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Integration and Excision of pMC7105 in *Pseudomonas syringae* pv. *phaseolicola*: Involvement of Repetitive Sequences†

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The site for integration of pMC7105 into the chromosome of *Pseudomonas syringae* pv. *phaseolicola* has been mapped to a 2.6-kilobase-pair (kb) *Bgl*II-*Eco*RI fragment on this 150-kb indigenous plasmid. Selected excision plasmids resulting from imprecise excision of pMC7105 were used to identify one of the plasmid-chromosome juncture fragments and to characterize the mechanism of recombination from the chromosome. A 14.2-kb *Bam*HI plasmid-chromosome juncture fragment has been identified in pEX8060 (234 kb), an excision plasmid which carries approximately 90 kb of chromosomal sequences to the left of the site of integration. This fragment contains a portion of the 2.6-kb *Bgl*II-*Eco*RI fragment as well as chromosomal sequences. Blot hybridization with a probe made from selected fragments of pMC7105 revealed three distinct repetitive sequences, RS-I, RS-II, and RS-III, on this plasmid. The 2.6-kb fragment, to which the site of integration maps, also contains RS-II. Five copies of RS-II are present in pMC7105, and more than 20 copies are present in the chromosome. Eight small excision plasmids were shown to result from recombination among fragments of pMC7105 that contain common repetitive sequences. The results indicate that integration and excision of pMC7105 occur through general recombination at homologous repetitive sequences.

Many phytopathogenic bacteria have been shown to contain indigenous plasmids (4, 6, 13, 21). A strain of *Pseudomonas syringae* pv. *phaseolicola*, a pathogen of common bean (*Phaseolus vulgaris*), harbors a 150-kilobase-pair (kb) plasmid, pMC7105, that can replicate autonomously or integrate into the bacterial chromosome (7). Imprecise excision of the integrated form of pMC7105 has resulted in the formation of plasmids that range in size from approximately 35 to 270 kb; some contain large segments of chromosomal DNA (7, 22; L. J. Szabo and D. Mills, submitted for publication). The site on pMC7105 for chromosome integration was recently mapped to a *Bam*HI fragment that has homology with 7 of the other 18 *Bam*HI fragments of pMC7105 and to some chromosomal fragments identified in the excision plasmids (Szabo and Mills, submitted for publication).

There is strong evidence that the formation of F-prime plasmids in *Escherichia coli* K-12 occurs through recombination between homologous insertion sequence (IS) elements (14, 20). IS elements are widely distributed among many bacterial species (for a review, see reference 16), and their occurrence among phytopathogenic pseudomonads was recently demonstrated (4).

In this paper we present evidence for three distinct repetitive sequences on pMC7105, of which two are present in the chromosome of *P. syringae* pv. *phaseolicola*. One is at the site of integration on pMC7105, and multiple copies of this repetitive sequence are present in the chromosome. Finally, the repetitive sequences appear to act as sites for recombination in the formation of excision plasmids.

(The data presented in this paper are a portion of the work done by L.J.S. in partial fulfillment of the Ph.D. degree at Oregon State University, Corvallis.)

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. The bacterial strains and plasmids used in this study are listed in Table 1. Plasmid pMC7105 was used as a reference standard in this study and was isolated from strain LR721. The culture conditions and media have been described elsewhere (Szabo and Mills, submitted for publication). The pseudomonads were cultured in MaNY medium (6), and *E. coli* strains were grown in LB medium (9). Drug selection media were supplemented with ampicillin, chloramphenicol, and rifampin (each 100 μg/ml), streptomycin (50 μg/ml), and tetracycline (12 μg/ml) when appropriate.

DNA isolation. Plasmid DNA was isolated from *P. syringae* pv. *phaseolicola* by the alkaline lysate procedure of Currier and Nester (8) as modified by Cantrell et al. (3). Cultures were grown to an absorbancy at 660 nm of 0.6 to 0.7, and samples of cells from 500 ml of culture were suspended in 100 ml of TE (50 mM Tris, 20 mM EDTA, pH 8.0). After the addition of 6 ml of sodium dodecyl sulfate (20%, wt/vol) and 12 ml of pronase (Sigma Chemical Co.; 5 mg/ml, predigested for 90 min at 37°C), the cells were lysed with gentle mixing at 37°C. The lysates were adjusted to pH 12.3 to 12.4 with 6.5 ml of 3 M NaOH and gently mixed for 5 min. The lysate was adjusted to pH 8.5 with 16 ml of 2 M Tris-hydrochloride (pH 7.0) and then gently mixed for 10 min. NaCl (3%, wt/vol) was added, and the lysates were extracted with 0.5 volume of salt-saturated phenol (3% [wt/vol] NaCl) followed by extraction with an equal volume of chloroform. The DNA was precipitated with an equal volume of ethanol, suspended in TE, and purified further by two rounds of cesium chloride-ethidium bromide equilibrium centrifugation. The plasmid DNA was precipitated in ethanol after extraction of ethidium bromide (10) and suspended in TEN (6 mM Tris, 0.1 mM disodium EDTA, 10 mM NaCl, pH 7.4).

