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IS*1327*, a New Insertion-Like Element in the Pathogenicity-Associated Plasmid of *Erwinia herbicola* pv. *gypsophilae*

Amnon Lichter[1], Shulamit Manulis[2], Lea Valinsky[2], Baruch Karniol[1], and Isaac Barash[1]

Department of Botany, Tel Aviv University, Tel Aviv 69978, Israel; and Department of Plant Pathology, ARO, the Volcani Center, P.O.B. 6, Bet Dagan 50250, Israel

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The pathogenicity-associated plasmid (pPATH) of *Erwinia herbicola* pv. *gypsophilae* was previously shown to be exclusively present in pathogenic strains and to contain a gene cluster encoding phytohormone biosynthesis. Sequence analysis of the DNA region located downstream from the cytokinin biosynthetic gene (*etz*) revealed homology to insertion sequences (IS) of the IS*6* family. Southern blot analysis performed on plasmid DNA of *E. herbicola* pv. *gypsophilae* revealed the presence of six copies of this insertion-like element, which was designated as IS*1327*. Only pathogenic strains contained IS*1327* and restriction fragment length polymorphism was observed among gypsophila and beet pathovars of *E. herbicola*. Nonpathogenic deletion derivatives of pPATH contained fewer copies of IS*1327*, suggesting its presence in the deleted region. One copy of IS*1327* (IS*1327*-R) was located 2.8 kb downstream from the IS element adjacent to the *etz* (IS*1327*-L) in a direct repeat.

Additional keywords: gall formation, phytopathogenic bacteria.

Erwinia herbicola pv. *gypsophilae* induces gall formation on gypsophila plants (Cooksey 1986) whereas another pathovar of *E. herbicola* is pathogenic on table beet as well as gypsophila (Burr et al. 1991). The genes specifying indole-3-acetic acid (IAA) and cytokinin biosynthesis have been isolated from *E. herbicola* pv. *gypsophilae* (Clark et al. 1993; Lichter et al. 1995b; Lichter et al. 1995a). These genes are clustered on a native plasmid designated as pPATH, which is present only in pathogenic strains (Lichter et al. 1995a; Lichter et al. 1995b; Manulis et al. 1991). Insertional inactivation of the IAA or cytokinin biosynthetic genes resulted in smaller galls (Clark et al. 1993; Lichter et al. 1995a).

The presence of various insertion sequence (IS) elements in phytopathogenic bacteria has been demonstrated (Coplin 1989; Galas and Chandler 1989). A model system for evolution of *Agrobacterium tumefaciens* Ti plasmids based on a stepwise accumulation of insertions, deletions, and inversions mediated by various IS elements has been proposed (Otten et al. 1992). Analyses of the homology and organization of the various genes in the T-DNA including *iaa*M, *iaa*H, and *ipt* suggest that they may have originated from other bacteria, e.g., *Pseudomonas syringae* pv. *savastanoi*, by horizontal gene transfer using IS elements. The IAA biosynthetic genes of *P. syringae* pv. *savastanoi* were found to undergo insertions and deletions involving IS*51*, IS*52*, and IS*53* (Yamada et al. 1986; Soby et al. 1994). Thus, IS elements could mediate genomic rearrangements that would lead to the evolution of new plasmids affecting bacterial pathogenicity.

Bacterial ISs from the IS*6* family have been identified in diverse bacterial species but not in phytopathogenic bacteria. IS*6* elements were discovered in the gram-negative bacterium *Proteus vulgaris* (i.e., IS*26*) and later in additional gram-negative and gram-positive bacteria (Mollet et al. 1983; Galas and Chandler 1989; Murphy 1989) . These elements are very small in size (756 to 920 bp) and possess an open reading frame (ORF) encoding a transposase (TnpA) that is flanked by inverted repeats. Evolutionary relatedness among the various IS*6* elements has been demonstrated (Kato et al. 1994). Members of the IS*6* family have been found to constitute composite transposons containing direct repeats of the IS element (Mollet et al. 1983; Murphy 1989). The intervening sequence commonly contains genes for antibiotic resistance (Galas and Chandler 1989; Murphy 1989).

During elucidation of the genomic structure of the pPATH in *E. herbicola* pv. *gypsophilae* we observed the presence of repetitive DNA that was identified as an insertion-like element. The present study was undertaken to characterize this IS element and to determine its distribution in pathogenic *E. herbicola* strains.

The presence of repetitive DNA on the pPATH was inferred from an ambiguous hybridization pattern observed during mapping of pLA150, a cosmid derivative of pPATH (Lichter et al. 1995a; Lichter et al. 1995b). This cosmid clone had previously been shown to contain a cluster of the IAA and cytokinin biosynthesis genes (*iaa*M, *iaa*H, and *etz*, respectively).

