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Genomic Fluidity of *Bordetella pertussis* Assessed by a New Method for Chromosomal Mapping

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The genomic organization of *Bordetella pertussis* strains has been examined by using a new method. This method does not depend on the prior determination of a restriction map of the bacterial chromosome but is based on the ability to measure directly the distance between two genes. This is accomplished through the integration at each gene of a suicide vector containing a cleavage site for the intron-encoded endonuclease I-*Sce*I, which is not otherwise found in the chromosome. Integration is mediated by homologous recombination between the chromosomal and cloned plasmid copies of a gene of interest. Digestion with I-*Sce*I gives rise to a fragment the size of which represents the distance between the two genes. Multiple pairwise determinations within a set of genes provide sufficient information to derive a map of the relative gene positions. Mapping a set of 11 to 13 genes for five strains of *B. pertussis* and one strain of *B. parapertussis* revealed extensive divergence of gene order between *B. pertussis* Tohama I, *B. pertussis* 18-323, and *B. parapertussis* ATCC 15311. Less extensive divergence of gene order was observed between *B. pertussis* Tohama I and *B. pertussis* Tohama III, BP165, and Wellcome 28, with most of the observed differences explainable by large inversions.

Knowledge of the structure and content of bacterial genomes has increased dramatically in recent years. Several bacterial genomes have been completely sequenced, and a much greater number have been mapped. This has provided valuable tools for the genetic analysis and manipulation of bacterial chromosomes, together with comprehensive basic data concerning the composition and structure of bacterial genes and genomes. These studies are ushering in an era of new approaches to the study of bacterial genetics.

In spite of these advances, relatively few comparative studies have been performed in which the chromosomal structure of several different strains of the same species, or different species of the same genus, have been analyzed (6). The comparative studies which have been performed have given widely varying pictures of the degree of conservation of gene order in the various groupings of bacterial strains examined. At one end of the spectrum, conservation of genomic organization is seen among different species or even different genera. For example, such conservation has been observed among different species of *Borrelia*, between *Mycoplasma hominis* and *Clostridium perfringens*, and among the enteric organisms *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* (12, 25–27). At the other end of the spectrum, great divergence of gene order can be seen, even among members of the same species. Such divergence has been observed among strains of *Bacillus cereus* and *Leptospira interrogans* (5, 39).

An appreciation of genomic variability is an essential aspect of our understanding of the basic forces acting in the evolution of bacterial chromosomes. By introducing genetic diversity at a higher level of genetic organization than single-base-pair mutations, chromosomal rearrangements contribute to the genetic variation in populations which is the raw material upon which natural selection acts to forward evolution (1). For example, chromosomal rearrangements can lead to loss of function through deletion or to gain of function by novel juxtapositions of promoters and genes. Duplications provide the opportunity for divergence of function by accumulation of mutational change. It has recently been proposed that a selective force in the evolution of bacterial operons results from the horizontal transfer of fortuitous proximal arrangements of genes of related function in a scenario which depends on spontaneous chromosomal reassortment (13). The recently described "pathogenicity islands" in a number of virulent bacteria provide a beautiful example of the evolution of clusters of related genes (8).

To perform a comparative analysis of the genomic organization of *Bordetella pertussis*, we have developed a new method for the analysis of chromosomal structure. This method is relatively rapid and simple to perform, and it does not rely on the prior derivation of a restriction map to allow the location of genes within the context of such a map. Instead, it directly measures the distances between genes or sequences of interest in a manner similar to that described recently by Mahillon et al. (16). This method is termed "chromosomal surveying" because, by analogy with the geographical counterpart, emphasis is on the spatial relationships of a set of points to each other rather than their relationship to details of the genetic terrain in which they are located.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains and plasmids used in this study are described in Table 1. *E. coli* strains were grown on L agar or in L broth (19) supplemented with antibiotics as appropriate. Concentrations of antibiotics were 100 μg/ml for ampicillin, 10 μg/ml for gentamicin sulfate, and 10 μg/ml for kanamycin sulfate. *E. coli* DH5α, which was used as a transformation host for all cloning steps, was obtained from Bethesda Research Laboratories. *B. pertussis* strains were grown on Bordet-Gengou agar (Difco) containing 1% proteose peptone (Difco) and 15% defibrinated sheep blood. Concentrations of antibiotics used routinely were 10 μg/ml for gentamicin sulfate, 10 μg/ml for kanamycin sulfate, 100 μg/ml for streptomycin sulfate, 50 μg/ml for rifampin, and 50 μg/ml for nalidixic acid. Concentrations of streptomycin sulfate and nalidixic acid used to isolate spontaneously occurring resistant derivatives were 200 and 100 μg/ml, respectively.