Total DNA was isolated by a modified procedure of Comai and Kosuge (5). Cells from 100-ml cultures were washed with 10 ml of TE, suspended in 8 ml of TEN, and incubated

TABLE 1. Bacterial strains and plasmids

Strain	Genotype or phenotype[a]	Plasmid characteristics			
		Plasmid	kb	Resistance specified	Source or comments
P. syringae pv. *phaseolicola*					
LR700	Wild type	pMC7105	151	None	(7)
LR705	Nal^r	pMC7105	151	None	M. Curiale
LR712	ser	pMC7105	151	None	M. Curiale
LR716	Loc^{cs} Str^r	pMC7105	151	None	(7)
LR719	Loc^{cs} Str^r	pMC7105 chrom Ω		None	(7)
LR721	ser Rif^r	pMC7105	151	None	Szabo and Mills[b]
LR743	Loc^{cs} Str^r	pMC7113	52	None	(7)
LR744	Loc^{cs} Str^r	pMC7114	73	None	(7)
LR745	Loc^{cs} Str^r	pMC7115	87	None	(7)
PP806	Loc^{cs} Str^r	pEX8060	234	None	Szabo and Mills[b]
PP807	Loc^{cs} Str^r	pEX8070	151	None	Szabo and Mills[b]
PP808	Loc^{cs} Str^r	pEX8080	35	None	Szabo and Mills[b]
PP809	Loc^{cs} Str^r	pEX8090	73	None	Szabo and Mills[b]
PP810	Loc^{cs} Str^r	pEX8100	152	None	Szabo and Mills[b]
PP812	Loc^{cs} Str^r	pEX8120	268	None	Szabo and Mills[b]
PP813	Loc^{cs} Str^r	pEX8130	103	None	Szabo and Mills[b]
PP814	Loc^{cs} Str^r	pEX8140	52	None	Szabo and Mills[b]
E. coli K-12					
HB101	r_B^- m_B^- *leu pro thi lacY recA endo-1* Str^r			None	H. Evans
HB101		pBR322	4.4	Ap^r Tc^r	(2)
HB101		pAB0008	12.4	Ap^r	pBR322 with *Bam*HI-8 of pMC7105[b]
HB101		pAB0010	8.7	Ap^r	pBR322 with *Bam*HI-10 of pMC7105, this study
HB101		pAB2103	5.0	Ap^r	pBR322 with *Bam*HI-*Bgl*II fragment from *Bam*HI-8, this study

[a] Ap^r, Ampicillin resistance; Nal^r, nalidixic acid resistance; Rif^r, rifampin resistance; Str^r, streptomycin resistance; Tc^r, tetracycline resistance; Ser, serine auxotrophy; Loc^{cs}, low-cobalt, cold sensitive, morphology mutant.
[b] Submitted for publication.

for 30 min on ice after the addition of 1 ml of lysozyme (10 mg/ml) and 1 ml EDTA (0.25 M, pH 8.0). Cells were lysed by the addition of sodium dodecyl sulfate (0.5 ml of a 20% solution) and pronase (Sigma; 1 ml of a solution [5 mg/ml], predigested for 90 min at 37°C). This mixture was incubated at 37°C for 2 h with gentle shaking. The cleared lysate was extracted twice with 6 ml of salt-saturated phenol (3% NaCl) and twice with 12 ml of chloroform. The chloroform-lysate mixtures were heated at 56°C for 5 min to help dissociate the protein that was complexed with the DNA. The DNA was precipitated by the addition of 2 volumes of isopropanol, collected on a glass rod, redissolved, and precipitated two more times. The precipitated DNA was further purified by cesium chloride-ethidium bromide centrifugation. The DNA was precipitated after the extraction of ethidium bromide and suspended in TEN. Recombinant plasmid DNA was isolated by the cleared lysate procedure of Kahn et al. (15).

Restriction analysis and electrophoresis. The restriction endonucleases *Eco*RI and *Bam*HI were purified in our laboratory by a procedure obtained from P. Myers (Cold Spring Harbor Laboratories, personal communication) and by the method of Wilson and Young (24), respectively. These and other restriction endonucleases purchased from Bethesda Research Laboratories, Inc., were used for restriction mapping of DNA by double digestion as described by Davis et al. (10). Typically, 2 to 3 μg of plasmid DNA was digested with 6 to 10 U of restriction enzyme in a total volume of 40 μl.

Plasmid and whole cell DNA were separated by electrophoresis through a horizontal slab gel of 0.7% agarose submersed in Tris-acetate running buffer at 1.5 V/cm for 26 to 30 h. Small fragments from the double digestion were analyzed on 0.7 to 1.4% agarose slabs. The gels were stained with ethidium bromide and photographed with Polaroid type 55 Land film and a Kodak yellow filter (no. 15) under UV light. *Hin*dIII and *Xba*I fragments of lambda DNA were used as molecular weight standards. Individual DNA fragments from pMC7105 were recovered from agarose gels by electroelution into dialysis bags (17).

