomal DNA fragments and probes used in this study. (A) Probe 1 comprises a *Cla*I-*Hpa*I fragment of cosmid p11-11, which harbors a repeat present in *B. pertussis* and *B. parapertussis*. See also reference 16. The IS*481* probe (probe 2) was generated by means of PCR with primers 1 and 4, as indicated. (B) Chromosomal DNA fragments harboring the repeated DNA sequence from *B. parapertussis*. Designations of plasmids are indicated on the left. pRPP4 contains a DNA fragment obtained by means of inverse PCR using primers V and T, as indicated. The location of the repeated DNA elements is indicated by open bars. pEMBL18 sequences are represented by shaded bars.

*Hin*dIII site of pEMBL18. After transformation, colonies were screened with a probe derived from IS*1001* (probe 3; Fig. 1), and one positive clone was identified. The clone appeared to contain a plasmid, designated pRPP4, with a 1.8-kb *Hin*dIII DNA fragment derived from *B. parapertussis* (Fig. 1).

Primers A (5′-CGCCGCTTGATGACCTTGATA-3′) and Z (5′-CACCGCCTACGAGTTGGAGAT-3′), corresponding to nucleotides 1231 to 1210 and 733 to 754 of IS*1001*, respectively (see Fig. 4), and primer R (5′-TTTTTTGGT TCATCGCGC-3′), based on the sequence of the terminal inverted repeat of the element, were used in testing various strains for the presence of IS*1001* by means of PCR. A DNA fragment internal to IS*481* (probe 2; Fig. 1) was generated by PCR using primers 1 (5′-GGGGTCACCGCGCCGACTGT-3′) and 4 (5′-GGGCCTGATGCTCGTAGCGC-3′), derived from the sequence of IS*481* (15) and corresponding to nucleotides 208 to 228 and 496 to 476, respectively.

Sequence and computer analysis. For DNA sequencing, appropriate fragments were cloned and sequenced in both directions. Sequence reactions were performed according to Craxton (6), with *Taq* polymerase (Promega Corp., Madison, Wis.), dye-labeled M13 primers, or dye-labeled dideoxynucleotides with primers A, Z, V, T, and R (see above), using a model 370 automated sequencer (Applied Biosystems). Searches for homology were carried out by using FastA (21) in GenBank and GenPept 70.0, Swissprot 21.0, and EMBL 30.0 data banks.

Nucleotide sequence accession number. The IS*1001* sequence has been submitted to the EMBL data bank under accession number X66858.

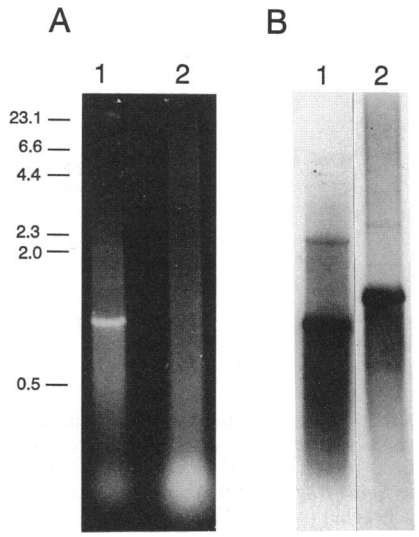

FIG. 2. Visualization of repetitive DNA in *B. pertussis* and *B. parapertussis* after self-hybridization and S1 nuclease treatment. (A) A 1% agarose gel loaded with self-annealed DNA from *B. pertussis* Tohama (lane 1) and *B. parapertussis* B24 (lane 2). (B) Southern blots of the heteroduplex DNA of each strain. For *B. pertussis* heteroduplex DNA (lane 1), an internal fragment of IS*481* (probe 2; Fig. 1) was used as a probe. For the *B. parapertussis* heteroduplex DNA (lane 2), the 1.3-kb heteroduplex product was purified by gel electrophoresis and used as a probe. The sizes (in kilobases) and positions of *Hin*dIII-digested lambda marker DNA are indicated on the left.

