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Streptococcus Plasmid pAMα1 Is a Composite of Two Separable Replicons, One of Which Is Closely Related to *Bacillus* Plasmid pBC16

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A tetracycline resistance plasmid of *Streptococcus faecalis*, pAMα1, is shown to contain two independent sets of replication functions, separated from each other on either side by short (300- to 400-base-pair) sequences of homology. The homologous sequences are oriented as direct repeats and therefore permit the dissociation of pAMα1 into its component replicons, referred to here as pAMα1Δ1 and pAMα1Δ2, as the reciprocal products of a simple intramolecular recombination. pAMα1Δ1 is a 4.6-kilobase plasmid which carries the *tet* gene, and pAMα1Δ2 is a 5.1-kilobase plasmid which carries no known selectable marker. pAMα1Δ1 is shown to replicate efficiently in *Bacillus subtilis* and to confer tetracycline resistance on *Bacillus* hosts. We demonstrate by restriction mapping analysis that pAMα1Δ1 is virtually identical to a 4.6-kilobase tetracycline resistance plasmid of *Bacillus cereus*, pBC16, which is known to show extensive homology to plasmid isolates from *Staphylococcus* species (such as pUB110), as well as from other *Bacillus* species. The pAMα1Δ1-pBC16-pUB110 replicon thus exists naturally in at least three different gram-positive genera, indicating that these plasmids have a high degree of interspecific functional adaptability and supporting the view that plasmid DNA is commonly exchanged among many species of gram-positive bacteria in their natural environments.

pAMα1 (Fig. 1A) is a 9.6-kilobase (kb), nonconjugative plasmid of *Streptococcus faecalis* which confers amplifiable tetracycline resistance (Tc^r). The tetracycline resistance determinant (*tet*) is located on a 4.2-kb segment of pAMα1, which is flanked by homologous sequences of ca. 380 base pairs (referred to as RS sequences), oriented as direct repeats; the remaining 4.7-kb portion of the plasmid carries no known selectable marker (30). Clewell and his co-workers (6) have shown that amplification is probably accomplished by a series of "uneven recombinations" between RS sequences within a replicating molecule or by intermolecular recombination at RS homology to generate tandem duplications of the *tet*-containing segment (Fig. 1B).

Intramolecular recombination at RS homology (Fig. 1C) is also possible. This would dissociate pAMα1 into two circular molecules, each containing a single RS sequence: a 4.6-kb molecule carrying the *tet* determinant (Fig. 1E) and a 5.1-kb molecule without a drug resistance gene (Fig. 1D). Yagi and Clewell (31) have identified the 5.1-kb dissociation product as an autonomous replicon in *S. faecalis*, leading them to conclude that the replication functions of pAMα1 probably reside in this region of the plasmid; no autonomous replicon corresponding with the 4.6-kb *tet*-containing dissociation product could be detected, however, despite an intensive search, indicating that this plasmid species may not have the capacity to replicate in *S. faecalis*.

In the present work, we found that the 4.6-kb dissociation product (referred to here as pAMα1Δ1) functions as an autonomous replicon in *Bacillus subtilis*. This demonstrates that pAMα1 is actually a composite of two functional replicons (which are separable by a simple intramolecular recombination between RS sequences) and that at least one of the component replicons (pAMα1Δ1) is capable of replication in *B. subtilis*. In addition, we found very unexpectedly that pAMα1Δ1 is virtually identical in its profile of restriction endonuclease recognition sites to a *tet*-containing *Bacillus cereus* plasmid of the same size, pBC16. Interestingly, Polak and Novick (22) have recently reported substantial regions of homology between pBC16 and a *Staphylococcus aureus* replicon, pUB110, which carries a kanamycin resistance (Km^r) determinant; pUB110 and pBC16 were found to be replication incompatible and differed in their

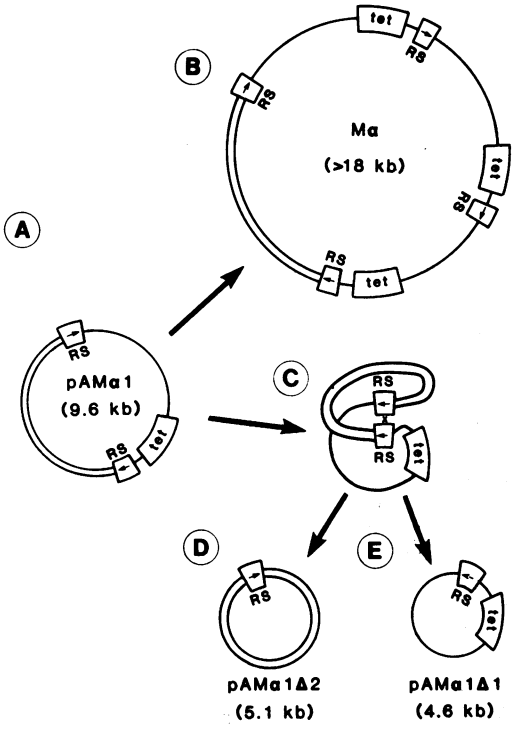

FIG. 1. Schematic diagram of pAMα1 (A), showing amplified (B) and deleted forms (D and E) of the plasmid that can result from different kinds of recombination events involving homologous RS sequences (RS) which flank the tetracycline resistance gene (*tet*) (see text).