DNA labeling and blot hybridization. Purified DNAs were labeled with [^{32}P]dCTP (800 Ci/mol) to 2×10^7 to 7×10^7 cpm/μg with nick translation kits purchased from Bethesda Research Laboratories or New England Nuclear Corp. DNA fragments were transferred to diazobenzyloxymethyl (DBM) paper, and hybridizations were carried out in 50% formamide buffer (1) at 42°C with 0.5×10^7 to 1.5×10^7 cpm of probe. The blots were hybridized for 16 to 20 h and then washed as previously described (1), and autoradiography was performed with Kodak no-screen or X-Omat X-ray film.

Molecular cloning. Equimolar concentrations of *Bam*HI-digested pMC7105 and either pBR322 or pBR325 DNA (12) were ligated together in L-buffer (66 mM Tris [pH 7.6], 6.6 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ATP) (3) with 50 U of T4 ligase (Bethesda Research Laboratories) per ml at 12.5°C for 10 to 20 min and then diluted 20- to 50-fold with L-buffer and incubated an additional 18 to 20 h. *E. coli* HB101 was prepared and transformed with recombinant plasmids as described by Morrison (19).

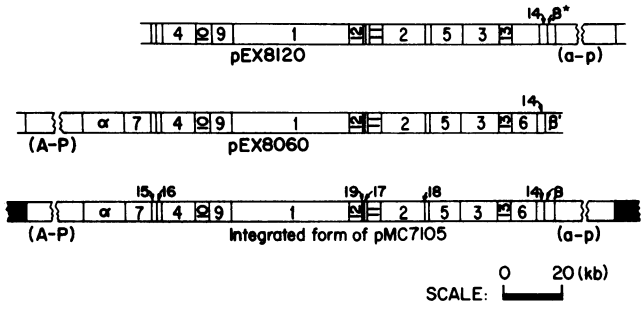

FIG. 1. Proposed linear map of the integrated form of pMC7105 and two excision plasmids with chromosomal sequences from either side of the site of integration. The chromosomal fragments to the left of the site of integration are designated by uppercase letters; fragments to the right are indicated by lowercase letters. Fragments in which excision occurred are indicated by the open bars (□); solid bars (■) represent chromosomal sequences beyond the site of integration that are not detected in these excision plasmids. The left and right plasmid-chromosome juncture fragment, *Bam*HI-α and *Bam*HI-β, respectively, contain portions of *Bam*HI-8 of pMC7105. *Bam*HI-β* and *Bam*HI-β′ are fragments that differ from *Bam*HI-β. See the text for complete details.

RESULTS

To map the site of integration of pMC7105, which resides within *Bam*HI fragment 8 (8.1 kb), it was essential to identify and characterize at least one of the two plasmid-chromosome juncture fragments. pEX8060, a 234-kb excision plasmid in our collection (Table 1), appears to have been excised at a chromosomal site arbitrarily designated as being to the left of the site of integration, and at a second site within the right plasmid-chromosome juncture fragment, *Bam*HI-β (Fig. 1). This plasmid contains a portion of *Bam*HI-β (*Bam*HI-β′), all of the pMC7105 sequences, and approximately 90 kb of chromosomal sequences to the left of the site of integration, including the left plasmid-chromosome juncture fragment, *Bam*HI-α. Previous results (Szabo and Mills, submitted for publication) showed that *Bam*HI-8 has homology with seven (1, 2, 4, 5, 7, 12, and 14) other *Bam*HI fragments from pMC7105 and with several chromosomal fragments present in pEX8060 and other excision plasmids. These results precluded its use as a labeled probe to specifically identify the plasmid-chromosome juncture fragments. However, a 14.2-kb *Bam*HI fragment from pEX8060, which corresponds to none of the 19 *Bam*HI fragments of pMC7105, shows very intense hybridization with the *Bam*HI-8 probe (Szabo and Mills, submitted for publication). This fragment was surmised to either have multiple copies of the repeat sequence on *Bam*HI-8 or to contain a large portion of *Bam*HI-8 sequences. To distinguish between these two possibilities, the 14.2-kb fragment was extracted from the gel by electroelution and subjected to comparative restriction analysis with *Bam*HI-8.