RESULTS

Analysis of repeated sequences in *B. pertussis* and *B. parapertussis*. To detect repeated DNA sequences in the genomes of *B. pertussis* and *B. parapertussis*, we used the approach described by Ohtsubo and Ohtsubo (22). The method is based on the observation that reiterated DNA sequences rapidly form heteroduplexes after denaturation and renaturation of genomic DNA containing these sequences. After renaturation, S1 nuclease treatment is used to remove single-stranded DNA generally derived from single- or low-copy-number genes. Heteroduplex end products of *B. parapertussis* B24 and *B. pertussis* Tohama are depicted in Fig. 2. Three distinct bands of 1, 1.5, and 2 kb were visible after self-annealing of *B. pertussis* DNA (Fig. 2A, lane 1). The 1- and 2-kb bands (Fig. 2B, lane 1) hybridized with a labeled fragment of IS*481* (probe 2; Fig. 1). Since IS*481* comprises approximately 1 kb, these hybridizing fragments probably represent single and tandem copies of IS*481*, respectively.

No distinct bands could be observed in self-annealed *B. parapertussis* DNA when the same quantity of DNA as used for *B. pertussis* was loaded (Fig. 2A, lane 2). However, when the amount of *B. parapertussis* heteroduplex product was increased, a faint band at 1.3 kb could be detected (not shown). When this 1.3-kb heteroduplex product was purified by gel electrophoresis and used as a probe, a distinct band of 1.3 kb could be detected on a Southern blot containing *B. parapertussis* heteroduplex product (Fig. 2B, lane 2). No bands were detected when labeled *B. parapertussis* heteroduplex products were hybridized with *B. pertussis* Tohama heteroduplex products (not shown). These results indicated that the genome of *B. parapertussis* B24 contains a repeated sequence comprising approximately 1,300 bp.

A repeated DNA sequence adjacent to one copy of IS*481* has been found to occur in nine copies in *B. parapertussis* and four copies in *B. pertussis* (16). A probe derived from this repeat (probe 1; Fig. 1) was used in Southern blots to determine its relationship with the 1.5- and 1.3-kb repeats observed in *B. pertussis* and *B. parapertussis*, respectively. No hybridization was observed (not shown), indicating that the 1.5- and 1.3-kb repeats represented unidentified repeated elements.

Cloning of the repeated DNA sequence from *B. parapertussis*. To obtain a specific probe for the repeated sequence detected in *B. parapertussis*, the 1.3-kb heteroduplex DNA product was purified by gel electrophoresis and labeled with digoxigenin. The labeled probe was subsequently used in Southern blots containing *Sma*I-restricted *B. parapertussis* chromosomal DNA (Fig. 3). The probe hybridized to at least 22 *Sma*I fragments, confirming its repetitive nature in *B. parapertussis*. A number of these *Sma*I fragments with sizes of 0.5 to 1.8 kb were purified by gel electrophoresis and inserted into pEMBL18. After transformation of the ligation mixture, *E. coli* colonies were analyzed with the *B. parapertussis* heteroduplex probe. Of approximately 75 colonies, 1 hybridized strongly with the heteroduplex probe. Plasmid DNA was isolated from this colony, and the plasmid, designated pRPP2, was found to contain a 1.1-kb *Sma*I insert derived from *B. parapertussis* (Fig. 1B). Southern blot analysis of *B. parapertussis* DNA digested with *Cla*I revealed that the 1.1-kb *Sma*I fragment hybridized to multiple DNA fragments. In contrast, the 1.1-kb *Sma*I fragment hybridized to only one fragment when *B. pertussis* DNA digested with *Cla*I was analyzed (not shown). To investigate whether pRPP2 contained *B. parapertussis*-specific sequences, the 0.3- and 0.8-kb *Sma*I-*Sph*I fragments of pRPP2,

