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Imprecise Excision of Plasmid pE194 from the Chromosomes of *Bacillus subtilis* pE194 Insertion Strains

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Plasmid pE194 has been shown to be rescued by integration after cultivation of infected *Bacillus subtilis recE4* cells at a restrictive high temperature. The plasmid is also spontaneously excised from the chromosome at a low frequency by precise or imprecise excision (J. Hofemeister, M. Israeli-Reches, and D. Dubnau, Mol. Gen. Genet. 189:58–68, 1983). We have investigated nine excision plasmids, carrying insert DNA 1 to 6 kbp in length, either in a complete pE194 or in a partially deleted pE194 copy. Type 1 (additive) excision plasmids have the left- and right-junction DNAs preserved as 13-bp direct repeats (5′-GGGGAGAAAACAT-3′) corresponding to the region between positions 864 and 876 in pE194. In type 2 (substitutive) excision plasmids, a conserved 13-bp sequence remains only at the right junction while the left junction has been deleted during the excision process. The type 3 excision plasmid carries at each junction the tetranucleotide 5′-TCCC-3′, present in pE194 between positions 1995 and 1998. Although we isolated the excision plasmids from different integration mutants, the insert DNAs of eight independently isolated plasmids showed striking sequence homology, suggesting that they originated from one distinct region of the *B. subtilis* chromosome. Thus, we postulate that imprecise excision of pE194 occurs most frequently after its translocation from the original insertion site into a preferred excision site within the host chromosome. The imprecise excision from this site occurs at excision breakpoints outside the pE194-chromosome junctions in a chromosomal region which remains to be investigated further.

Under restrictive growth conditions, the staphylococcal plasmid pE194 integrates into the *Bacillus subtilis* chromosome by a *recE*-independent Campbell-like recombination using several plasmid integration (Int) sites (10, 12). After integration, the erythromycin (EM) resistance plasmid is chromosomally inherited; at low frequency, it causes auxotrophic traits by gene disruption. Auxotrophic pE194 insertion mutants revert with a frequency of 10^{-8} to 10^{-9}, and the resulting prototrophs are characterized by either (i) a total loss of pE194 plasmid DNA, (ii) translocation of the pE194 copy into a new chromosomal location, (iii) excised pE194 without detectable changes, or (iv) excision plasmids containing a chromosomal DNA insert. In order to further investigate the mechanism and specificity of the spontaneous imprecise excision of pE194, nine excision plasmids isolated from six *B. subtilis* pE194 integration mutants were characterized by sequencing of recombinant DNA junctions, by restriction site mapping, and by DNA hybridization.

MATERIALS AND METHODS

Bacterial strains and plasmids. *B. subtilis* strains used in this study are listed in Table 1. Plasmids pE′a1, pE′a2, pE′a3, pE′lys, pE′g3, pE′f, pE′pur, pE′d1, and pE′d2 are excision plasmids carrying chromosomal DNA isolated from the appropriate strains (Table 2). *Escherichia coli* BMH71-18 (16) was used as a recipient for the cloning vector pUC19.

Growth conditions and transformation. Media used were TBY (10 g of Difco tryptone, 5 g of yeast extract, and 5 g of NaCl per liter), NBY (10 g of peptone, 5 g of yeast extract, and 5 g of NaCl per liter), NBY agar, and MSM (4) enriched with appropriate growth supplements. Five micrograms of EM per milliliter was used to select for resistance to macrolide-lincosamide-streptogramin B antibiotics. Competent cells of *B. subtilis* were prepared and transformed as described previously (6). EM-resistant (Em^r) plasmid transformants were selected by overlay after cultivation at 32°C for 90 min.

Isolation of *B. subtilis* DNA. Large-scale purification of plasmid DNA was performed by cesium chloride-ethidium bromide density gradient centrifugation as described previously (8). In rapid plasmid preparations for screening, 1.5-ml cultures were used as described before (3, 10). Chromosomal DNA of *B. subtilis* was isolated according to the procedure of Dubnau and Davidoff-Abelson (6).

Restriction endonuclease digestion and gel electrophoresis. Restriction enzymes purchased from Boehringer Mannheim (*Taq*I, *Cfo*I, *Sau*3A, *Bcl*I, *Hpa*I, and *Cla*I) and from ZIMET Jena (*Hin*fI and *Eco*RI) were used according to the specifications of the suppliers. DNA was analyzed by electrophoresis using either 0.8 to 1.8% agarose or 5% polyacrylamide gels.

DNA labelling and DNA hybridization. Probes were prepared by the oligolabelling procedure (7) using [α-^{32}P]dATP. DNA samples were transferred from agarose gels to BA85 nitrocellulose membranes (Schleicher & Schuell) by Southern blotting (17). Hybridization was performed at 65°C as described by Maniatis et al. (13).