Identification of *Bam*HI-α in pEX8060. *Bam*HI-8 of pMC7105 was cloned into the *Bam*HI site of pBR322, and the resulting plasmid, pAB0008, was used to map the five internal *Eco*RI restriction sites on *Bam*HI-8 (Fig. 2). Double digestion of pAB0008 with *Bam*HI and *Eco*RI produced six fragments from *Bam*HI-8 and two vector fragments (Fig. 3A). A comparison of *Eco*RI fragments from the 14.2-kb *Bam*HI-α fragment and *Bam*HI-8 revealed three common fragments of 1.8, 1.4, and 0.6 kb (Fig. 3A, lanes 2 and 3); however, fragments smaller than 0.6 kb were not visible. The 3.2-kb *Bam*HI-*Eco*RI fragment from *Bam*HI-8 (Fig. 2) clearly was not present in the 14.2-kb fragment from pEX8060. The absence of this terminal fragment and presence of an adjacent fragment (0.6 kb) and the right terminal fragment (1.4 kb) from *Bam*HI-8 (Fig. 2) strongly suggested that recombination had occurred within the 3.2-kb fragment. This was confirmed by transferring the fragments shown in Fig. 3A to DBM paper and probing with pAB0008 (Fig. 3B). The 14.2-kb fragment was confirmed to be the left plasmid-chromosome juncture fragment, *Bam*HI-α, because it contains five of six internal fragments that comprise *Bam*HI-8. Other fragments from *Bam*HI-α also have homology with *Bam*HI-8. One of these should contain chromosomal sequences as well as a portion of the 3.2-kb fragment extending from the *Eco*RI site leftward to the site of integration. The apparent homology of other internal fragments from *Bam*HI-

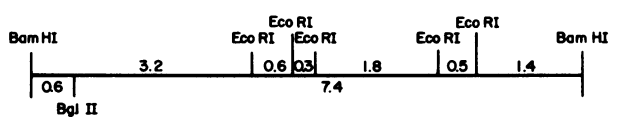

FIG. 2. Partial restriction map of *Bam*HI fragment 8 from pMC7105. *Eco*RI and *Bgl*II endonuclease sites are shown, and the corresponding fragment sizes are given in kb.

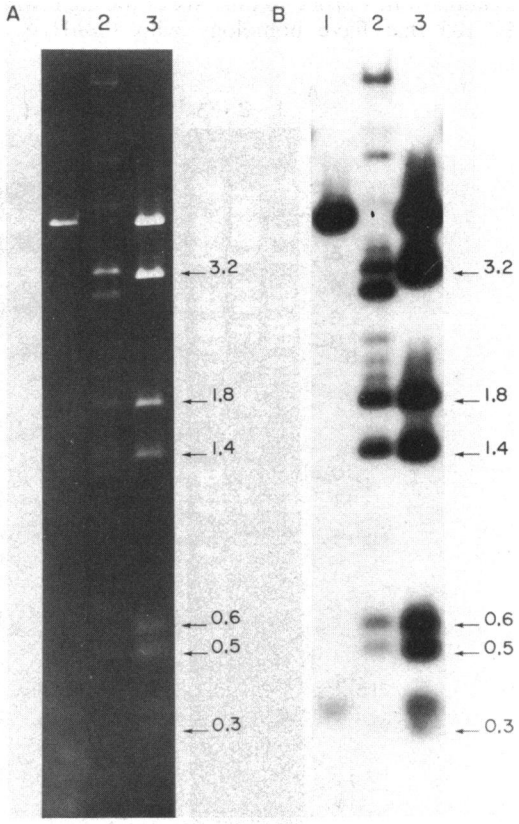

FIG. 3. Comparative restriction analysis of *Bam*HI-8 from pMC7105 and a 14.2-kb *Bam*HI fragment from pEX8060. (A) Ethidium bromide-stained 1% agarose gel showing fragments derived by *Bam*HI-*Eco*RI double digestion of (lane 1) pBR322 and (lane 2) pAB0008 and (lane 3) *Eco*RI digestion of the purified 14.2-kb *Bam*HI fragment from pEX8060. (B) Autoradiogram of fragments shown in A that were blotted to DBM paper and hybridized with ^{32}P-labeled pAB0008 probe. Arrows identify *Eco*RI fragments derived from *Bam*HI-8.

α may represent products of incomplete digestion or fragments that have homology with the repetitive sequence on *Bam*HI-8.

Discovery of two repetitive sequences and the site of integration in the 3.2-kb internal fragment from *Bam*HI-8. A single *Bgl*II site on *Bam*HI-8 maps within the 3.2-kb *Bam*HI-*Eco*RI fragment (Fig. 2), and we sought to determine whether recombination with the chromosome occurred at a site within the 0.6-kb *Bam*HI-*Bgl*II fragment or the adjacent 2.6-kb *Bgl*II-*Eco*RI fragment. Recombination within the 0.6-kb region would leave a portion of this fragment in both *Bam*HI-α and *Bam*HI-β, whereas recombination within the 2.6-kb region would place the 0.6-kb fragment entirely within *Bam*HI-β. Since pEX8060 contains only a portion of *Bam*HI-β, another plasmid, pEX8120, which contains 134-kb of chromosomal DNA from the right side of the site of integration (Fig. 1), was selected. The *Bam*HI restriction fragments of pMC7105, pEX8060, and pEX8120 (Fig. 4A) were transferred to DBM paper and probed with pAB2103, a pBR322 derivative which contains the 0.6-kb *Bam*HI-*Bgl*II fragment. This probe has no homology with *Bam*HI-α of pEX8060, but, in addition to fragments of pMC7105, which were known to have homology, it hybridizes with one other fragment in each excision plasmid (Fig. 4B). These fragments were designated *Bam*HI-β′ and *Bam*HI-β*, respectively, in pEX8060 and pEX8120. Of significance was the hybridization of this probe to only six of the eight fragments of pMC7105 that have homology with *Bam*HI-8. These results indicate that the site of recombination is within the 2.6-kb *Bgl*II-*Eco*RI fragment and that there is more than one repetitive sequence on *Bam*HI-8. The repetitive sequence on the 0.6-kb *Bam*HI-*Bgl*II fragment was designated RS-I.