Construction of plasmid vectors. pSS1898 was constructed as follows. The suicide vector pJM703.1 was modified by the addition of the oligonucleotide

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant features	Source and/or reference(s)
E. coli K-12		
DH5α	High-efficiency transformation	Bethesda Research Laboratories
SM10	Conjugation-proficient donor	29
SM10(λ*pir*)	λ*pir* lysogen of SM10	20
B. pertussis		
Tohama I	Patient isolate, Japan, approx 1954	9
BP536	Str^r Nal^r derivative of Tohama I	32
18-323	ATCC type strain, intracerebral challenge strain	ATCC, 10
BP977	Str^r Nal^r derivative of 18-323	This study
165	Patient isolate, United States, approx 1950	17, 23
BP1013	Str^r derivative of 165	This study
Wellcome 28	Patient isolate, The Netherlands, approx 1969	24, 28
BB112	Str^r derivative of Wellcome 28	38
Tohama III	Avirulent-phase patient isolate	37
BP369	Str^r Rif^r derivative of Tohama III	37
B. parapertussis		
ATCC 15311	ATCC type strain	ATCC
BP998	Rif^r derivative of ATCC 15311	This study
Plasmids		
pSS1894	pBR322-based vector; *gen amp oriT*	18
pJM703.1	R6K *oriV* (Pir dependent) suicide plasmid; *amp oriT*	20
pSS1898	Mapping vector; R6K *oriV gen oriT*	This study
pSS1577	pBR322-based vector; *kan amp rpsL oriT*	33
pSS1884	Derivative of pSS1577 containing I-*Sce*I and I-*Ppo*I sites	This study
D2BpUC9Cam	pUC9Cam containing approx 250-bp *oriT* fragment	36
pBR322	Cloning vector; *amp tet* ColE1 *oriV*	4
pSS1914	Mapping vector; ColE1 *oriV kan amp oriT*	This study
Plasmids used for mapping		
pSS1919	10.0-kb *Eco*RI *fhaB* fragment cloned into pSS1898	This study
pSS1921	17.5-kb *Eco*RI *prn* fragment cloned into pSS1898	This study
pSS1923	14.0-kb *Eco*RI *cya′* and upstream fragment cloned into pSS1898	This study
pSS1924	6.7-kb *Eco*RI *aroA* fragment cloned into pSS1898	This study
pSS1925	7.7-kb *Eco*RI *por* fragment cloned into pSS1898	This study
pSS1926	4.7-kb *Eco*RI *ptx* fragment cloned into pSS1898	This study
pSS1927	17.0-kb *Eco*RI *recA* fragment cloned into pSS1898	This study
pSS1930	4 kb-*Eco*RI *bvg* downstream fragment cloned into pSS1898	This study
pSS1931	10.0-kb *Eco*RI *fhaB* fragment cloned into pSS1914	This study
pSS1932	17.5-kb *Eco*RI *prn* fragment cloned into pSS1914	This study
pSS1933	14.0-kb *Eco*RI *cya′* and upstream fragment cloned into pSS1914	This study
pSS1934	4.5-kb *Eco*RI *fimX* fragment cloned into pSS1914	This study
pSS1935	4.7-kb *Eco*RI *ptx* fragment cloned into pSS1914	This study
pSS1936	17.0-kb *Eco*RI *recA* fragment cloned into pSS1914	This study
pSS1937	0.83-kb *Eco*RI *vrg6* fragment cloned into pSS1914	This study
pSS1938	17.5-kb *Eco*RI *fim2* fragment cloned into pSS1914	This study
pSS1939	4.0-kb *Eco*RI *bvg* downstream fragment cloned into pSS1914	This study
pSS1940	0.85-kb *Eco*RI *fim3* PCR fragment cloned into pSS1914	This study
pSS2058	2.7-kb *Apo*I *′cya′* fragment cloned into pSS1914	This study
pSS2083	1.8-kb *Not*I *dnt* fragment cloned into pSS1577 derivative, I-*Sce*I added	This study

linker 5′-GATCTCGCGGCCGCGA-3′ at the *Bam*HI site between the *mob*RP4 fragment and pBR322 sequences to create pSS1869. This resulted in the destruction of that *Bam*HI site and the introduction of a *Not*I site at this position. As a result, a 2.3-kb *Eco*RI-*Not*I fragment containing *ori*R6K and *mob*RP4 could be liberated from pSS1869. This fragment was joined to the *Eco*RI-*Not*I fragment of pSS1894 (18), containing a gentamicin resistance gene, to create pSS1898.