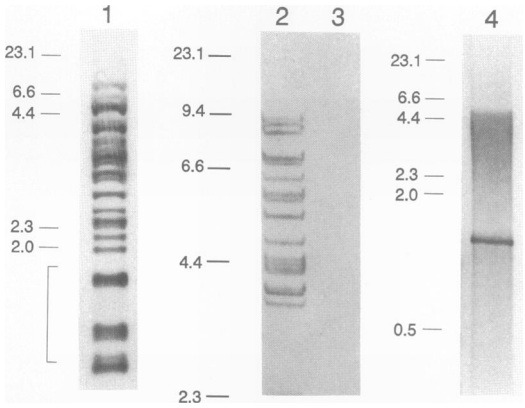

FIG. 3. Detection of repeated sequences in *B. parapertussis* B24. Shown is a Southern blot of *Sma*I-digested *B. parapertussis* DNA hybridized to the purified heteroduplex product of *B. parapertussis*. The *Sma*I fragments that were extracted from the gel are indicated by a bracket (lane 1). *Cla*I-digested *B. parapertussis* DNA (lane 2), *Cla*I-digested *B. pertussis* DNA (lane 3), and *B. parapertussis* heteroduplex DNA (lane 4), hybridized to probe 3. The sizes (in kilobases) and positions of *Hin*dIII-digested lambda marker DNA are indicated on the left.

designated probes 3 and 4, respectively (Fig. 1), were hybridized with *B. pertussis* and *B. parapertussis* DNA. The 0.3-kb *Sma*I-*Sph*I fragment hybridized to a unique *Cla*I fragment of *B. pertussis* and *B. parapertussis* (data not shown). In contrast, the 0.8-kb *Sma*I-*Sph*I fragment hybridized to approximately 19 *Cla*I fragments of *B. parapertussis* chromosomal DNA (Fig. 3, lane 2). No hybridization was observed with *B. pertussis* DNA when this fragment was used as a probe (Fig. 3, lane 3). As expected, the 0.8-kb *Sma*I-*Sph*I fragment was found to hybridize with the 1.3-kb *B. parapertussis* heteroduplex product (Fig. 3, lane 4).

Since the 0.8-kb *Sma*I-*Sph*I fragment in pRPP2 contained only part of a larger 1.3-kb repeated DNA element, attempts were made to obtain a complete copy of the repeated sequence. Chromosomal DNA was digested with *Pst*I or *Sph*I and inserted into the appropriate restriction sites of pEMBL18. After transformation, *E. coli* colonies were screened with probe 3 (Fig. 1). From two positive clones, plasmids (designated pRPP1 and pRPP3) that contained a 1.8-kb *Sph*I fragment and a 1.2-kb *Pst*I fragment, respectively, were isolated. To identify the termini of the repeated sequence unequivocally, inverse PCR was used to clone *B. parapertussis* DNA flanking a repeated element (see Materials and Methods). Amplified DNA was inserted into pEMBL18 and transformed into *E. coli*. One plasmid, designated pRPP4 (Fig. 1), containing a 1.8-kb *B. parapertussis* DNA fragment that hybridized to probe 3 was selected.

Sequence of the repeated DNA sequence from *B. parapertussis*. All clones summarized in Fig. 1B were sequenced on both strands except the right terminus of the repeated element located in pRPP4, which was sequenced on one strand only. Plasmids pRPP2 and pRPP3 were each found to contain the right part of the repeated sequence which was truncated by a *Sma*I site and a *Pst*I site, respectively. By comparison of the sequences of pRPP2 and pRPP3, the right terminus of the element could be determined. The 1.8-kb *Sph*I fragment in pRPP1 was found to harbor a complete copy of the repeated sequence, and by sequence comparison of pRPP1 and pRPP4, the left terminus of the element was determined. The entire DNA sequence of the repeated element is shown in Fig. 4. Small differences were observed between the various copies, which occurred mainly in the left part of the element. The element was found to comprise 1,306 bp, with terminal inverted repeats of 15 bp, and to have a G+C content of 60%. Four ORFs longer than 100 amino acids could be identified (Fig. 5). Examination of DNA regions flanking the element revealed duplications of four to six bases in the target DNA (Fig. 6). Thus, the repeated sequence from *B. parapertussis* has properties consistent with bacterial IS elements and was designated IS*1001*.