Sequence analysis of the downstream DNA region flanking the *etz* gene (Figs. 1 and 2) revealed DNA sequence similarity

Nucleotide and/or amino acid sequence data have been submitted to the EMBL, GenBank, and DDJB as accession number X87144.

The designation of IS*1327* was obtained from Esther M. Lederberg, Plasmid Reference Center, Stanford University, CITY, CA.

Corresponding author: Isaac Barash, Department of Botany, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel; Tel: 972-3-6409847; Fax: 972-3-6409380; E-mail: isaaci@ccsg.tau.ac.il

to various IS elements (Fig. 3). Deduced amino acids of the sequence data demonstrated the presence of an ORF between positions 51 and 366. An overlapping reading frame was identified between positions 250 and 756, but its first methionine residue was located only at position 616 (Fig. 2). Both reading frames exhibited homology to transposase (TnpA) genes related to IS elements of the IS*6* family (Kato et al. 1994). This IS-like element was designated as IS*1327*. The two deduced translational products designated as IS*1327*A and IS*1327*B were compared with IS*26*, the proto-

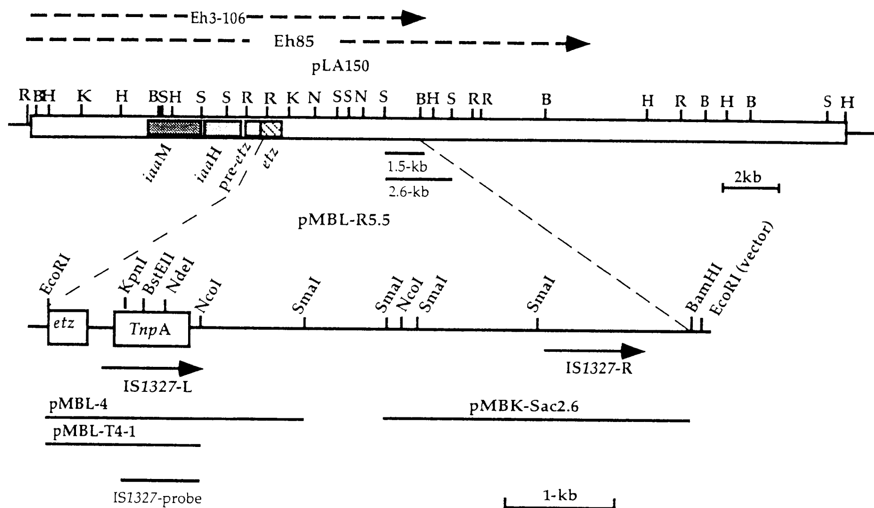

Fig. 1. A restriction map of pLA150 and pMBL-R5.5. The plasmid pMBL-R5.5 was cloned from the cosmid pLA484 (Lichter et al. 1995b) and is aligned to the map of pLA150. Part of the *etz* open reading frame and the putative *Tnp*A (transposase) of IS*1327* are illustrated as an open box. Arrows signify tahe location and orientation of the two IS*1327* copies on pLA150. The various subclones used for sequencing of IS*1327*-L were derived from pMBL-4 and pMBL-R5.5 (Table 1). pMBL-T4-1 was used to generate the IS*1327*-specific probe for Southern hybridizations and pMBK-Sac2.6 was employed for location and partial sequencing of IS*1327*-R. Broken arrows above pLA150 illustrate the deleted portion of pPATH for the specified mutants.

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      IR-L
1     CGGCTCTGTCGCATTAAGGAGTCGTCAGATAACATACTGTTTTTTTGCGATGTCCCTGCCGATGTCTCTTATCCGAAAAGCCTTCAAACGCCTGCATTA
                                                                M  S  L  P  M  S  L  I  R  K  A  F  K  R  L  H  Y

101   TCCCGTCGATATTATTGCCCAGTGCGTTCGCTGGTATCTGGCTTACTCACTCAGCTTGCGAAATCCTGAAGAGATAATGGCTGAGCGGGGGATTGTCGTT
       P  V  D  I  I  A  Q  C  V  R  W  Y  L  A  Y  S  L  S  L  R  N  P  E  E  I  M  A  E  R  G  I  V  V
                                          KpnI
201   GATCATTCCACGCTTCACCGCTGGCTCATCCGGCTGGTACCACGCTTAGATAAGGCGTTCCGCCGGTATAAACGTAATCCTGGCCGACGGTGGCGAATGG
      D  H  S  T  L  H  R  W  L  I  R  L  V  P  R  L  D  K  A  F  R  R  Y  K  R  N  P  G  R  R  W  R  M  D
                         fra eshit?
301   ACGAAACCTACATCAAAATCAGGGGGGCAGTGGAAATACCTGTACCGGGCAGTTGACAGTACGGGGCAGACCATCGACCTCCTGCTGGTCGCTAAGCGTG
        E  T  Y  I  K  I  R  G  A/E-W  K  Y  L  Y  R  A  V  D  S  T  G  Q  T  I  D  L  L  L  V  A  K  R  D