To show that the 2.6-kb *Bgl*II-*Eco*RI fragment carries the site of integration and to determine whether it carries a repetitive sequence, it was isolated from an agarose gel by electroelution, labeled, and used to probe the blot of *Bam*HI fragments shown in Fig. 4A. This probe hybridized to four (1, 7, 8, and 12), eight (1, 7, 12, A, B, C, α, and β′), and five (1, 12, a, jk, and β*) *Bam*HI fragments of pMC7105, pEX8060, and pEX8120, respectively (Fig. 4C). It hybridized to *Bam*HI-7 and *Bam*HI-12, which show no homology with RS-I. The resolution of the autoradiogram was not sufficient to determine whether hybridization occurred with *Bam*HI fragments j, k, or both, and therefore the doublet jk is indicated. Each excision plasmid is lacking an intact copy of *Bam*HI-8, and pEX8120 is also lacking a complete copy of *Bam*HI-7 of pMC7105. At least four (A, B, C, and either a or jk) chromosomal *Bam*HI fragments in these plasmids have homology with this probe. Since excision of pEX8120 occurred within *Bam*HI-7, which contains this repetitive sequence, either a, j, or k should be the fusion product formed by excision, rather than a chromosomal *Bam*HI fragment which contains a copy of this repetitive sequence. *Bam*HI-α, *Bam*HI-β*, and *Bam*HI-β′ hybridized to this probe as expected, since the probe carries the site of integration. Furthermore, the 2.6-kb fragment carries a second unique

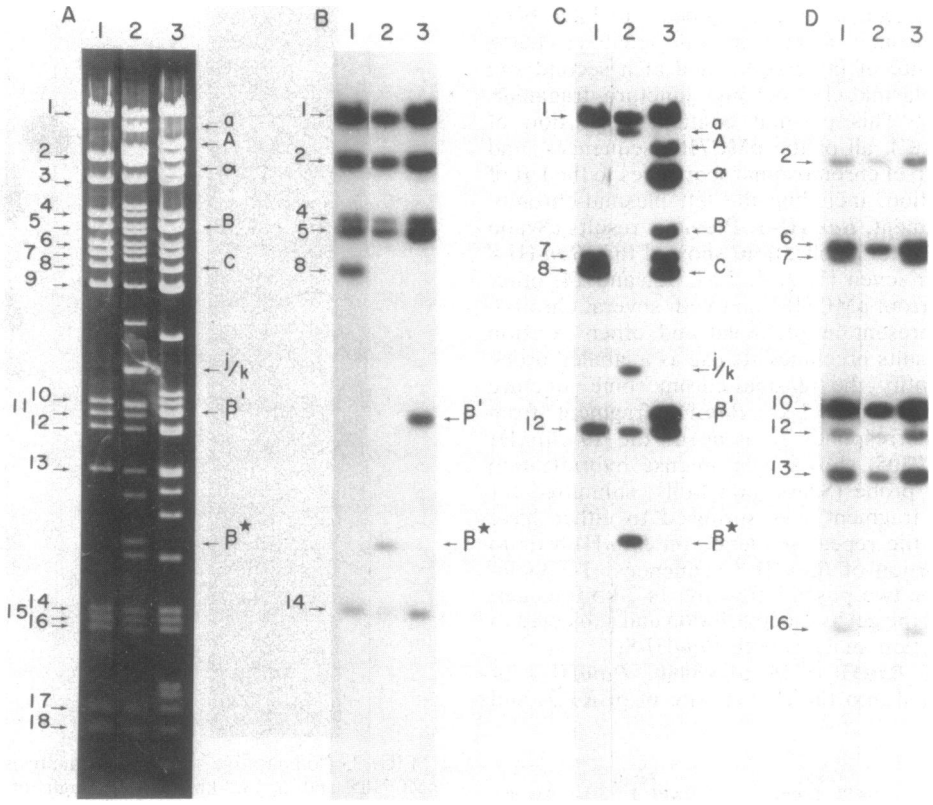

FIG. 4. Agarose gel electrophoresis of *Bam*HI-digested pMC7105 (lane 1), pEX8120 (lane 2), and pEX8060 (lane 3). (A) Ethidium bromide-stained 0.7% agarose gel. Autoradiograms of the DBM blotted fragments shown in panel A were hybridized with the following ^{32}P-labeled plasmids: pAB2103 (B); 2.6-kb *Bgl*II-*Eco*RI fragment from *Bam*HI-8 (C); pAB0010 (D). Numbers refer to *Bam*HI fragments from pMC7105; letters refer to the chromosomal fragments contained within pEX8060 (uppercase) and pEX8120 (lowercase); *Bam*HI-α refers to the left plasmid-chromosome juncture fragment; *Bam*HI-β′ and *Bam*HI-β*, respectively, refer to putative derivatives of the right plasmid-chromosome juncture fragment in pEX8060 and pEX8120. See the text for details.

repetitive sequence from *Bam*HI-8, hereafter referred to as RS-II.