DNA sequencing. DNA fragments were sequenced by the chemical-degradation method of Maxam and Gilbert (14), modified by the rapid solid-phase method as described previously (2, 15). DNA fragments were 3′ end labelled with [α-^{32}P]dATP or [α-^{32}P]dCTP.

RESULTS

Newly isolated pE194 insertion mutants. In order to obtain additional *B. subtilis* strains with different chromosomal

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TABLE 1. Bacterial strains

Strain	Chromosomal genotype[a]	Reference
BD431	*trpC2 thr-5* pE194-*cop6*	20
BD224	*trpC2 thr-5 rec4*	9
BD886 (strain a)	*trpC2 thr-5 recE4* [*lys*::pE194]	10
BD889 (strain d)	*trpC2 thr-5* [pE194]	10
BD891 (strain f)	*trpC2 thr-5* [pE194]	10
BD892 (strain g)	*trpC2 thr-5* [pE194]	10
GB700	*trpC2 thr-5* [*his*::pE194]	This work
GB701	*trpC2 thr-5* [*lys*::pE194]	This work
GB702	*trpC2 thr-5* [*pur*::pE194]	This work
GB703	*trpC2 thr-5* [*cas*::pE194]	This work

[a] Brackets indicate integration of plasmid pE194 into the chromosome of *B. subtilis*.

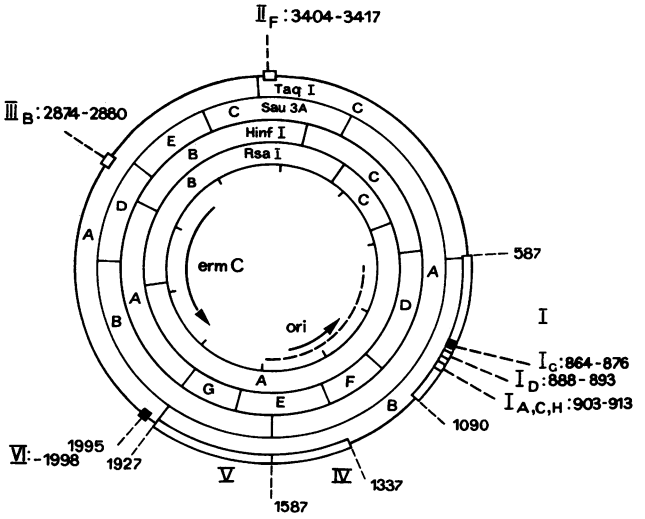

FIG. 1. Restriction site and fragment map of pE194. The locations of *ori* and *ermC* are shown together with the directions of replication and transcription. The dashed line denotes the essential replication region of pE194. The five Int sites (Int-I through -V) for *recE*-independent chromosomal integration are indicated by open boxes; the Int sites (I_G and VI) occupied by chromosomal insert DNA in excision plasmids are shown by black boxes.

pE194 insertions, we isolated auxotrophic insertion mutants from strain BD431 as described earlier (10). In addition to using a *cys*::pE194 mutant isolated earlier (10), we isolated the mutants GB700 (*his*::pE194), GB701 (*lys*::pE194), GB702 (*pur*::pE194), and GB703 (aa::pE194), which on MSM medium required the addition of histidine, lysine, purine, and a complex amino acid mixture, respectively. Chromosomal insertion of pE194 was confirmed by cotransformation of the pE194 Em^r gene and the auxotrophic trait (data not shown) by restriction mapping and by Southern hybridization studies. These experiments confirmed (i) that in each mutant one copy of pE194 had been integrated at one distinct location of the chromosome and (ii) that several pE194 Int regions were involved in the crossover event causing insertion (Table 2; Fig. 1). The pE194 Int-IV region between bp 1337 and 1587, which is affected in mutant GB702, has not been described before. Regions Int-I and Int-V coincide with pE194 Int sites described earlier (10, 12) (Fig. 1).

Isolation of pE194 excision plasmids. Two of the newly isolated strains (GB701 and GB702) and four previously described strains (BD886, BD889, BD891, and BD892) (10) were used for the screening of excised pE194 derivatives as described earlier (10). Single colonies from each of the six strains were used to inoculate EM medium. After growth at 32°C, the cultures were subjected to the usual plasmid isolation procedure including cesium chloride-ethidium bromide density centrifugation. A plasmid band was visible under UV irradiation only in CsCl gradients loaded with BD892 DNA. The gradients of the other five strains without visible evidence of the presence of plasmid DNA were fractionated and tested by transformation of competent *recE4* cells (BD224) to EM resistance. Samples from the plasmid DNA fraction from BD892 gave approximately 10^4 transformants, while samples from the other five strains gave only about 10 transformants under comparable conditions, indicating different amounts of excised plasmid DNA in various lysates. In addition to pE194, some transformants from each strain carried larger plasmids, indicating the incorporation of additional DNA probably from the host chromosome. These excision plasmids are pE′a1, pE′a2, and pE′a3 from strain BD886, pE′d1 and pE′d2 from strain BD889, pE′f from strain BD891, pE′g3 from strain BD892, pE′pur from strain GB702, and pE′lys from strain GB701 (Table 2).