Distribution of IS*1001*. To investigate whether IS*1001* could be used as a specific probe for the detection of *B. parapertussis* in clinical samples, the host range of this IS element was determined. For this purpose, chromosomal DNA was isolated from 10 *B. parapertussis*, 2 *B. pertussis*, and 2 *B. bronchiseptica* human clinical isolates (Table 1). The DNA was digested with *Sph*I (not shown) and *Pst*I and analyzed by means of Southern blotting using probe 3 (Fig. 7). All 10 *B. parapertussis* strains hybridized with this probe, and the hybridization patterns were identical to the pattern observed with the *B. parapertussis* B24 (Fig. 7). DNA fragments of the *B. pertussis* and *B. bronchiseptica* strains did not hybridize with IS*1001* DNA. Twenty additional *B. pertussis* strains and one additional *B. bronchiseptica* strain, all human clinical isolates, were analyzed for the presence of IS*1001* by means of PCR using primers A and Z (Fig. 4). All 21 strains were found to be negative with the PCR. In addition to the three human isolates of *B. bronchiseptica* referred to above, a number of *B. bronchiseptica* strains derived from animals were analyzed for the presence of IS*1001*-like elements by means of Southern blotting using probe 3 and with use of *Pst*I (not shown) and *Sph*I (Fig. 8) to cleave chromosomal DNA. It appeared that three strains isolated from dogs did not contain IS*1001*-like elements. However, the three pig strains and a rabbit strain were found to contain at least four copies of an IS*1001*-like element. Interestingly, a different hybridization profile was observed among these strains.

DISCUSSION

IS elements have been described for a large number of bacterial species (8). These elements are generally species specific and have often been found by chance or through their ability to affect gene expression by transposition and insertional inactivation (26). A purposeful search for IS elements that occur in multiple copies can be performed by the approach we describe here. The method is based on the formation of heteroduplex molecules by reiterated DNA sequences after denaturation and renaturation of chromosomal DNA (22). Removal of single-stranded DNA, generally derived from single- or low-copy-number genes, yields a heteroduplex product that is enriched for repeated sequences. When this procedure was applied to *B. pertussis* DNA, which is known to contain approximately 80 copies of an insertion element, designated IS*481* (16, 17), three distinct bands with molecular size of 1, 1.5, and 2 kb were detected after agarose gel electrophoresis of the enriched sequences. The 1- and 2-kb bands hybridized to IS*481*, indicating that they represented single copies and tandem repeats of this element, respectively. The third band contains an as yet unidentified repeated sequence of *B. pertussis*.

Results obtained with *B. parapertussis* chromosomal DNA allowed the detection of repeated DNA present in approximately 20 copies per genome if agarose gel electrophoresis was used to visualize the enriched sequences. To