restriction maps only in the drug-resistance-containing regions. We interpret the discovery of highly homologous replicons in isolates from three different genera to be evidence of extensive natural exchange of plasmid DNA among gram-positive bacteria.

MATERIAL AND METHODS

Plasmids and bacterial strains. *S. faecalis* strains harboring pAMα1 and pAMα1::Tn*917* in the JH2-2 genetic background (29, 31) were kindly provided to us by D. B. Clewell. *B. subtilis* 168 strains harboring plasmids pBC16 and pUB110 were obtained from the *Bacillus* Genetic Stock Center at Ohio State University. *B. subtilis* 168 derivatives were used as recipients in all plasmid DNA transformation experiments.

Preparation of plasmid DNA. Plasmid DNA was isolated from *B. subtilis* bacteria grown to the late log phase in LB broth (Luria-Bertani broth [18]) as described previously (32). Plasmid DNA was isolated from *S. faecalis* in a similar way, except that the growth medium was supplemented with 10 mM DL-threonine (Sigma Chemical Co.), the lysozyme concentration was increased to 5 to 10 mg/ml, and the time of incubation with lysozyme was extended to 60 to 90 min.

Transformation and selection. Transformation-competent *B. subtilis* bacteria were prepared, stored, and transformed as described by Dubnau and Davidoff-Abelson (8). Plasmid transformants were selected for Tc^r or macrolide-lincosamide-streptogramin B resistance (MLS^r) on LB agar plates, using a double soft-agar overlay to provide a period of expression or induction before selection as described previously (32). Because the MLS^r phenotype conferred by Tn*917* is erythromycin inducible (29), transformants were plated together with 0.1 ml of a 10-μg/ml erythromycin solution in 2.5 ml of LB soft agar as the first overlay. After 2 h at 37°C (or 3 h at 33°C), another LB soft-agar overlay was added, which contained 0.1 ml of a 400-μg/ml erythromycin solution and 0.1 ml of a 10-mg/ml lincomycin solution. To select for Tc^r, no drug was added to the first LB soft-agar overlay, and 0.1 ml of a 4-mg/ml tetracycline solution was added to the second overlay.

Restriction enzyme analysis. Restriction endonucleases purchased from New England Biolabs and Bethesda Research Laboratories were used according to the instructions of the manufacturers. Restriction digestions were analyzed by electrophoresis in horizontal 0.8% agarose or vertical 7.5% polyacrylamide slab gels, as described by Segall and Losick (25).

Restriction endonuclease mapping. The relative positions of *Hpa*II cleavage sites in pAMα1, pAMα1Δ1, pAMα1Δ2, and pAMα1::Tn*917* were determined from the analysis of enzyme digestions run to completion or prematurely terminated at various times, as described previously (21). *Bam*HI, *Eco*RI, *Pvu*II, *Pst*I, *Hin*dIII, and *Xba*I cleavage sites were then ordered by using those enzymes individually, in combination with each other, and in combination with *Hpa*II. *Mbo*I, *Taq*I, and *Hpa*I cleavage sites of pAMα1Δ1 were ordered by analyzing partial digests of restriction fragments radioactively end labeled with ^{32}P, as described by Smith and Birnstiel (26).

Heteroduplex analysis. DNA heteroduplex molecules were constructed and spread using standard procedures (7, 14) modified as described previously (15). After adsorption to copper grids, DNA molecules were shadowed with platinum-palladium (80:20) and observed with a Philips 300 electron microscope at magnifications of ×9,433 and 12,726. All molecular size determinations used ϕX174 replicative form II DNA as a double-stranded reference and ϕX174 virion DNA as a single-stranded reference.

RESULTS

Introduction of pAMα1 and pAMα1::Tn*917* into *B. subtilis*. Tn*917* is a 5.4-kb transposon of *S. faecalis* which confers resistance to erythromycin and other macrolide-lincosamide-streptogramin B antibiotics. Our interest in the structure and replication properties of pAMα1 in *Bacillus* species was a consequence of experiments in which we unsuccessfully attempted to introduce pAMα1::Tn*917* into *B. subtilis* by transformation. Our inability to obtain either Tc^r or MLS^r transformants of *B. subtilis* with pAMα1::Tn*917* plasmid DNA was difficult to reconcile with successful efforts in the Clewell

laboratory (5) to obtain Tc^r transformants of *B. subtilis* with pAMα1 itself.