Inserts and junctions of type 1, type 2, and type 3 plasmids. The sizes of the insert DNAs of the nine excision plasmids

TABLE 2. Comparison among excision plasmids on the basis of altered pE194 restriction fragments, and positions of chromosomal insert DNAs within the pE194 sequence compared with pE194 sites involved in integration in the appropriate strains

Integration strain	pE194 site used for integration (bp)	Excision plasmid	Altered restriction fragment(s) obtained with:				Position of chromosomal insert in excision plasmids (bp)
			*Hin*fI	*Taq*I	*Sau*3A	*Cfo*I	
BD886	903–913[a]	pE′a1	B, C, D	A, B, C	A, C, E	A, B	3136–876
		pE′a2	B, C, D	B, C	A, C	A, B	3485–876
		pE′a3	D	B	A	B	864–876
BD889	888–935[a]	pE′d1	D	B	A	B	864–876
		pE′d2	A	A	B	A	1995–1998
BD891	3404–3417[a]	pE′f	D	B	A	B	864–876
BD892	587–1090[b]	pE′g3	D		A	B	864–876
GB702	1337–1587[b]	pE′pur	D	B	A	B	864–876
GB701	587–1090[b]	pE′lys	B, C, D	A, B, C	A, C, E	A, B	3136–876
GB700	1587–1927[b]	Not tested					
GB703	587–1090[b]	Not tested					

[a] Determined by sequencing (6).
[b] Determined by restriction mapping (10; this work).