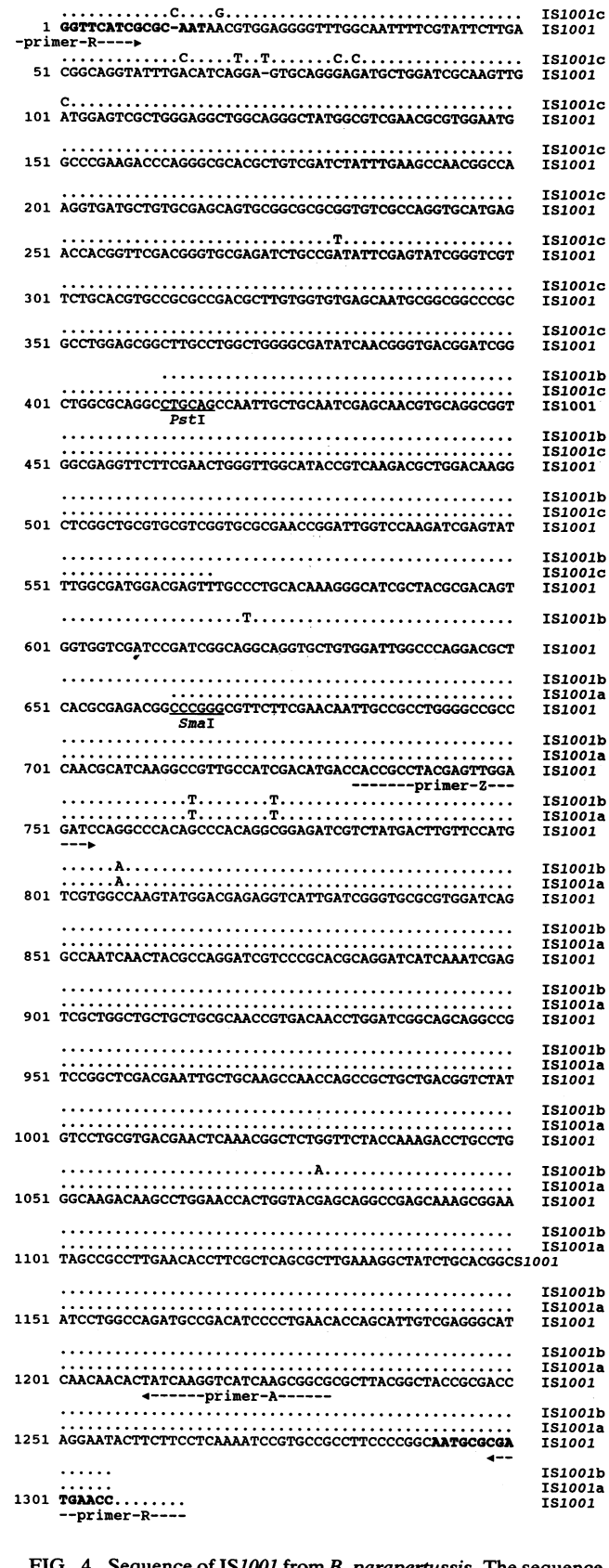

FIG. 4. Sequence of IS1001 from *B. parapertussis*. The sequence is based on the element found in pRPP1 and is numbered from the outer end of the left terminal inverted repeat. Both left and right inverted repeats are in bold, and restriction sites mentioned in the text are indicated. Partial sequences of a number of independently isolated IS1001 copies are also shown. For these sequences, only differences with IS1001 are indicated. Identical bases are represented by dots. The IS1001a, -b, and -c sequences are based on copies of the element found in pRPP2, pRPP3, and pRPP4, respectively. PCR primers A, Z, and R are indicated.

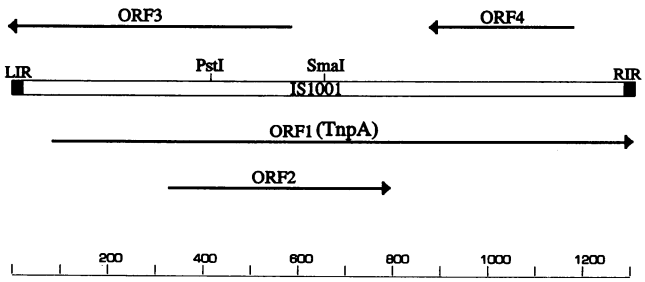

FIG. 5. Locations of ORFs in IS1001. IS1001 is represented by an open bar. The left terminal inverted repeat (LIR), right terminal inverted repeat (RIR), and relevant restriction sites are indicated. The lines below IS1001 represent ORFs translated from left to right. ORFs depicted above IS1001 are read in the opposite direction. The scale indicates base pairs.

facilitate detection of repeated sequences present in fewer copies, the number of cycles of denaturation and renaturation may be increased. Alternatively, the labeled heteroduplex products may be used as a probe to identify repeated sequences in Southern blots containing heteroduplex end products or to identify restricted chromosomal DNA. The latter approach was used successfully to clone and isolate a repeated sequence, designated IS1001, from the genome of *B. parapertussis*.