We were able to confirm the observations of Clewell and his colleagues that *B. subtilis* is transformable with pAMα1, obtaining ca. 2×10^2 Tc^r transformants per ml of competent cells (see above), using 1 μg of plasmid DNA per ml (a relatively low transformation efficiency). As in their work, moreover, we found that none of the *B. subtilis* transformants contained the 9.6-kb form of pAMα1 used in the transformations. Instead, each contained a deleted form, which we refer to here as pAMα1Δ1, estimated from the mobility of supercoiled molecules in agarose gels to be less than 5.0 kb (data not shown). When digested with *Eco*RI, pAMα1Δ1 was found to retain the two *Eco*RI recognition sites of pAMα1 (Fig. 2), which were known to be within the amplifiable portion of the plasmid (31). From the mobility of the *Eco*RI digestion products of pAMα1Δ1 (Fig. 2), we estimated the plasmid to be ca. 4.6 kb in size. In the course of many subsequent transformations of pAMα1Δ1 into new *B. subtilis* recipients, no further deletions of the plasmid have been observed.

The transformation of plasmid DNA into *B. subtilis* is sometimes greatly enhanced when the transformation recipient already contains a plasmid with some homology to the transforming DNA (11). For this reason, we attempted to use pAMα1Δ1 already present in the transformation recipients as a "resident homology" to enhance the transformation of *B. subtilis* by pAMα1::Tn*917*. When a pAMα1Δ1-containing *B. subtilis* strain was grown to transformation competence, it was found to be transformable to MLS^r at moderately high efficiency (more than 10^4 transformants per ml), using pAMα1::Tn*917* plasmid DNA. The plasmid content of such MLS^r transformants was complex, but included a prominent species of ca. 10 kb (the expected size of pAMα1Δ1::Tn*917*), as well as a prominent pAMα1Δ1-size species (data not shown). When plasmid DNA prepared from MLS^r transformants was used to transform plasmidless *B. subtilis* strains, MLS^r transformants were obtained at moderate frequencies (<10^4/ml); these contained a simpler profile of plasmid species, with a 10-kb form (presumed to be pAMα1Δ1::Tn*917*) remaining the most prominent, and invariably retained a pAMα1Δ1-size species, despite the absence of a Tc^r selection. In the absence of tetracycline and macrolide-lincosamide streptogramin B antibiotics, MLS^r transformants rapidly lost MLS^r but stably maintained Tc^r, with a gradual loss of all plasmid species except pAMα1Δ1.

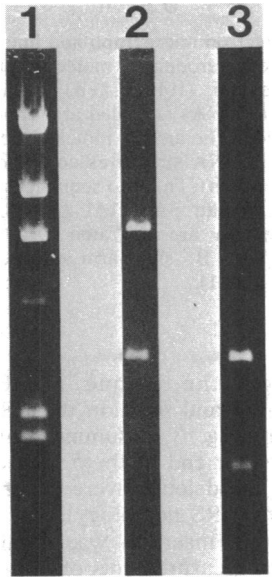

FIG. 2. *Eco*RI digests of pAMα1 (lane 2) and pAMα1Δ1 (lane 3) separated electrophoretically in a 0.8% agarose gel. A *Hin*dIII digest of lambda DNA was run in lane 1.

Restriction endonuclease mapping analysis of pAMα1, pAMα1Δ1, pAMα1::Tn*917*, and pAMα1Δ1::Tn*917*. As diagrammed in Fig. 1, a circular, *tet*-containing molecule 4.6 kb in size is expected as one of the dissociation products of pAMα1 when recombination occurs between different RS sequences within the same plasmid. The origin of pAMα1Δ1 is thus easily understood if the amplifiable portion of pAMα1 contains the information required for autonomous replication in *B. subtilis* in addition to the information required for conferring Tc^r. As an initial test of the hypothesis that pAMα1Δ1 is, in fact, identical to the 4.6-kb dissociation product (species E in Fig. 1), we mapped several restriction endonuclease recognition sites in pAMα1 and pAMα1Δ1 (a restriction map of pAMα1 recently published by Burdett et al. [3] is in agreement with ours for sites mapped in common). The results (Fig. 3) indicated that the deletion suffered by pAMα1 in generating pAMα1Δ1 probably involved the excision of a single, continuous 5.1-kb segment, the endpoints of which must be at or very near the positions of the RS sequences in pAMα1 (determined relative to the *Eco*RI sites by Yagi and Clewell [30]). A simple restriction analysis of pAMα1::Tn*917* and pAMα1Δ1::Tn*917* (data not shown) allowed us to determine the approximate location of the transposon insertion in this plasmid (shown on the map in Fig. 3) and to verify that the 10-kb plasmid species present in *B. subtilis* bacteria transformed to MLS^r by pAMα1::Tn*917* actually was pAMα1Δ1::Tn*917*. A detailed restriction map of Tn*917* will be presented elsewhere (J. B.