Comparison of the right plasmid-chromosome juncture fragment from the integrated form of pMC7105 and in pEX8120. To verify that *Bam*HI-β* (2.5 kb) of pEX8120 was the right juncture fragment, *Bam*HI-digested whole cell DNA from strains with the integrated and autonomously replicating form of pMC7105 was probed with pAB2103. Unexpectedly in strain LR719, which contains the integrated form of pMC7105, the 0.6-kb *Bam*HI-*Bgl*II fragment hybridized with a single 4.2-kb nonplasmid fragment (Fig. 5). We have confirmed (data not shown) that this 4.2-kb fragment, designated *Bam*HI-β, is present in three other sibling strains of LR719 and also in PP808, a strain with a small excision plasmid, pEX8080, whose excision did not involve either of the plasmid-chromosome juncture fragments. The hybridization of labeled pAB2103 probe with whole cell DNA from strain LR716, which has the autonomous replicating form of pMC7105, confirmed that RS-I is confined to pMC7105. It hybridized to six fragments of pMC7105, as expected, but to none of the chromosomal fragments (Fig. 5B, lane 3).

Correlation between sites for excision and presence of common repetitive sequences. The presence of RS-II within the 2.6-kb *Bgl*II-*Eco*RI fragment that contains the plasmid site for integration (Fig. 4C) was suggestive that repetitive sequences act as sites for recombination in the formation of excision plasmids. Fragments which recombined to produce several small excision plasmids have been deduced from the *Bam*HI restriction map of pMC7105; the distribution of repetitive sequences around pMC7105 are illustrated in Fig. 6. The formation of pEX8080 involved recombination between *Bam*HI-4 and *Bam*HI-1, each of which contains RS-I. The formation of pEX8070, pEX8090, pMC7114, and pMC7115 resulted from recombination within fragments that carry RS-II.

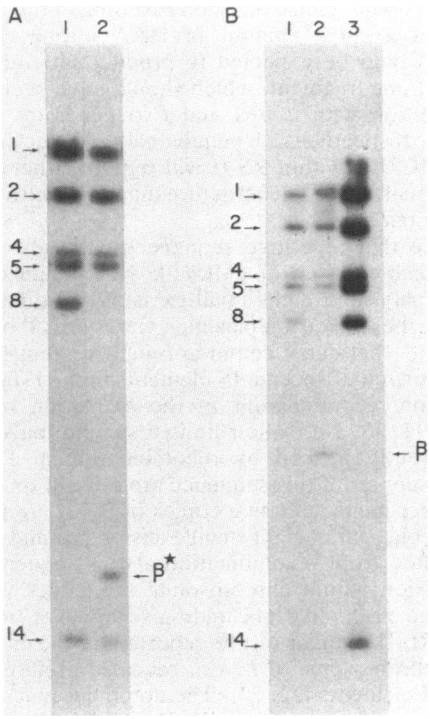

FIG. 5. Autoradiograms of DBM blots containing *Bam*HI-digested DNA probed with ^{32}P-labeled pAB2103. (A) pMC7105 (lane 1), pEX8120 (lane 2). (B) Whole cell DNA extracts of LR716 (lane 1) and LR719 (lane 2) and control pMC7105 (lane 3). Numbers refer to *Bam*HI fragments from pMC7105. The right plasmid-chromosome juncture fragment of the integrated form of pMC7105 and a derivative from pEX8120 are designated *Bam*HI-β and *Bam*HI-β*, respectively.

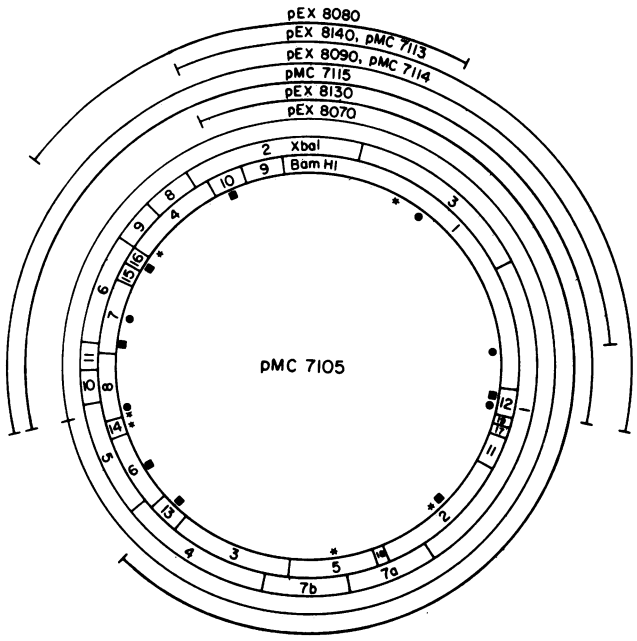

FIG. 6. Restriction maps of pMC7105 showing the distribution of three repetitive sequences and the *Bam*HI fragments in which excision occurred to produce eight excision plasmids. Symbols used to identify the repetitive sequences are as follows: (*) RS-I; (●) RS-II; (■) RS-III. The precise location of a repetitive sequence within a particular fragment has not been determined, with the exception of RS-I and RS-II in *Bam*HI-8. Also, two copies of RS-II are present on *Bam*HI-1, and they map within *Xba*I-1 and *Xba*I-3.