Sequence analysis of IS1001 revealed the presence of poly(A) or poly(T) sequences flanking both left and right termini of the element. This finding suggests that stretches of A's or T's represent preferential insertion sites for IS1001. Since *B. pertussis* has a very high G+C content of approximately 67% (14), the number of potential insertion sites for IS1001 is probably very limited in this species. Specificity for particular sequences may be a means by which an IS element regulates its copy number to prevent an adverse effect on its host. Analysis of the regions flanking IS1001 suggests that insertion results in duplication of approximately six bases of the target sequence. Apart from a complete copy, various fragments of IS1001 were isolated and sequenced. Comparison of the sequences revealed small differences which occurred mainly in the left part of IS1001. When the DNA sequence of IS1001 was used to search the EMBL and GenBank data bases, no striking homologies were found. However, one of the ORFs observed in IS1001 (ORF1; Fig. 5) showed homology with a putative transposase, designated TnpA, encoded by IS1096 which is found in *Mycobacterium smegmatis* (Fig. 9). In view of the observed homology, it seems likely that ORF1 codes for a protein involved in transposition, and we have designated this ORF *tnpA*. Interestingly, like all copies of IS1001 analyzed, IS1096 appeared to be inserted into an A+T-rich DNA region (5). *M. smegmatis* is a commensal of the respiratory tract, and it is conceivable that genetic exchange occurred between *M. smegmatis* and *B. parapertussis* or between these two species and a third resident of the respiratory tract.

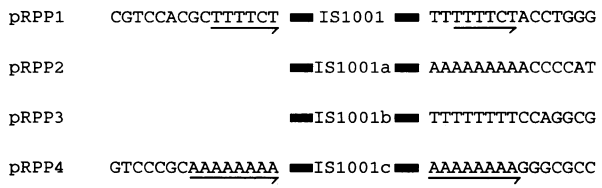

FIG. 6. Flanking sequences of isolated copies of IS*1001*. Designations of plasmids containing the IS*1001* copies are indicated on the left. Sequences found flanking IS*1001* are indicated, and direct repeats are underlined.

IS*1001*-like sequences were not found in *B. pertussis* or in *B. bronchiseptica* strains isolated from humans and dogs. However, in *B. bronchiseptica* strains isolated from pigs and a rabbit, a repeated element that hybridized to IS*1001* was found. Upon testing by means of PCR using primer R or primers A and Z (see Materials and Methods), DNA fragments with the expected sizes were generated with these latter strains, indicating a high degree of homology with IS*1001* (not shown). Based on phylogenetic relationships, *B. pertussis* and *B. parapertussis* are considered descendants of *B. bronchiseptica* (19). The distribution of IS*1001*-like elements in the genus *Bordetella* may suggest that *B. parapertussis* has evolved from a particular *B. bronchiseptica* clone carrying an IS*1001*-like element. Alternatively, it is possible that *B. parapertussis* and *B. bronchiseptica* have acquired the IS*1001*-like element independently. The absence of common hybridization bands between the two species suggests different primary insertion sites and indicates independent acquisition. Analysis of more *B. bronchiseptica* strains for the presence of IS*1001*-like elements and determination of their nucleotide sequences may provide further insight into the evolution of *Bordetella* species.

Because of their mobile nature, IS elements can be located at different sites in the chromosome, and often variable copy numbers are found (9). Thus, probes derived from IS elements are potentially useful for distinguishing strains of a single species by means of restriction fragment length polymorphism (28). No restriction fragment length polymorphism was observed in *B. parapertussis* when IS*1001* was used as a probe, suggesting a clonal divergence of this species, which may have evolved relatively recently. In contrast, different hybridization profiles were observed in all four *B. bronchiseptica* strains harboring the IS*1001*-like element. This finding suggests that these *B. bronchiseptica* strains are genetically more diverse than the *B. parapertus-*

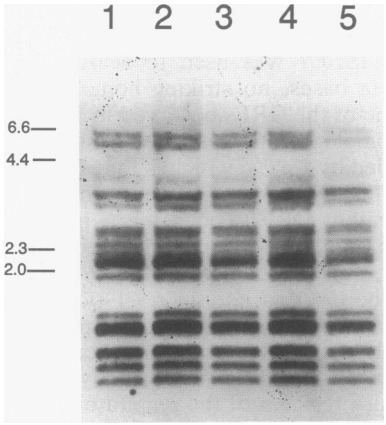

FIG. 7. Southern blot of chromosomal DNA from *B. parapertussis* clinical isolates. A fragment derived from IS*1001* (probe 3; Fig. 1) was used as a probe. Chromosomal DNA was digested with *Pst*I. Lanes: 1, strain 841805; 2, strain 840750; 3, strain 840994; 4, strain 841637; 5, strain 850038. The remaining *B. parapertussis* strains (Table 1) showed identical hybridization patterns. *B. pertussis* Tohama and Wellcome 28 and two *B. bronchiseptica* human isolates (Table 1) did not hybridize with this probe (not shown). The sizes (in kilobases) and positions of *Hin*dIII-digested lambda marker DNA are indicated on the left.