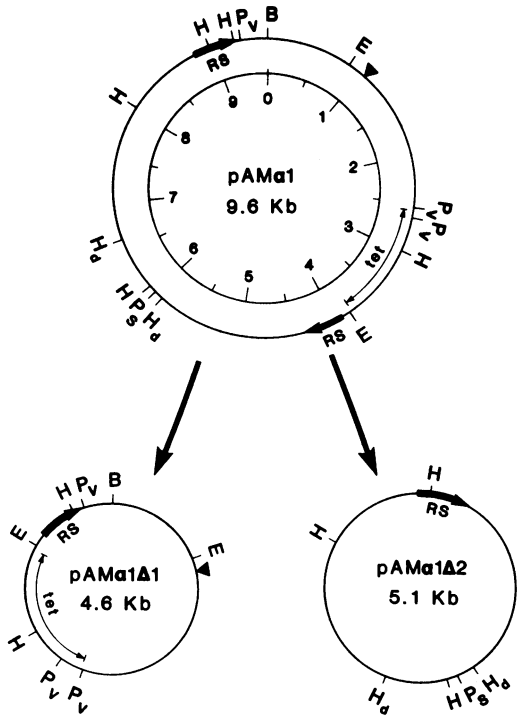

FIG. 3. Restriction maps of pAMα1, pAMα1Δ1, and pAMα1Δ2. Restriction sites are indicated as follows: B, *Bam*HI; E, *Eco*RI; H_d, *Hin*dIII; H, *Hpa*II; P_s, *Pst*I; and P_v, *Pvu*II. The approximate locations of the *tet* genes (*tet*) of pAMα1 and pAMα1Δ1 were determined from the data of Burdett et al. (3) and Polak and Novick (22). Symbols: ▲, insertion sites of the transposon in pAMα1:Tn*917* and pAMα1Δ1::Tn*917*; ⟶, positions of the RS sequences (31; see text). Size coordinates are given for pAMα1 in kb.

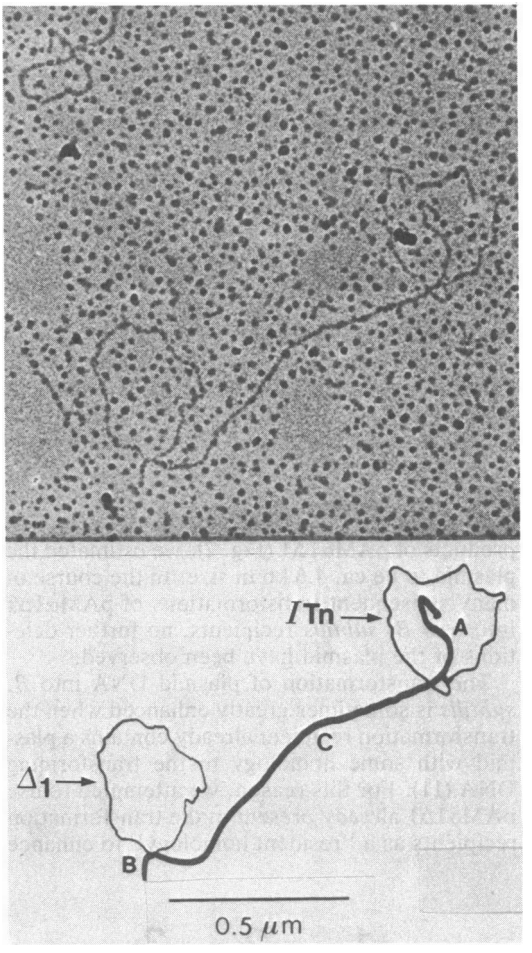

FIG. 4. Electron micrograph and line drawing of a representative heteroduplex molecule prepared with *Bam*HI-digested (linearized) pAMα1Δ1 and pAMα1::Tn*917* DNAs annealed at a concentration of 50% formamide. The arrows indicate the locations of single-stranded DNA structures corresponding either to Tn*917* sequences (*I*Tn) or to sequences deleted from pAMα1 to generate pAMα1Δ1 (Δ_1). Letters mark duplex regions that are separated by Δ_1 and *I*Tn: A, long-arm duplex; B, short-arm duplex; C, middle duplex (see Table 1).