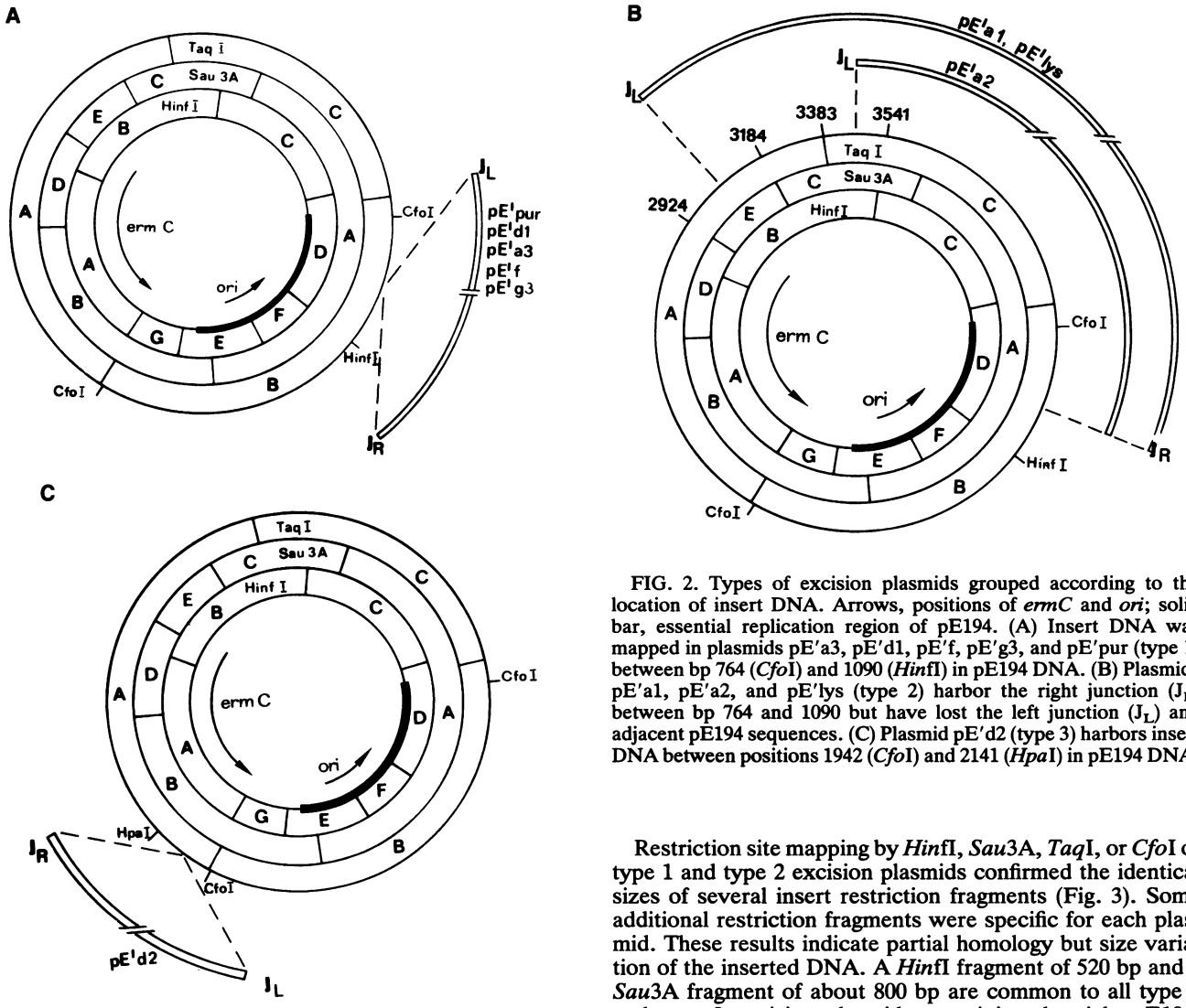

FIG. 2. Types of excision plasmids grouped according to the location of insert DNA. Arrows, positions of *ermC* and *ori*; solid bar, essential replication region of pE194. (A) Insert DNA was mapped in plasmids pE'a3, pE'd1, pE'f, pE'g3, and pE'pur (type 1) between bp 764 (*Cfo*I) and 1090 (*Hin*fI) in pE194 DNA. (B) Plasmids pE'a1, pE'a2, and pE'lys (type 2) harbor the right junction (J_R) between bp 764 and 1090 but have lost the left junction (J_L) and adjacent pE194 sequences. (C) Plasmid pE'd2 (type 3) harbors insert DNA between positions 1942 (*Cfo*I) and 2141 (*Hpa*I) in pE194 DNA.

varied from about 1 to 6 kb. The recombinant character of all nine plasmids was confirmed by restriction mapping with various restriction enzymes. Three types of excision plasmids were distinguished on the basis of the altered pE194 fragments (Table 2). Type 3 plasmid pE'd2 contains the insert in a pE194 region between positions 1942 (*Cfo*I site) and 2141 (*Hpa*I site) (Fig. 2C). In both type 2 plasmids (pE'a1, pE'a2, and pE'lys) and type 1 plasmids (pE'pur, pE'd1, pE'a3, pE'f, and pE'g3), the right pE194-chromosome junction is located between positions 764 (*Cfo*I site) and 1090 (*Hin*fI site) of pE194 (Fig. 2A and B). However, the locations of their respective left pE194-chromosome junctions differ. In type 1 plasmids, the left pE194-chromosome junction is also located between bp 764 and 1090 of pE194 (Fig. 2A) and the chromosomal insert seems to be present in addition to the complete pE194 sequence. In type 2 plasmids, two or three adjacent restriction fragments, including the integrational left junctions, are lacking (Fig. 2B; Table 2). The left junction of the chromosomal insert is located between positions 3383 and 3541 (pE'a2) or between positions 2924 and 3184 (pE'a1 and pE'lys) (Fig. 2B) of pE194, indicating distinct excision breakpoints within the pE194 plasmid.

Restriction site mapping by *Hin*fI, *Sau*3A, *Taq*I, or *Cfo*I of type 1 and type 2 excision plasmids confirmed the identical sizes of several insert restriction fragments (Fig. 3). Some additional restriction fragments were specific for each plasmid. These results indicate partial homology but size variation of the inserted DNA. A *Hin*fI fragment of 520 bp and a *Sau*3A fragment of about 800 bp are common to all type 1 and type 2 excision plasmids containing the right pE194-chromosome junction (Fig. 3). In type 1 plasmids pE'pur, pE'd1, pE'a3, and pE'f, the left junction is located on an identical 540-bp *Hin*fI fragment or on a 1,050-bp *Sau*3A fragment (Fig. 3).

The insert DNAs of the eight type 1 and type 2 excision plasmids are further characterized by a unique *Eco*RI site about 250 bp from the right junction (Fig. 3). In addition, in type 1 plasmids pE'pur, pE'a3, pE'd1, and pE'f, a unique *Bcl*I site is located about 300 bp from the left junction of the insert DNA. The restriction site mapping suggests at least the partial identity of insert DNAs of type 1 and type 2 excision plasmids (Fig. 3).

Nucleotide sequence of pE194 junctions. In type 1 and type 2 excision plasmids, the insert DNA is located between positions 764 and 1090, whereas in type 3 plasmid pE'd2 the insert DNA is located between positions 1942 and 2141 (Fig. 2). The fragments carrying the left and right junctions of various type 1 (pE'pur, pE'g3, pE'a3, pE'f, and pE'd3) and type 3 (pE'd2) plasmids were isolated and sequenced (2, 15) in order to characterize the DNA involved in the recombination event.