pSS1914 was constructed as follows. The complementary oligonucleotides 5′-CTAGATAGGGATAACAGGGTAATT-3′ and 5′-CTAGAATTACCCTG TTATCCCTAT-3′ encoding an I-*Sce*I site were added at the *Xba*I site of pSS1577 to create pSS1876. The complementary oligonucleotides 5′-CTAGTT TAAACATTTAAATTAATTAAG-3′ and 5′-CTAGCTTAATTAATTTAAAT GTTTAAA-3′ containing *Pac*I, *Swa*I, and *Pme*I sites were then added to the *Spe*I site of pSS1876 to create pSS1880. In this insertion, the *Spe*I site is maintained only to one side of the oligonucleotide. pSS1884 was created by the addition of the complementary oligonucleotides 5′-CTCTCTTAAGGTAGCTTAAT-3′ and 5′-TAAGCTACCTTAAGAGAGAT-3′ at the *Pac*I site of pSS1880. This insertion resulted in the addition of an I-*Ppo*I site and the maintenance of the *Pac*I site to only one side of the oligonucleotide insertion. Plasmid D2BpUC9Cam, which was generously provided by V. Waters (36), was used as the source of an approximately 250-bp *Bam*HI-*Hin*dIII fragment containing a functional *oriT* of RP4 which was cloned between the *Bam*HI and *Hin*dIII sites of pBR322 (4) to create pSS1910. The *Hin*dIII site in pSS1910 was destroyed, and an *Spe*I site was added, by the addition of the self-complementary oligonucleotide 5′-AGCTCA CTAGTG-3′ at the *Hin*dIII site to create pSS1911. The *Eco*RI-*Spe*I fragment from pSS1884 containing the kanamycin resistance gene, as well as the *Pac*I,

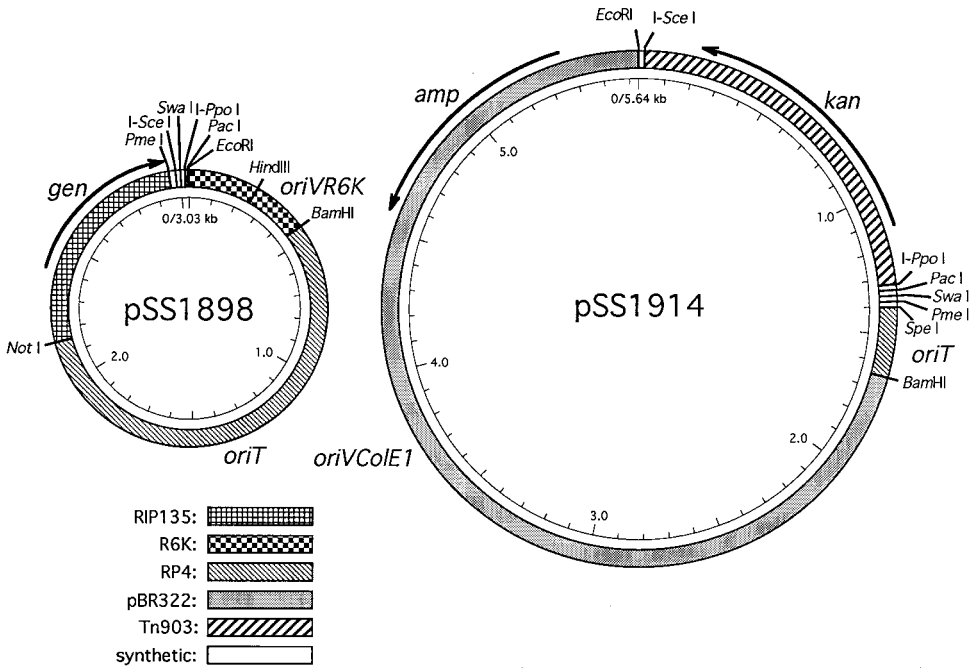

FIG. 1. Maps of two cloning vectors used for chromosomal surveying. Shaded areas indicate the sources of different sequences with reference to the key provided. Note that sequence homology between the two vectors is restricted to approximately 250 bp containing *oriT* of RP4.

*Swa*I, *Pme*I, I-*Sce*I, and I-*Ppo*I sites, was cloned between the *Eco*RI and *Spe*I sites of pSS1911 to create pSS1914.

Cloning of chromosomal genes for mapping. Plasmids created by cloning fragments of *B. pertussis* chromosomal DNA into pSS1898 and pSS1914 and used for mapping are listed in Table 1. *Eco*RI chromosomal fragments of *B. pertussis* Tohama I containing genes for pertactin (*prn*), 5-enolpyruvylshikimate-3-phosphate synthase (*aroA*), porin (*por*), pertussis toxin (*ptx*), RecA homolog (*recA*), truncated adenylated cyclase toxin operon with upstream sequences (*cya*), and the fimbrial genes *fimX* and *fim2* were obtained from a clone bank in pSS1577 as previously described (33). An *Apo*I fragment internal to the *cya* operon was subsequently subcloned from the *cya* fragment described above. The 10.0-kb *Eco*RI fragment containing *fhaB* and the 4.0-kb *Eco*RI fragment containing sequences downstream of the *bvg* operon were derived from pUW21-26 (31). Plasmid pDB101 (kindly provided by David Beattie) served as a source of the 829-bp *Eco*RI fragment containing the *vrg6* gene. A 0.85-kb *Eco*RI fragment of the *fim3* gene was generated by PCR. These fragments were cloned into pSS1898 and/or pSS1914 for the purposes of this work. To create pSS2083, pWEC32 (35) (kindly provided by Allison Weiss) was first modified by the addition of the linker 5′-AATTGGCGCGCC-3′ to the *Eco*RI site within the *dnt* gene to create a *Not*I fragment, which was subsequently cloned into a pSS1577 derivative in which the *Eco*RI site had been converted to a *Not*I site by using the same oligonucleotide. An I-*Sce*I site was subsequently added at the *Xba*I site by using the complementary oligonucleotides described above, to create pSS2083.