Excision plasmids pEX8130 and pMC7113 were formed by recombination between *Bam*HI-10 and *Bam*HI-12, whereas recombination occurred in *Bam*HI-10 and *Bam*HI-13 to produce pEX8140 (Fig. 6). *Bam*HI-10, *Bam*HI-12, and *Bam*HI-13 were not previously known to carry a common repetitive sequence, although *Bam*HI-12 was known to carry RS-II (Fig. 4C). Recombination at a common repetitive sequence in these fragments would necessarily involve a third repetitive sequence. To prove this hypothesis, *Bam*HI-10 was cloned into pBR322, and the resulting plasmid (pAB0010) was labeled by nick translation and hybridized to the DBM blot containing *Bam*HI digests of pMC7105, pEX8060, and pEX8120 (Fig. 4A). Hybridization was observed to seven (2, 6, 7, 10, 12, 13, and 16) fragments of pMC7105 and pEX8060, and six (2, 6, 10, 12, 13, and 16) fragments of pEX8120 (Fig. 4D). As predicted, *Bam*HI fragments 10, 12, and 13 have sequence homology, indicating that pMC7105 contains a third repetitive sequence, which was designated RS-III.

Three excision plasmids were formed by recombination between sequences within pMC7105 and sequences within the chromosome. The plasmid fragment in which recombination occurred in the formation of pEX8100, pEX8060, and pEX8120 was *Bam*HI-1, *Bam*HI-β′ (which contains a portion of *Bam*HI-8), and *Bam*HI-7, respectively. Each of these fragments carries RS-II, although *Bam*HI-1 and *Bam*HI-7

also carry RS-I and RS-III, respectively. Since RS-I is not present in the chromosome (Fig. 5B), RS-II is the probable chromosomal and plasmid site for recombination in both pEX8100 and pEX8060. Although *Bam*HI-7 contains RS-III, recombination produced a fusion fragment in pEX8120, which does not contain RS-III (Fig. 4D). Hence, the probable site of recombination involved RS-II.

Distribution of RS-II and RS-III. To determine the approximate number of copies of RS-II and RS-III in the bacterial chromosome, labeled probes of fragments containing RS-II and RS-III DNA were hybridized to DBM blots containing *Eco*RI-digested DNA from four strains that were cultured separately for at least 3 years in our laboratory. RS-II hybridized to approximately 30 fragments in each of the four strains (Fig. 7A). However, in strain LR700, hybridization was observed to all the fragments detected in the other three strains, plus an additional fragment. Hybridization of this probe to *Eco*RI-digested pMC7105 DNA blotted on DBM paper revealed six copies in the plasmid. Thirteen fragments in LR700, LR705, and LR712 showed homology with RS-III, whereas LR716 contained 12 of these fragments and 1 additional fragment (Fig. 7B). pMC7105 contains eight copies of RS-III (Fig. 4D).

DISCUSSION

The evidence presented here supports the hypothesis that integration and excision of the pMC7105 replicon occurs through homologous recombination between repetitive sequences. This system of integration and excision appears analogous, in most respects, to that of the F-factor in *E. coli* K-12. Integration of the F-factor occurs through recombination between homologous IS elements contained on F and the bacterial chromosome (11, 20). The formation of F-prime plasmids also appears to be mediated by IS elements (9).

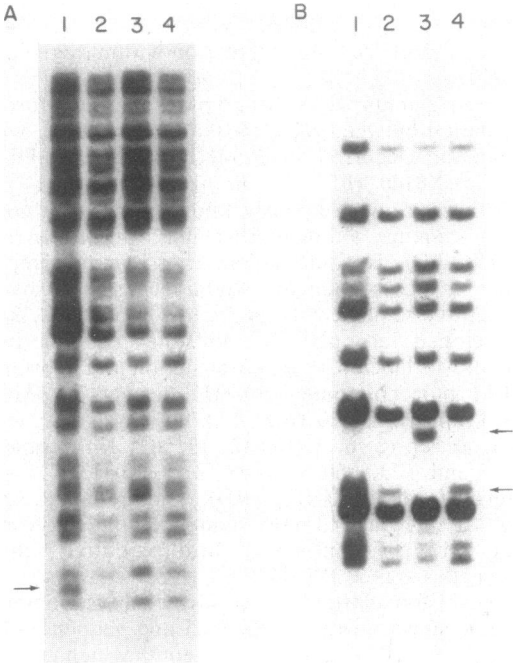

FIG. 7. Autoradiograms showing fragments that hybridized with ^{32}P-labeled RS-II (A) and RS-III (B). Lanes: LR700 (1); LR705 (2); LR712 (3); LR716 (4). Arrows indicate fragments that are present or absent in some strains.