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Heteroduplex analysis of pAMα1::Tn*917* and pAMα1Δ1. If pAMα1Δ1 were generated by a single, continuous 5.1-kb deletion, a heteroduplex of pAMα1::Tn*917* and pAMα1Δ1, each linearized by *Bam*HI digestion (a unique site in both molecules), would display two single-stranded loops: one of transposon size (with a short stem formed by the terminal inverted repeats) and one of deletion size. The double-stranded portion of the heteroduplex would be of pAMα1Δ1 size. In an actual experiment of this kind, several such structures were observed, one of which is shown in the photograph in Fig. 4. An analysis of the measurements made of the double-stranded portions and the single-stranded loops of several heteroduplex molecules is summarized in Table 1 and is in good agreement with the predicted values. These data place the site of the transposon insertion at ca. 1.0 kb from the unique *Bam*HI site in pAMα1::Tn*917* and position the RS sequences as shown in Fig. 3 (assuming that these are located at either end of the 5.1-kb deletion, as expected if the deletion were generated by recombination at RS homology). The position of the transposon insertion was confirmed independently by measurements of *Bam*HI-digested pAMα1-pAMα1::Tn*917* heteroduplex molecules (data not shown).

Heteroduplex analysis and restriction mapping of the 5.1-kb dissociation product of pAMα1. The

TABLE 1. Analysis of pAMα1Δ1-pAMα1::Tn*917* heteroduplex molecules

Class of molecules[a]	Arbitrary length (cm)	φX174 units	kb	No. of molecules measured
φX174 replicative form II	4.74 ± 0.1[b]	1.0	5.384	8
φX174 virion	3.77 ± 0.2	0.8		5
pAMα1Δ1-pAMα1::Tn*917*				
Long-arm duplex	0.93 ± 0.02	0.20[c]	1.05 ± 0.02	7
Short-arm duplex	0.23 ± 0.02	0.05[c]	0.27 ± 0.02	7
Middle duplex	3.05 ± 0.14	0.64[c]	3.45 ± 0.16	7
*I*Tn heteroduplex	3.62 ± 0.4	0.96[d]	5.17 ± 0.57	5
Δ_1 heteroduplex	3.37 ± 0.4	0.89[d]	4.80 ± 0.57	6

[a] Magnification, ×9,433.
[b] Ninety-five percent confidence level.
[c] φX174 replicative form II standard used.
[d] φX174 virion standard used.

5.1-kb dissociation product of pAMα1 (Fig. 1D), which we refer to here as pAMα1Δ2, is known to function in *S. faecalis* as an autonomous replicon (5, 30) and may be recovered from Tc^s segregants of pAMα1-containing cells (pAMα1Δ2 is called αd in publications from the Clewell laboratory). A heteroduplex of pAMα1 and pAMα1Δ2, each linearized by digestion with *Pst*I (Fig. 2), should display one single-stranded loop 4.6 kb in size and two double-stranded arms that together total 5.1 kb. The sizes of the double-stranded arms would reflect the distances from the *Pst*I site in pAMα1 to its two RS sequences. The arms should be 3.1 and 2.0 kb in size, respectively, if the RS sequences were correctly positioned on the map in Fig. 3 by the analysis presented above of pAMα1::Tn*917*-pAMα1Δ1 heteroduplexes. A pAMα1Δ2 dissociation derivative of pAMα1 was isolated as described by Clewell et al. (6) and annealed with pAMα1 to generate heteroduplex molecules. Several molecules were observed with the structure of the one shown in Fig. 5, measurements were made of the single-stranded loops and the double-stranded arms, and the results are summarized in Table 2. Of the molecules analyzed, double-stranded arms averaged 3.0 ± 0.11 and 1.88 ± 0.11 kb, in good agreement with predicted values.

A restriction map was prepared for pAMα1Δ2, and the results (Fig. 3) confirmed that pAMα1Δ1 and pAMα1Δ2 derive from separate halves of pAMα1.