Bacterial conjugations. The plasmids used for mapping are listed in Table 1. Plasmids were mobilized into *Bordetella* strains by conjugations performed as described previously (30) except that plasmids were transferred after transformation into *E. coli* SM10, which contains a chromosomal integration of the *tra* genes of RP4. SM10 (λ*pir*), which contains the *pir* gene of plasmid R6K to allow replication of JM703.1 derivatives, was used for transfer of the pSS1898 derivatives. The pSS1898 derivatives were introduced into target strains, using selection for gentamicin resistance and counterselection by streptomycin, rifampin, or nalidixic acid resistance, depending on the strain. Exconjugants arising from these matings were restreaked on Bordet-Gengou medium containing gentamicin and used as recipients in matings with *E. coli* donors harboring the pSS1914 derivatives or pSS2038. Selection was for gentamicin and kanamycin resistance, and counterselection was as described above. Colonies arising from the second mating were pooled for the preparation of samples for pulsed-field gel electrophoresis (PFGE).

PFGE. Bacterial suspensions were embedded in agarose, and chromosomal DNA was liberated as described previously (33). Digestion with I-*Sce*I was performed as directed by the manufacturer (Boehringer Mannheim). PFGE was performed as described previously (33), using a CHEF-DRII apparatus (Bio-Rad). The switch time was ramped from 50 to 200 s over the 32-h run time at 200 V, and the size standards were *Saccharomyces cerevisiae* chromosomes obtained from New England BioLabs. If I-*Sce*I fragments which were below the range of these markers were obtained, gels were run with a switch time ramped from 1 to 50 s, and a ladder of bacteriophage lambda concatemers (New England BioLabs) was used as size standards (Fig. 3b).

Mapping of I-*Ceu*I sites. The locations of the I-*Ceu*I sites in the chromosomes of BP369, BP536, BP1013, and BB112 were mapped by analyzing chromosomal DNA samples of these strains harboring the different pSS1898 derivatives inserted at their respective genomic locations. These samples were digested with I-*Ceu*I and I-*Sce*I and subjected to PFGE as described above. The locations of I-*Ceu*I sites could be deduced from the sizes of the fragments produced in these double digests.

RESULTS

Description of the chromosomal surveying method and its validation on *B. pertussis* Tohama I. The chromosomal surveying approach uses two plasmid suicide vectors which were designed to function independently of each other. These two vectors are shown in Fig. 1. The vegetative origins of replication for these plasmids are both unable to function in *Bordetella* strains, that of pSS1898 because the *pir* gene is not present and that of pSS1914 because ColE1 derivatives are naturally unable to replicate in members of this genus. The two plasmids contain different antibiotic resistance markers to allow selection for both vectors in the same strain. The extent of DNA sequence similarity between these two vectors is limited to the 250-bp *oriT* fragment of pSS1914. A set of 13 chromosomal fragments, containing all or part of previously characterized genes and operons of *B. pertussis*, with various amounts of flanking sequences, were cloned into one or both of these vectors as listed in Table 1. An example of how these plasmids are used is presented schematically in Fig. 2. Successive integration of two plasmids, mediated by homologous recombination, at the locations of the cloned DNA sequences results in the incorporation of two cleavage sites for the intron-encoded restriction endonuclease I-*Sce*I. This site is 18 bp in length (21) and is not found elsewhere in the *Bordetella* chromosome; thus, subsequent cleavage of chromosomal DNA with I-*Sce*I gives rise to a fragment the size of which represents the distance