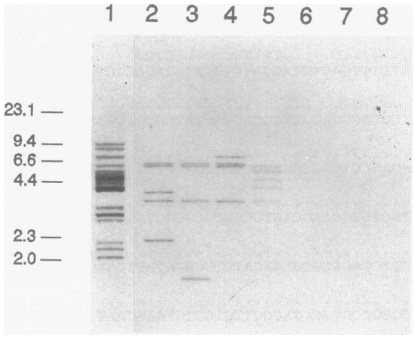

FIG. 8. Southern blot of chromosomal DNA from *B. parapertussis* and *B. bronchiseptica* digested with *Sph*I and hybridized with IS*1001* (probe 3). Lanes: 1, *B. parapertussis* B24; 2 to 4, *B. bronchiseptica* B14, B15, and B16, respectively (pig isolates); 5, *B. bronchiseptica* B171, a rabbit isolate; 6 to 8, *B. bronchiseptica* B19, B20, and B81 (dog isolates). The sizes (in kilobases) and positions of *Hin*dIII-digested lambda marker DNA are indicated on the left.

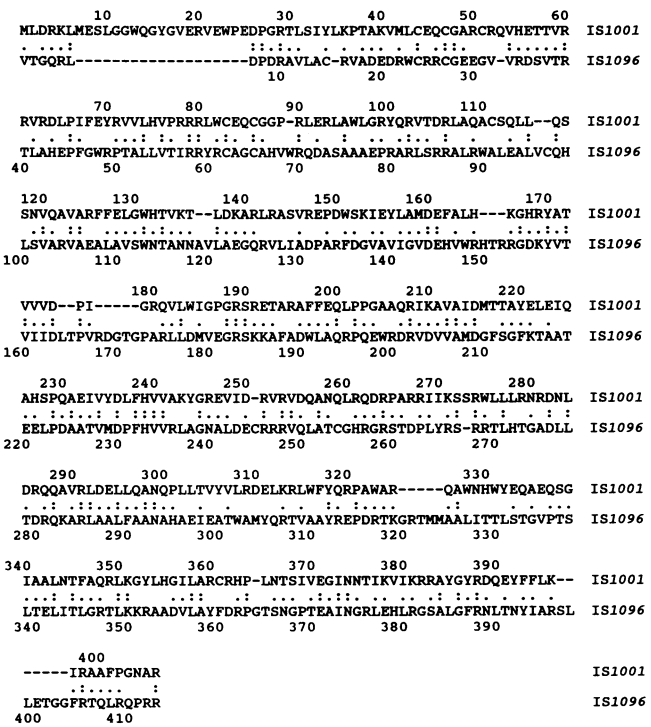

FIG. 9. Homology between proteins encoded by IS*1001* and IS*1096* of *M. smegmatis*. Dashes indicate gaps introduced to increase the number of matches. Identical and similar amino acids are represented by double and single dots, respectively. The percentage of identity between the two sequences is 20.5.

sis strains analyzed. A similar conclusion was reached on the basis of multilocus enzyme genotypes (20).

One of the aims of this study was to isolate a repeated sequence that could be used to identify *B. parapertussis* in clinical samples. The occurrence of IS*1001*-like sequences in some *B. bronchiseptica* strains does not limit the usefulness of IS*1001* as a tool for identifying *B. parapertussis*, since *B. bronchiseptica* is very rarely isolated from humans. Moreover, the three human isolates that we analyzed did not harbor IS*1001*-like sequences. We are currently comparing diagnosis of pertussis based on serology and culturing with a PCR-based diagnosis, using IS*481*- and IS*1001*-specific sequences.

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