The five type 1 plasmids contain at their right and left junctions the directly repeated 13-bp sequence 5'-GGG

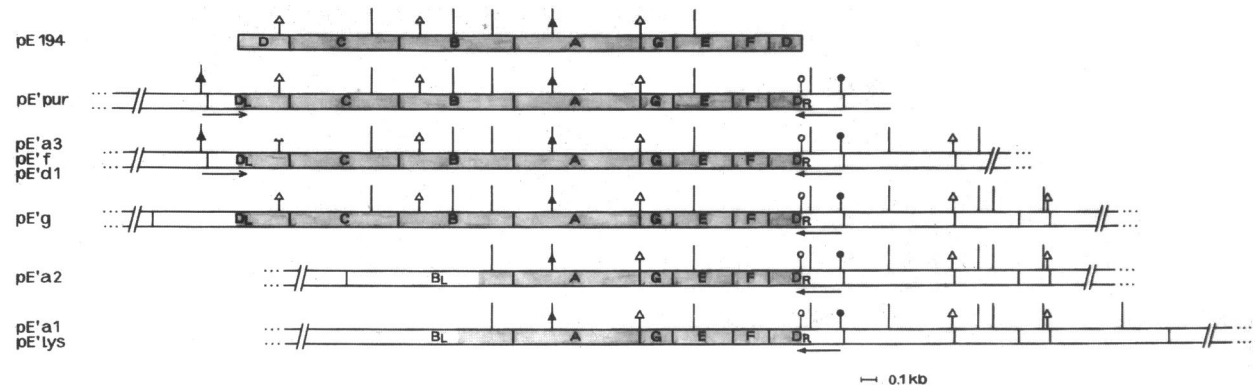

FIG. 3. Comparison of restriction patterns of chromosomal insert DNAs of type 1 and type 2 plasmids. The schema summarizes data of restriction mapping and Southern hybridization of excision plasmids with ^{32}P-labelled pE194 DNA. The *Hin*fI fragments are boxed and designated A through G according to Horinouchi and Weisblum (11). Horizontal arrows, DNA fragments determined by sequencing. Symbols for cleavage sites: |, *Sau*3A; △, *Taq*I; ●, *Eco*RI; ▲, *Bcl*I; ○, *Sma*I. L and R, left and right junctions.

GAGAAAACAT-3′ (Fig. 4A), which has its counterpart between bp 864 and 876 in the pE194 sequence determined by Horinouchi and Weisblum (11). Type 3 plasmid pE′d2 carries the tetranucleotide 5′-TCCC-3′, corresponding to the pE194 sequence between bp 1995 and 1998, at both junctions

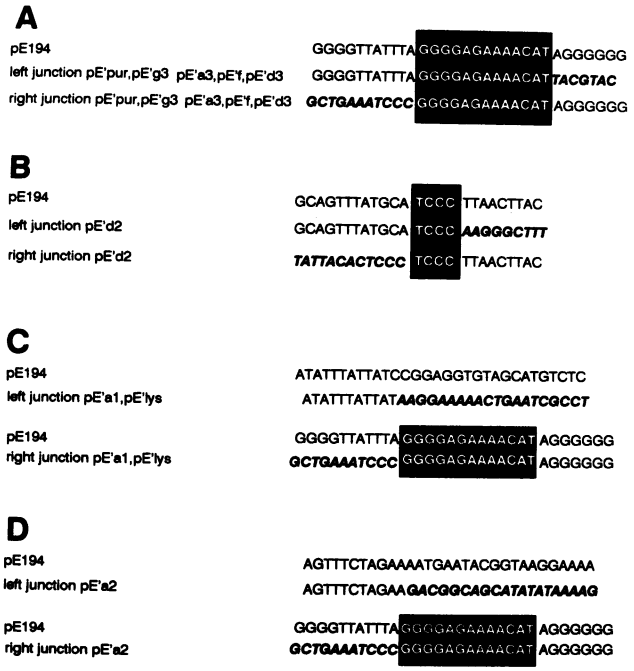

FIG. 4. Nucleotide sequences of plasmid-chromosome junctions of various excision plasmids. The nucleotide sequences of left and right junctions are shown to be aligned to their recombination sites on plasmid pE194. Only one strand of each partner is presented in the 5′→3′ direction. Duplicated core sequences within the junctions and their pE194 recombination sites are boxed. (A) Left- and right-junction sequences of type 1 plasmids. Recombination appears to have occurred within a 13-bp sequence located in pE194 between bp 864 and 876. (B) Junction sequences of type 3 plasmid pE′d2. Recombination appears to have occurred within a 4-bp sequence located in pE194 between bp 1995 and 1998. (C) Left- and right-junction sequences of type 2 plasmids pE′a1 and pE′lys. (D) Left- and right-junction sequences of type 2 plasmid pE′a2.