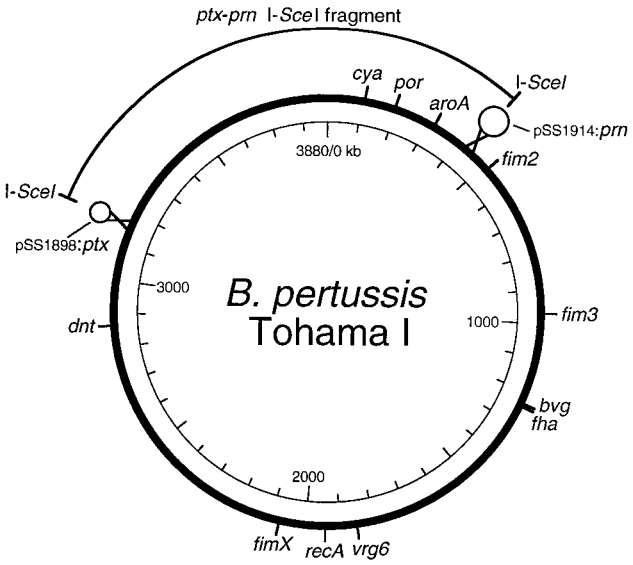

FIG. 2. Schematic representation of the chromosomal surveying approach. Two subsequent integrations of derivatives of pSS1898 and pSS1914 have been directed to the *ptx* and *prn* loci, respectively. Upon digestion with I-*Sce*I, a fragment is liberated, the size of which represents the distance between the two loci. By further measurement of the distances between other pairs of genes, the information to derive a map is obtained.

between the two genes. In this way, the approach described here is similar to that recently described by Mahillon et al., in which transposons bearing I-*Sce*I sites were used to subdivide the *E. coli* chromosome (16). To collect enough information to derive the relative positions of a set of genes, multiple pairwise measurements of intergenic distance must be performed. A set of 26 pairwise combinations provides enough information to unequivocally determine the map positions for 13 markers in *B. pertussis* Tohama I. Results of the PFGE performed to determine the sizes of the fragments generated by these combinations are shown in Fig. 3, and a schematic interpretation of how these measurements are used to support the map of *B. pertussis* Tohama I so derived is shown in Fig. 4. Comparing this map to that previously determined for Tohama I by a different method (33) shows that the two maps are essentially identical.

Comparison of the maps of *B. pertussis* Tohama I, *B. pertussis* 18-323, and *B. parapertussis* ATCC 15311. For the purposes of comparison to Tohama I, *B. pertussis* 18-323, which is the ATCC (American Type Culture Collection) type strain for *B. pertussis*, and ATCC 15311, which is the type strain for *B. parapertussis*, were subjected to this mapping procedure. The maps derived for these strains are shown in Fig. 5 and 6, respectively. The most striking finding from this analysis is that there are no discernible similarities in genomic organization in this set of three maps. Another feature of the *B. pertussis* 18-323 map relative to that of Tohama I became apparent when the *cya* locus was mapped. After integration of pSS1933, two bands were consistently generated in the PFGE analysis, which indicated two possible locations for this locus. As the chromosomal fragment in pSS1933 includes part of the *cya* operon as well as sequences upstream of this operon in Tohama I, it appeared that the upstream sequences were separated from the *cya* operon in *B. pertussis* 18-323. Consistent with this hypothesis, pSS2058, which contained an *Apo*I fragment internal to the *cya* operon, was found to integrate at only one of the two possible locations. The other location is designated *cus* (cyclase upstream sequences) on the map of 18-323. The ability of this method to simultaneously give information on the locations of multiple occurrences of a homologous sequence suggests that it could be extended to allow the mapping of repeated sequence elements such as insertion sequences.

A striking feature of the *B. parapertussis* ATCC 15311 map is the presence of nearly a megabase of additional DNA compared to Tohama I. This finding was supported by summing the sizes of *Xba*I and *Spe*I fragments derived from the two strains (data not shown). The slightly larger size of *B. pertussis* 18-323 is within the error of the method and is probably not significant. It should also be noted that the *fimX* locus could not be mapped in 18-323 and the *fimX* and *fim3* loci could not be mapped in ATCC 15311. In these cases, the recovery of exconjugants was very low, and the sites of integration of the