Tc^s segregants were found to arise in pAMα1::Tn*917*-containing *S. faecalis* populations at the same level as in pAMα1-containing populations, and these contained a plasmid species identical to pAMα1Δ2.

pAMα1Δ1 is highly homologous to pBC16. pBC16 is a 4.6-kb *tet*-containing plasmid originating from a German isolate of *B. cereus* (1) which is now known to be a typical representative of a family of closely related Tc^r-

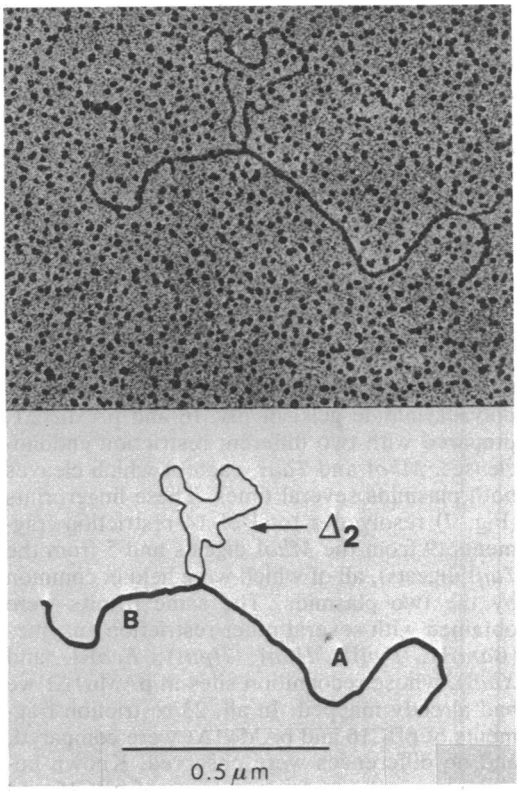

FIG. 5. Electron micrograph and line drawing of a representative heteroduplex molecule prepared with *Pst*I-digested (linearized) pAMα1Δ2 and pAMα1 DNAs annealed at a concentration of 50% formamide. The arrow indicates the location of a single-stranded DNA structure corresponding to deleted sequences from pAMα1 to generate pAMα1Δ2 (Δ_2). Letters mark duplex regions on either side of Δ_2: A, long-arm duplex; B, short-arm duplex (see Table 1).

TABLE 2. Analysis of pAMα1Δ2-pAMα1 heteroduplex molecules

Class of molecules[a]	Arbitrary length (cm)	φX174 units	kb	No. of molecules measured
φX174 replicative form II	5.74 ± 0.1[b]	1.0	5.384	7
φX174 virion	4.73 ± 0.2	0.82		6
pAMα1Δ2-pAMα1::Tn*917*				
Long-arm duplex	3.28 ± 0.12	0.57[c]	3.08 ± 0.11	8
Short-arm duplex	2.00 ± 0.12	0.35[c]	1.88 ± 0.11	8
Δ_2 heteroduplex	4.08 ± 0.48	0.86[d]	4.63 ± 0.5	6

[a] Magnification, ×12,726.
[b] Ninety-five percent confidence level.
[c] φX174 replicative form II standard used.
[d] φX174 virion standard used.

conferring replicons widely dispersed among such *Bacillus* species as *B. licheniformis*, *B. subtilis*, *B. sphaericus*, and *B. stearothermophilus* (2, 22). Polak and Novick (22) have mapped several restriction sites in pBC16, which allowed them to identify extensive homology between this plasmid and a Km^r-conferring plasmid of *S. aureus*, pUB110. Some of their data are reproduced in Fig. 6, where it can be seen that the restriction maps of the two plasmids are superimposable over all areas except the regions in which the heterologous drug resistance determinants are located. By inspection of these data, it was apparent that our restriction map of pAMα1Δ1 was identical to that of pBC16 for all restriction sites characterized by both laboratories. To assess better the extent of homology, we compared the restriction "fingerprints" (i.e., the profiles of restriction fragments resolved on polyacrylamide gels) of pBC16 and pAMα1Δ1, prepared with two different restriction endonucleases. *Mbo*I and *Taq*I, each of which cleaves both plasmids several times. These fingerprints (Fig. 7) resolved a total of 14 restriction fragments (9 from the *Mbo*I digests and 5 from the *Taq*I digests), all of which were held in common by the two plasmids. The same results were obtained with several other restriction enzymes (*Bam*HI, *Pvu*II, *Hpa*I, *Hpa*II, *Eco*RI, and *Xba*I), whose recognition sites in pAMα1Δ1 we had already mapped. In all, 23 restriction fragments of pBC16 and pAMα1Δ1 were compared, and no differences were observed. Known homologies in the restriction maps of pBC16 and pUB110 are summarized in Fig. 6.