(Fig. 4B). In both type 1 and type 3 plasmids, the pE194 sequences next to the recombination sites are perfectly retained without deletions of pE194 DNA, thus indicating the additive character of those plasmids. Type 2 plasmids pE′a1, pE′lys, and pE′a2 also carry the conserved 13-bp sequence 5′-GGGGAGAAAACAT-3′ at their right junctions (Fig. 4C and D), whereas the pE194 sequence at the left junctions has been deleted because of the excision. Plasmid pE′a2 has its left excision point between coordinates 3485 and 3486 of pE194 (Fig. 4D); plasmids pE′a1 and pE′lys have this junction between nucleotides 3136 and 3137 (Fig. 4C). Thus, substitutive plasmid pE′a2 has a deletion of the pE194 sequence constituting the region from bp 3486 to 876, and plasmids pE′a2 and pE′lys have a deletion from bp 3137 to 876.

Sequencing the common *Eco*RI site region of the insert DNA (Fig. 3), i.e., 265 bp upstream from the right junction, we found the sequences to be identical in all eight type 1 and type 2 plasmids. Moreover, 250-bp insert fragments adjacent to the left junctions and comprising the unique *Bcl*I site (Fig. 3) were also sequenced in type 1 plasmids pE′pur, pE′f, and pE′a3. They also were homologous.

Origin of chromosomal insert DNA. The uniformity of inserts of the various type 1 and type 2 plasmids prompted the question of whether the inserts originate from one unique sequence or multiple but identical sequences of the *B. subtilis* chromosome.

A ^{32}P-labelled *Sma*I-*Eco*RI fragment of 265 bp, found in all eight type 1 and type 2 plasmids (Fig. 3), and ^{32}P-labelled pE194 were hybridized to *Eco*RI-restricted chromosomal DNA of *B. subtilis* BD224, not being infected by pE194 DNA. One chromosomal *Eco*RI fragment of about 8 kbp hybridized with the 265-bp insert probe (Fig. 5A) but not with pE194 (Fig. 5B).

In order to investigate which of the original insertion mutants carries the pE194 copy in this 8-kbp *Eco*RI fragment, we analyzed *Eco*RI-restricted chromosomal DNAs from insertion strains BD891, BD889, BD886, GB702, and GB701 in Southern blots. The 265-bp insert probe also hybridized with one *Eco*RI fragment of 8 kbp, as was the case with the BD224 DNA, i.e., the chromosomal insert region of these mutant strains is not affected by the pE194 insertion. Only a 12-kbp *Eco*RI fragment of chromosomal DNA from mutant strain BD892 hybridized both with pE194

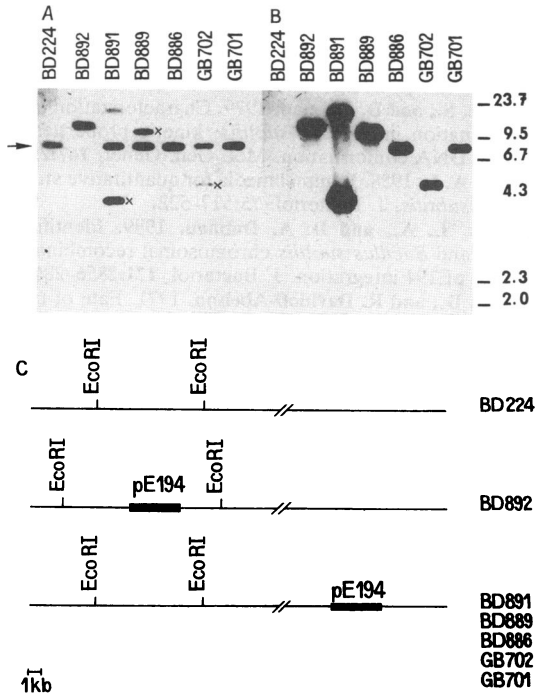

FIG. 5. Southern hybridization of digested chromosomal DNAs of various integration strains and of control strain BD224. *Eco*RI-restricted chromosomal DNA was electrophoresed on 0.8% agarose gels and transferred to a nitrocellulose filter. The blot was hybridized either with an [α-^{32}P]dATP-labelled 265-bp *Sma*I-*Eco*RI fragment derived from insert DNA (A) or with [α-^{32}P]dATP-labelled pE194 DNA (B). The 265-bp *Sma*I-*Eco*RI fragment was subcloned from plasmid pE'g3 into pUC19. Molecular sizes are given on the right, in kilobases. Arrow, chromosomal *Eco*RI fragment of about 8 kbp (see text); ×, unexpected signals (see text). (C) Schematic maps of the pE194 integration regions of the different strains.

and with the 265-bp insert probe (Fig. 5B and A, respectively); i.e., this fragment apparently consists of the 8-kbp chromosomal DNA fragment enlarged by the size (3.7 kbp) of the pE194 DNA. These findings suggest that the chromosomal integration site of pE194 in strain BD892 corresponds to the excision sites of the "pickup" DNA inserts in the eight excision plasmids (Fig. 5C).