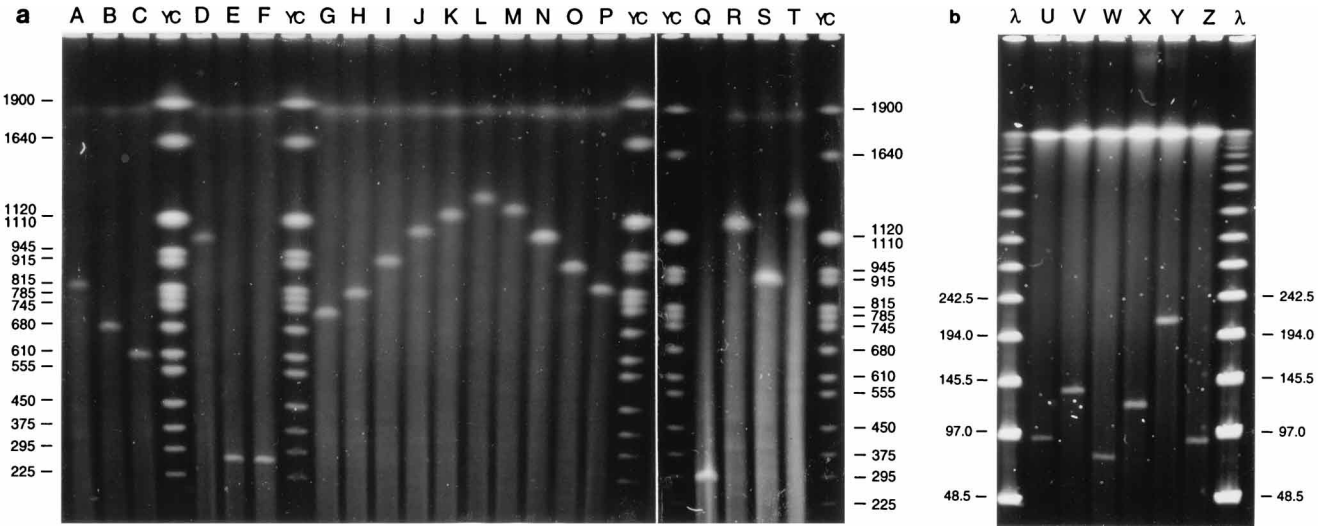

FIG. 3. PFGE showing a set of intergenic distance measurements which are sufficient to derive a map for *B. pertussis* Tohama I. The lanes are labeled with reference to Fig. 4. In panel a, YC stands for yeast (*S. cerevisiae*) chromosomes; in panel b, λ stands for a ladder of bacteriophage λ concatemers. Sizes of standards in kilobases are given to the left and right of each panel.

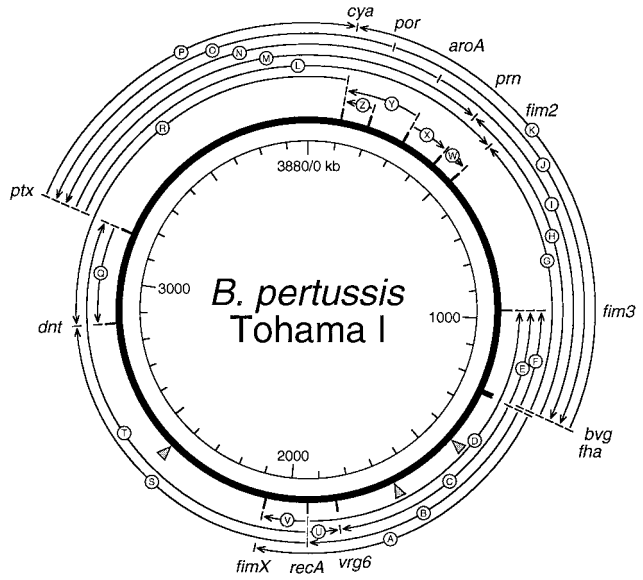

FIG. 4. Map of *B. pertussis* Tohama I derived by chromosomal surveying. Distances measured between pairs of genes are shown by arrows, and results of the PFGE performed to measure these distances are shown in Fig. 3. The origin of each arrow designates the site of integration of the pSS1898 derivative, and the head of each arrow designates the site of integration of the pSS1914 derivative or pSS2083. Shaded arrowheads show the presumptive locations of *rrn* operons as revealed by cleavage with I-*Ceu*I.

pSS1914 vector appeared to be randomly located, as no discrete bands were obtained in PFGE. These observations are consistent with the absence of these sequences in these strains.

Comparison of the maps of *B. pertussis* Tohama I, *B. pertussis* Tohama III, *B. pertussis* BP165, and *B. pertussis* Wellcome 28. In light of the striking divergence of genomic structure in the strains examined thus far, maps of three additional strains of *B. pertussis* were obtained. As shown in Fig. 7, the map of Tohama III is very similar to that of Tohama I but differs by a single inversion containing the *ptx* and *dnt* loci. Wellcome 28 and BP165 show a different inversion, one containing the *bvg* and *fha* loci (Fig. 8). In addition, in these two strains the *dnt* locus is found at a position much closer to *cya*, although the distance between *ptx* and *cya* is the same. This analysis suggests

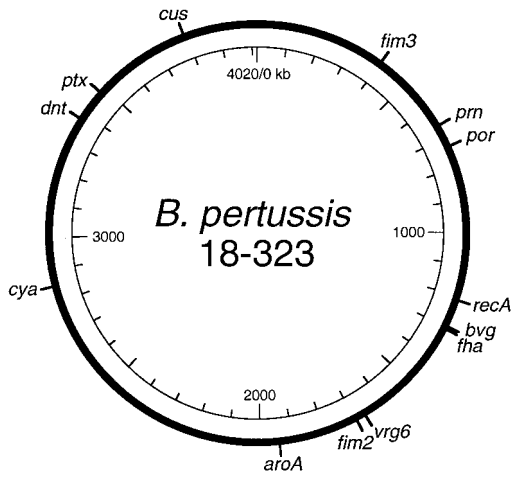

FIG. 5. Map of *B. pertussis* 18-323 derived by chromosomal surveying. Sequences upstream of the *cya* operon in *B. pertussis* Tohama I are denoted by *cus*.