DISCUSSION

Intramolecular recombination between the two RS sequences of pAMα1 in *S. faecalis* dissociates the plasmid into two circular molecules 5.1 and 4.6 kb in size. The 5.1-kb dissociation product (pAMα1Δ2) is known to function in *S. faecalis* as an autonomous replicon and is the plasmid species found in Tc^s segregants of pAMα1-containing strains (6, 31). The 4.6-kb *tet*-containing dissociation product represents

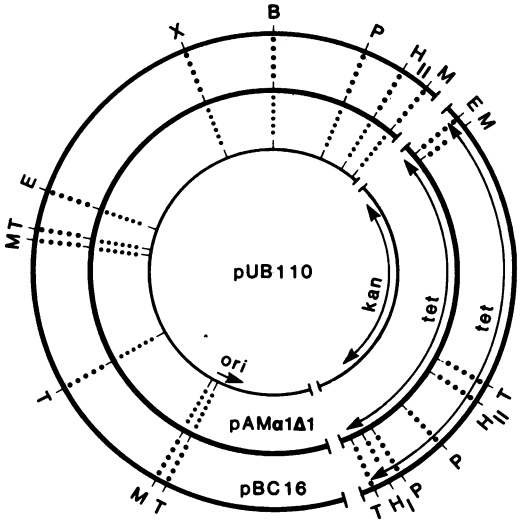

FIG. 6. Alignment of the restriction and genetic maps of pUB110, pBC16, and pAMα1Δ1. Restriction sites are indicated as in Fig. 3 except: H_I, *Hpa*I; H_{II}, *Hpa*II; M, *Mbo*I; P, *Pvu*II; T, *Taq*I; and X, *Xba*I; dotted lines connect conserved restriction sites. *ori* refers to the origin of DNA replication identified in pUB110 by Scheer-Abramowitz et al. (24), and the approximate locations of the antibiotic resistance determinants of the plasmids are indicated by regions labeled kan and tet (3, 22). These maps include data published previously by Polak and Novick (22), Scheer-Abramowitz et al. (24), and Jalanko et al. (13), as well as unpublished DNA sequence data supplied by McKenzie, T. Tanaka, and N. Sueoka. Four conserved *Mbo*I restriction sites within the antibiotic resistance regions of pBC16 and pAMα1Δ1 are not mapped, and several restriction sites in the *kan* gene of pUB110 are not listed.

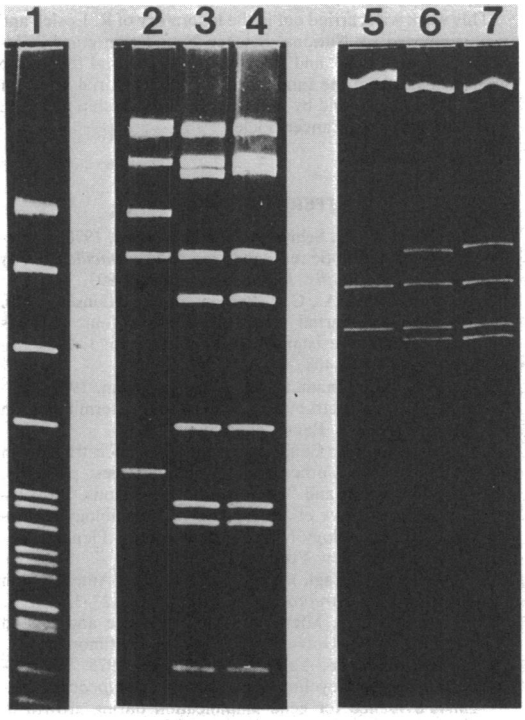

FIG. 7. *Taq*I and *Mbo*I restriction fingerprints of pUB110, pBC16, and pAMα1Δ1. Plasmids pUB110 (lanes 2 and 5), pBC16 (lanes 3 and 6), and pAMα1Δ1 (lanes 4 and 7) were digested with either *Mbo*I (lanes 2 to 4) or *Taq*I (lanes 5 to 7), and the fragments were separated electrophoretically in 7.5% polyacrylamide. An *Hpa*II digest of pBR322 was run in lane 1.

the amplifiable portion of pAMα1 and was not previously known to encode replication functions.

When pAMα1 was used to transform *B. subtilis* bacteria to Tc^r, a 4.6-kb replicon, pAMα1Δ1, was the only plasmid species detected in the transformants. Restriction mapping and heteroduplex analysis of pAMα1Δ1 revealed that this replicon was derived from pAMα1 by a single, continuous 5.1-kb deletion covering the portion of pAMα1 contained in pAMα1Δ2, with endpoints at or near the RS sequences; i.e., the pAMα1Δ1 replicon is apparently identical to the 4.6-kb *tet*-containing dissociation product of pAMα1. The RS sequences of pAMα1 therefore divide the plasmid into two separable domains, both with a set of replication functions and each active in a different genus.