In a previous study, (Szabo and Mills, submitted for publication), we demonstrated that integration of pMC7105 occurred within *Bam*HI fragment 8 and that this fragment had homology with seven other fragments from pMC7105. In this study we have demonstrated the presence of two repetitive sequences, RS-I and RS-II, on *Bam*HI-8 (Fig. 4B and C). RS-I was shown to have homology with the right plasmid-chromosome juncture fragment, but not the left fragment (Fig. 4B). These results indicated that the site of integration had to be outside RS-I. Furthermore, the absence of RS-I in chromosomal DNA indicated that this sequence could not be involved in integration through homologous recombination. RS-II was shown to reside within an internal 2.6-kb *Bgl*II-*Eco*RI fragment from *Bam*HI-8 that has homology with both juncture fragments (Fig. 4C). Furthermore, at least five copies of RS-II are found in pMC7105, and over 20 are present in the chromosome. These results strongly suggest that integration of pMC7105 occurred through homologous recombination at RS-II on this 2.6-kb fragment and at a similar sequence in the chromosome.

Further convincing evidence that integration and precise excision of pMC7105 occurred within RS-II sequences is revealed by the discovery of pEX8070, an excision plasmid that is indistinguishable from pMC7105 (Szabo and Mills, submitted for publication). If integration occurred by recombination within two homologous repetitive sequences on the plasmid and the chromosome, precise excision could result from a recombination event anywhere within the homologous repeated sequences. Previous results (Szabo and Mills, submitted for publication) showed that the *Bam*HI, *Eco*RI, and *Pst*I restriction profiles of pMC7105 and pEX8070 are identical. The imprecise excision resulting in the addition or deletion of a small amount of DNA during excision of pEX8070 would be expected to produce alterations in the mobility of one fragment, which should have been detected, since digestion with *Eco*RI and *Pst*I generates 47 and 33 fragments, respectively. Unequivocal evidence for integration of pMC7105 within RS-II will require either nucleotide sequence analysis or fine structure mapping of RS-II and the juncture fragments.

Evidence that repetitive sequences were also important for imprecise excision of pMC7105 was obtained from restriction analysis of eight small excision plasmids. Without exception, these excision plasmids were formed by excision in fragments that carry common repetitive sequences (Fig. 6). It is known that specific IS elements in the F-factor serve as sites for recombination in the formation of F-prime plasmids (11, 18, 20). In our limited sample, only pEX8080 was apparently formed by recombination at RS-I, even though six copies of this sequence are present on pMC7105. On the other hand, only five copies of RS-II are present on pMC7105, but four of eight small excision plasmids appear to have resulted from recombination at this sequence. Moreover, excision within chromosomal sequences which produced three prime-like plasmids also appears to have occurred at RS-II. Similar results reported for the excision of F from the chromosome of *E. coli* revealed preferential excision at IS*5* elements (12, 23). The argument that excision is occurring specifically within RS-II and RS-III is strengthened by the detection of identical excision plasmids from experiments which were greatly separated in time. pMC7113 and pMC7114 (Table 1) are identical to pEX8140 and pEX8090, respectively, but the latter plasmids were isolated approximately 1 year after the former.

It was significant that RS-I was found only on pMC7105 and not in the chromosome (Fig. 5). Since one copy of RS-I

resides on *Bam*HI-8 outside the site of integration, it was effectively used as a marker to identify the right plasmid-chromosome juncture fragment (Fig. 5). Whole cell DNA from four strains in which the right juncture fragment was present in the chromosome each had a single new 4.2-kb fragment which carried RS-I. We interpret this fragment to be the correct juncture fragment, *Bam*HI-β. The smaller 2.5-kb fragment, *Bam*HI-β*, from pEX8120 appears to be a modified juncture fragment whose origin is unknown. This was unexpected since pEX8120 carries chromosomal sequences entirely from the right side of the site of integration (Fig. 1). We have not detected the modification of any plasmid fragments among the 11 excision plasmids that have been analyzed. A comparison of the *Bam*HI restriction maps of chromosomal sequences from pEX8120 and the corresponding sequences from strain LR716 will be necessary to ascertain the nature of the modification of *Bam*HI-β that resulted in the formation of *Bam*HI-β*.

It remains to be determined whether pMC7105 contains additional repetitive sequences and whether the sequences identified here are common to other phytopathogenic pseudomonads. Comai and Kosuge (5) have recently identified a mobile element, IS*51*, in *P. syringae* pv. *savastanoi* that has caused a mutation to avirulence by insertion into a gene essential for indole acetic acid production on pIAA1. The discovery of IS*51* in that pathovar and RS-I, RS-II, and RS-III in this study may provide new approaches for the characterization of genetic systems in phytopathogenic bacteria.

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