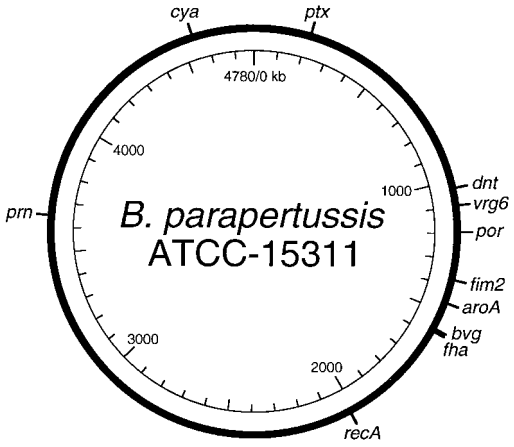

FIG. 6. Map of *B. parapertussis* ATCC-15311 derived by chromosomal surveying.

that even among more typical strains of *B. pertussis*, genomic structure is variable. The operons encoding rRNA were mapped presumptively by mapping cleavage sites for the intron-encoded restriction endonuclease I-*Ceu*I, which has been shown in other systems to be found in and only in *rrn* loci (14, 34). The three I-*Ceu*I sites which were found were not located at the predicted endpoints of the inversions. Thus, it appears that the rearrangements seen in these strains are not mediated by recombination between *rrn* loci as has been described previously for *Salmonella typhi* (15).

DISCUSSION

A comparison of the chromosomal locations of 12 genes has revealed that *B. pertussis* Tohama I, a typical laboratory strain, and 18-323, the ATCC type strain, lack any apparent conservation of gene order. Consistent with this divergence of genomic organization, sequences near the *cya* operon which are contiguous in Tohama I are separated in 18-323, and the *fimX*

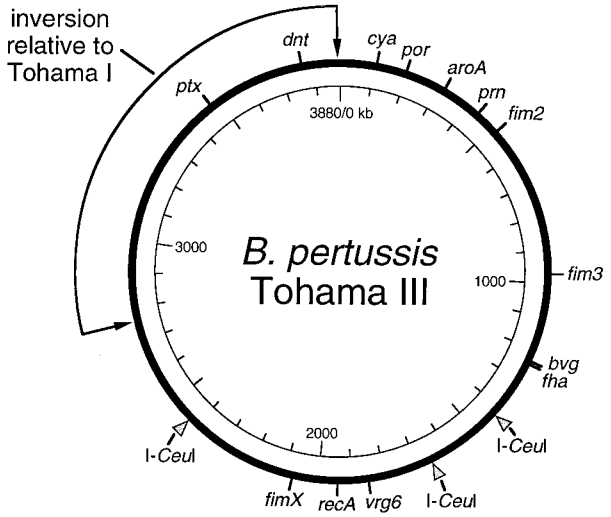

FIG. 7. Map of *B. pertussis* Tohama III derived by chromosomal surveying. Shaded arrowheads show the presumptive locations of *rrn* operons as revealed by cleavage with I-*Ceu*I. Endpoints of a hypothetical chromosomal inversion which could result in the creation of this map from that of *B. pertussis* Tohama I are indicated.

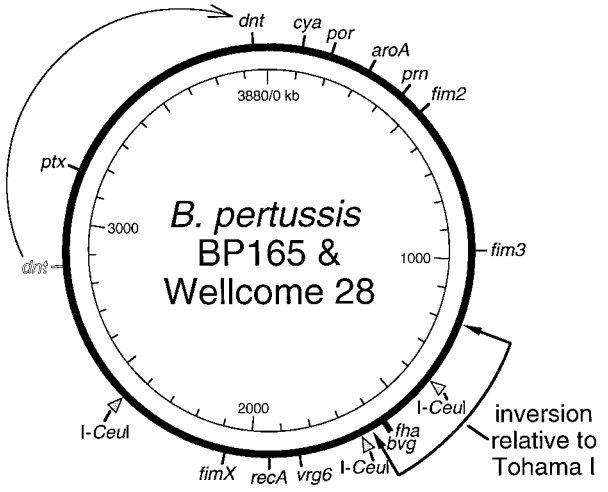

FIG. 8. Map of the *B. pertussis* strains BP165 and Wellcome 28 derived by chromosomal surveying. Shaded arrowheads show the presumptive locations of *rrn* operons as revealed by cleavage with I-*Ceu*I. Endpoints of a hypothetical chromosomal inversion which could result in apparent relocation of the *bvg* and *fha* loci and one I-*Ceu*I site are indicated. The translocation of the *dnt* locus relative to its position in *B. pertussis* Tohama I (in outline) is indicated by an arrow.