A derivative of pAMα1 that contains the MLS^r-conferring *S. faecalis* transposon Tn*917* (referred to here as pAMα1::Tn*917*) was found to be unable to transform plasmidless *B. subtilis* bacteria to either Tc^r or MLS^r. Heteroduplex analysis and restriction mapping revealed the transposon to be inserted at a site within the *tet*-containing domain of pAMα1 (the pAMα1Δ1 domain). Recombination between RS sequences in pAMα1::Tn*917* should therefore generate pAMα1Δ2 and pAMα1Δ1::Tn*917* as dissociation products. That such a recombination event is not inhibited by the presence of Tn*917* in the molecule is supported by the observation that Tc^s segregants containing only pAMα1Δ2 are equally abundant in populations of pAMα1- and pAMα1::Tn*917*-containing *S. faecalis* bacteria. The failure of pAMα1::Tn*917* to tansform *B. subtilis* thus tends to suggest that pAMα1Δ1::Tn*917* cannot replicate in *B. subtilis* (although other explanations are possible, of course). Nevertheless, when *B. subtilis* bacteria containing pAMα1Δ1 were used as recipients, pAMα1::Tn*917* transforming DNA produced MLS^r transformants very efficiently. Such transformants did contain a pAMα1Δ1::Tn*917* plasmid species, but they invariably contained pAMα1Δ1 as well. More significantly, when plasmid DNA was isolated from such transformants and used to transform a plasmidless *B. subtilis* recipient to MLS^r, pAMα1Δ1 was invariably cotransferred and became established in the new transformants along with pAMα1Δ1::Tn*917*. A likely interpretation of these observations is that Tn*917* has insertionally inactivated a gene whose product is required for the replication of pAMα1Δ1 in *B. subtilis* (but which is unnecessary for the replication of pAMα1 in *S. faecalis*) and that pAMα1Δ1 must be present to supply this gene product in trans for pAMα1Δ1::Tn*917* to be maintained stably in *B. subtilis*.

A question not clearly resolved in the present work is whether the two sets of replication functions contained in pAMα1 are actually restricted in their activity to one or the other genus: whether pAMα1Δ1 can replicate in *Streptococcus* species and whether pAMα1Δ2 can replicate in *Bacillus* species. If pAMα1Δ1 replicates independently in *S. faecalis*, but at a lower copy number than pAMα1Δ2 (or pAMα1), it may be too rare to have been detected in previous searches (31). Alternatively, pAMα1Δ1 replication functions may be expressed in *S. faecalis*, but may be suppressed in the presence of (the more stable?) pAMα1Δ2 (or pAMα1). The failure of pAMα1::Tn*917* to transform plasmidless *B. subtilis* strains could be explained as the failure of pAMα1Δ2 replication functions to be expressed, but might instead reflect the presence of a gene in pAMα1Δ2, having nothing to do with replication, whose product is toxic to *B. subtilis*.

A chance observation of similarities in the restriction maps of pAMα1Δ1 and the *tet*-containing *B. cereus* plasmid pBC16 led us to exam-

ine possible homologies between the two with greater sensitivity by comparing the patterns of restriction fragments generated when both were cleaved with different restriction endonucleases. Remarkably, no differences at all were detected, demonstrating a very high order of sequence homology between pAMα1Δ1 and pBC16. Because pBC16 is now known to share extensive homology with replicons from *Staphylococcus* species, such as pUB110 (22), we can conclude that close relatives of this replicon are distributed over at least three gram-positive genera.

We do not regard the discovery of homologous replicons in *Staphylococcus*, *Streptococcus*, and *Bacillus* species as very surprising in itself, since these genera have long been recognized to include members with natural mechanisms for efficient uptake of and transformation by both chromosomal and plasmid DNAs (9, 12, 17, 19, 20, 27, 28). Recent reports of conjugative plasmids in these bacteria that transfer across genus barriers, as well as species barriers (4, 10, 16, 23), further strengthen the conclusion that intergeneric exchange of genetic material in their natural environments must be relatively common among them. We were surprised, however, by the very high degree of sequence homology between pBC16 and pAMα1Δ1 apparent in their restriction fragment fingerprints. To find a perfect correspondence among 23 of 23 different restriction enzyme recognition sites in two plasmids is, in the most conservative interpretation, roughly equivalent to sequencing a total of more than 100 nucleotide base pairs from each, representing 23 scattered regions, and finding a perfect match. In addition, indistinguishable restriction fingerprints indicate a very high conservation of sequence homology between the sites tested and tempt one to conclude in this case that pAMα1Δ1 and pBC16 are virtually identical plasmids. Because most of the DNA in pAMα1Δ1 is likely to be nonessential in *S. faecalis* both for replication of pAMα1 and for amplification of the *tet*-containing segment, much greater sequence divergence might have been expected. We therefore interpret the high conservation of homology between pBC16 and pAMα1Δ1 as evidence that selective pressure for the dispersal of pBC16–pAMα1Δ1-associated Tc^r in *Streptococcus* species is probably very recent, perhaps as recent as the widespread use of tetracyclines as medical antibiotics and livestock feed supplements.

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