It is confusing that the 265-bp insert probe also hybridized weakly with the chromosomal DNA fragments carrying the inserted pE194 in strains BD891, BD889, and GB702 (Fig. 5A and B). Thus, it may be possible that the sequence of the 265-bp insert probe and that of pE194 are similar to a certain degree. pE194 DNA, restricted with various enzymes (*Hin*fI, *Sau*3A, *Alu*I, and *Taq*I), was hybridized to the 265-bp insert probe. Only pE194 DNA fragments, constituting the region from bp 2926 to 736, yielded weak signals (data not shown). Computer searches for sequence similarity of the 265-bp insert to pE194 gave only short stretches of 9 to 11 bp, possibly corresponding to the hybridization signals.

DISCUSSION

Six pE194 insertion strains of *B. subtilis* were carefully separated, and cultures from single colonies were used to isolate nine excision plasmids. Surprisingly, eight of these excision plasmids carry partly homologous insert DNAs, ranging from 1 to 6 kbp and apparently originating from one distinct chromosomal region. In these eight excision plasmids, the chromosomal insert is located within Int site I_G of pE194 in an additive or substitutive manner. In additive (type 1) excision plasmids, the left and right pE194-chromosome junctions are completely preserved. In substitutive (type 2) plasmids, only the right integration junctions remain intact; the pE194 sequence at the left junction is lost. Sequencing of the junctions revealed a 13-bp direct repeat 5'-GGGGAGAAAACAT-3', present at both border regions in type 1 plasmids and retained only at the right junction in type 2 plasmids.

The only type 3 plasmid, pE'd2, carries the insert also in an additive manner, but the insert is flanked by the directly repeated tetranucleotide 5'-TCCC-3' at both junctions within the pE194 Int-VI region. Both Int sequences (I_G and VI) are located either close to or within putative hairpin structures of single-stranded pE194 DNA (18, 19). Thus, previous results, characterizing junctions of various *B. subtilis* pE194 integration mutants as carrying short direct repeats of 2 to 15 bp and often being localized in or close to palindromic DNA regions, are confirmed (1a, 5).

In type 1 and type 2 excision plasmids, the chromosomal DNA was inserted close to the proposed pE194 replication region, which was mapped by deletion analysis between bp 765 and 1720 within pE194 (9). The insertion does not disturb the replication function of the plasmid DNA. Thus, we suggest that the inserted DNA is located between bp 864 and 876 at the end of the *rep* region and that this region is not essential for pE194 replication. However, the copy numbers of type 1 and type 2 plasmids are reduced (data not shown) compared with those of pE194 and type 3 plasmid pE'd2, in which the insert DNA is located outside the replication region. This reduction might indicate that insertions at the end of the *rep* region somehow influence essential functions for replication or copy number control but cannot inactivate them completely.

In order to explain the excision mechanism, we have to consider (i) that the insert DNAs of eight excision plasmids originate probably from the same chromosomal region, (ii) that all excision plasmids except pE'g3 carry the chromosomal insert DNA within a new Int site of pE194 distinct from the original integration site of pE194 (Table 2), and (iii) that in type 2 excision plasmids, the left-junction DNA is deleted.

We postulate that in some cells of a mutant population, pE194 has been translocated into an excision site. For type 1 and type 2 plasmids, this excision site is the original pE194 insertion target in mutant strain BD892. The chromosomal insert DNAs of type 1 and type 2 excision plasmids hybridized with the DNA fragment containing the integration target of mutant strain BD892. This so-called g region has previously been mapped near position 180 (10) within the chromosome of *B. subtilis*. We proposed it to be the preferred target of the postinsertional intrachromosomal translocation of pE194 and of imprecise pE194 excision. Thus, the high frequency of pE194 excision for strain BD892 (see Results), compared with frequencies for strains BD886, BD891, GB701, and GB702, is due to the fact that in the latter four strains pE194 must translocate from its original integration site into the g site before it can excise.

In short, we assume that pE194 excision in *B. subtilis* happens as outlined in Fig. 6. We postulate imprecise excision after translocation from one distinct chromosomal region. The excisional breakpoints lie either within the flanking chromosomal DNA regions (additive excision plasmids) or within the pE194 DNA and the flanking chromo-