gene, present in Tohama I, is apparently lacking in 18-323. That differences between these two strains were observed is not surprising. It has been appreciated for some time that 18-323 is not typical of *B. pertussis* strains. Significant differences between 18-323 and other, more typical isolates have been demonstrated by multilocus enzyme electrophoretic analysis and by DNA sequence analysis of the pertussis toxin operon (2, 22). The atypical nature of this strain may be related to the fact that it was originally selected for its usefulness in a murine model of infection (10). However, the degree of intraspecies divergence of genomic organization observed is remarkable.

In light of these results, it is not surprising that the map of the closely related human pathogen *B. parapertussis* is equally divergent. No conservation of gene order could be discerned between the ATCC type strain for this species and either Tohama I or 18-323. In keeping with these results, two genes, *fimX* and *fim3*, appear to be absent from this chromosome. Perhaps the most remarkable feature that distinguishes the *B. parapertussis* map is the presence of an additional megabase of DNA, capable of encoding approximately 1,000 additional genes. As the maps of the two species are so dissimilar, it is not possible to assign this additional DNA to a particular region or regions on the basis of this analysis.

Three additional strains of *B. pertussis*, Tohama III, Wellcome 28, and BP165, showed recognizable conservation of gene order relative to Tohama I and each other. Most differences which were observed were explainable by large inversions of segments of the chromosome. However, to explain the different location of the *dnt* locus in BP165 and Wellcome 28 relative to Tohama I, two successive inversions or a transposition event must be invoked. The similarity of the maps for BP165 and Wellcome 28 is striking in light of the fact that these two strains were apparently isolated from patients in two different continents, and approximately 20 years apart (17, 23, 24, 28). The relationships between the strains mapped in this study are generally consistent with the findings of studies using the independent methods of multilocus enzyme electrophoresis and DNA sequence analysis of the pertussis toxin operon to assess relatedness. By both of these methods, *B. parapertussis* and *B. pertussis* 18-323 were found to be significantly divergent from Tohama I or BP165 (2, 22).

Theoretically, the differences in genomic organization that we have observed could be due to great temporal separation between the isolates examined or to a relatively high rate of genomic rearrangements. The first possibility is highly unlikely, as solution DNA hybridization studies and multilocus enzyme electrophoretic studies clearly demonstrate that *B. pertussis* and *B. parapertussis* are closely related (11, 22). Another indication of the relatedness of these species is the observation that although *B. parapertussis* strains do not express pertussis toxin, they often contain the genes encoding this protein, which have been silenced by mutation (3). In light of the relatedness of these species, we must conclude that there is a relatively high degree of genomic fluidity. In keeping with this possibility, we have observed by PFGE several spontaneous chromosomal rearrangements in *B. pertussis* Tohama I passaged in the laboratory (data not shown). These rearrangements were observed at a frequency of approximately 1 in 100 colonies examined. In addition, a recent study which examined 70 isolates from a pertussis outbreak in Alberta, Canada, by PFGE revealed 14 distinguishable patterns of *Xba*I digestion (7). We are currently applying the techniques described here to assess whether the different patterns of *Xba*I digestion observed actually represent chromosomal rearrangements and thus whether variability in genomic organization is a feature of natural populations of *B. pertussis* as well.

The genomic mapping approach described here may be useful in the analysis of other bacterial species, especially those organisms in which appropriate restriction enzyme sites are not distributed evenly or occur with a frequency which precludes their use in restriction mapping. The ability to introduce plasmid DNA, by either conjugation, electroporation, or transformation, the presence of a sufficiently efficient homologous recombination system, and the absence of cleavage sites for I-*Sce*I or other enzymes with very rare cleavage sites are all that is required to implement this mapping approach. It should be noted that the vectors that we have used also contain sites for the intron-encoded restriction enzyme I-*Ppo*I, which was not used in this study. A number of additional restriction enzymes of the same class are now commercially available and could be incorporated into these vectors as well. For use in species in which ColE1 derivatives are able to replicate, we have constructed a vector with kanamycin resistance and the *pir*-dependent *oriV* of R6 which can be used in conjunction with pSS1898 in place of pSS1914 (not shown).

Although the time invested in performing this technique is not trivial, it is simple to perform, and information is gained without the derivation of a restriction map, thus saving a considerable amount of time. This time savings can be compounded by the simultaneous analysis of multiple strains by parallel processing of gel samples. By enabling the mapping of a number of strains, a more complete picture of the genomic stability or fluidity that different species and genera exhibit can emerge. Such information is complementary to the detailed type of information derived from genomic sequencing efforts usually directed at a single representative of a given species.

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