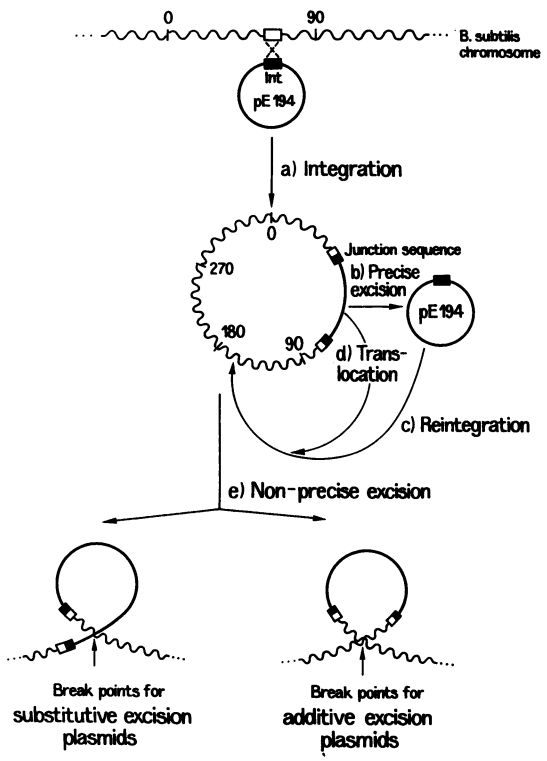

FIG. 6. Proposed model for integration and precise and imprecise (non-precise) excision of plasmid pE194 in *B. subtilis* after reintegration or translocation into an excision site.

somal DNA (substitutive excision plasmids) (Fig. 6). This hypothesis now has to be validated by sequencing the entire insert DNA and by functional characterization of the chromosomal excision region. So far, the nucleotide sequences of insert DNA of four excision plasmids have been determined (1). These data allow us to reconstruct the nucleotide sequence of the original chromosomal g region. Eleven putative open reading frames have been found within this DNA region. However, no significant homology between the 11 potential polypeptides and those in the SWISS-PROT protein sequence data bank was found (1).

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REFERENCES

1. **Bashkirov, I. V., et al.** Unpublished data.
1a. **Bashkirov, I. V., F. K. Khasanov, and A. A. Prozorov.** 1987. Illegitimate recombination in *B. subtilis*: nucleotide sequences at recombinant DNA junctions. Mol. Gen. Genet. **210**:578–580.
2. **Chuvpilo, S. A., and V. V. Kravchenco.** 1984. A simple and rapid method for sequencing DNA. FEBS Lett. **179**:34–36.
3. **Contente, S., and D. Dubnau.** 1979. Characterization of plasmid transformation in *Bacillus subtilis*: kinetic properties and the effect of DNA conformation. Mol. Gen. Genet. **167**:251–258.
4. **Demain, A. L.** 1958. Minimal media for quantitative studies with *Bacillus subtilis*. J. Bacteriol. **75**:517–522.
5. **Dempsey, L. A., and D. A. Dubnau.** 1989. Identification of plasmid and *Bacillus subtilis* chromosomal recombination sites used for pE194 integration. J. Bacteriol. **171**:2856–2865.
6. **Dubnau, D., and R. Davidoff-Abelson.** 1971. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. I. Formation and properties of the donor-recipient complex. J. Mol. Biol. **56**:209–221.
7. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
8. **Gryczan, T. J., S. Contente, and D. Dubnau.** 1978. Characterization of *Staphylococcus aureus* plasmids introduced by transformation into *Bacillus subtilis*. J. Bacteriol. **134**:318–329.
9. **Gryczan, T. J., J. Hahn, S. Contente, and D. Dubnau.** 1982. Replication and incompatibility properties of plasmid pE194 in *Bacillus subtilis*. J. Bacteriol. **152**:722–735.
10. **Hofemeister, J., M. Israeli-Reches, and D. Dubnau.** 1983. Integration of plasmid pE194 at multiple sites on the *Bacillus subtilis* chromosome. Mol. Gen. Genet. **189**:58–68.
11. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **150**:804–814.
12. **Khasanov, F. K., V. I. Bashkirov, and A. A. Prozorov.** 1985. Study of the integration of some plasmids into the *Bacillus subtilis* chromosome. Genetika **21**:1618–1626.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base specific chemical cleavages. Methods Enzymol. **65**:499–560.
15. **Rosenthal, A., R. Jung, and H.-D. Hunger.** 1986. Optimized conditions for solid-phase sequencing: simultaneous chemical cleavage of a series of long DNA fragments immobilized on CCS anion-exchange paper. Gene **42**:1–9.
16. **Rüther, U.** 1980. Construction of a new cloning vehicle, allowing direct screening of recombinant plasmids. Mol. Gen. Genet. **178**:475–477.
17. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
18. **te Riele, H., B. Michel, and S. D. Ehrlich.** 1986. Single-stranded plasmid DNA in *Bacillus subtilis* and *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **83**:2541–2545.
19. **te Riele, H., B. Michel, and S. D. Ehrlich.** 1986. Are single-stranded circles intermediates in plasmid DNA replication? EMBO J. **5**:631–637.
20. **Weisblum, B., M. Y. Graham, T. Gryczan, and D. Dubnau.** 1979. Plasmid copy number control: isolation and characterization of high-copy-number mutants of plasmid pE194. J. Bacteriol. **137**:635–643.