401   ATGCGGCAGCTGCTCTGCGTTTCTTCCGCAAGACTATCCGCAATAACGGGGAACCGGAGGTGGTAACCATTGATAAAAGCGGCGCGAATACCGCGGCTCT
        A  A  A  A  L  R  F  F  R  K  T  I  R  N  N  G  E  P  E  V  V  T  I  D  K  S  G  A  N  T  A  A  L

501   GGATACACTTAACGCGGGTAAAGGAGATGAAGAAAGCATTATCGTCAGGCAAAGCAGGTATCTGAATAATTTGATTGAGCAGGATCACCGGAACATCAAA
       D  T  L  N  A  G  K  G  D  E  E  S  I  I  V  R  Q  S  R  Y  L  N  N  L  I  E  Q  D  H  R  N  I  K

601   CGGCGGATACGACTGATGCTGGGGTTCAAATCATTTCGCCGGGCGCAGATGATACTGGCCGGCATTGAGTTAATCCATATGATACGCAAAGGGCAGTATA
      R  R  I  R  L  M  L  G  F  K  S  F  R  R  A  Q  M  I  L  A  G  I  E  L  I  H  M  I  R  K  G  Q  Y  K

701   AACATCCGCAGGGGGAACGGGTTGTCACCGGCAGAACAATTCTATCTTTTGATTGACTGAAAACAGATTACGCAGCTTTTGCTGACTTACTGCCGTTAAT
        H  P  Q  G  E  R  V  V  T  G  R  T  I  L  S  F  D  *
        IR-R                                            NcoI
801   GCGGCAGAGCCGTAAGTTTAGTGTCCTGATTATTCAACCACTACAAAGTGTCCATGG
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Fig. 2. Nucleotide sequence of IS*1327*. The nucleotide sequence of IS*1327*-L from the left inverted repeat to the *Nco*I site (underlined) is presented. The "corrected" version of the deduced amino acids translational product was obtained by artificially deleting a C residue at position 328. The deletion of this C residue according to the shift in homologous reading frames resulted in a change from alanine (A) to glutamate (E) residues, which is underlined. The inverted repeats, IR-L and IR-R, are also underlined except the central nucleotide that did not match. IS*1327*-L was sequenced by the dideoxy chain termination method using the Sequenase 2.0 kit (USB, Cleveland, OH) and [^{35}S]dATP (NEN, DuPont, Homberg, Germany). DNA for sequencing was prepared by Wizard minicolumns (Promega, Madison, WI) and sequencing was performed on both strands using pBluescript as the vector. Subclones for sequencing were generated by nested deletions of pMBL-4 using *Exonuclease III* (Promega) from the *Eco*RI site within the *etz* and downstream. A restriction map constructed from this region allowed serial subcloning of pMBL-4 using the *Nco*I, *Nde*I, *Bst*EII, *Hinc*II, *Kpn*I, and *Alu*I sites to generate the clones pMBL-T4-1 to 4, pMBL-2B, and pMBL-23B, respectively (Table 1). These clones were subsequently sequenced to generate the second strand of this region. The complete nucleotide sequence of 857 bp from position 1,450 of the *etz* sequence (Lichter et al. 1995a) until the first downstream *Nco*I site (Fig. 1) is presented. Further confirmation for the sequence corresponding to the shift between reading frames homologous to the TnpA was performed by automated sequencing using *Taq* DNA polymerase (Department for Biological Services, Weitzmann Institute, Rehovot, Israel).

type of the IS*6* family (Mollet et al. 1983), as well as with IS*240* from *Bacillus thuringiensis* (Delecluse et al. 1989) and IS*257* from *Staphylococcus aureus* (Murphy 1989). The amino acid sequence of IS*1327*A was similar to the N-terminal portion of *Tnp*A, whereas that of IS*1327*B was similar to the C-terminal portion of the *Tnp*A (Fig. 3). The shift in sequence similarity between the two reading frames was mapped to amino acids 89 to 92 of IS*1327,* suggesting a frameshift mutation due to insertion of an additional nucleotide (Figs. 2 and 3). Examination of the DNA data indicated the presence of four A and six G residues in this region that were responsible for an apparent conservation of the lysine (K) and glycine (G) residues of IS*1327*B. Generation of a complete ORF with the highest sequence similarity to the TnpA of the IS6 family could be obtained by deleting a C residue at position 328 (Figs. 2 and 3). Since a sequencing mistake could have generated the shift between the two reading frames with similarity to TnpA, this region was sequenced

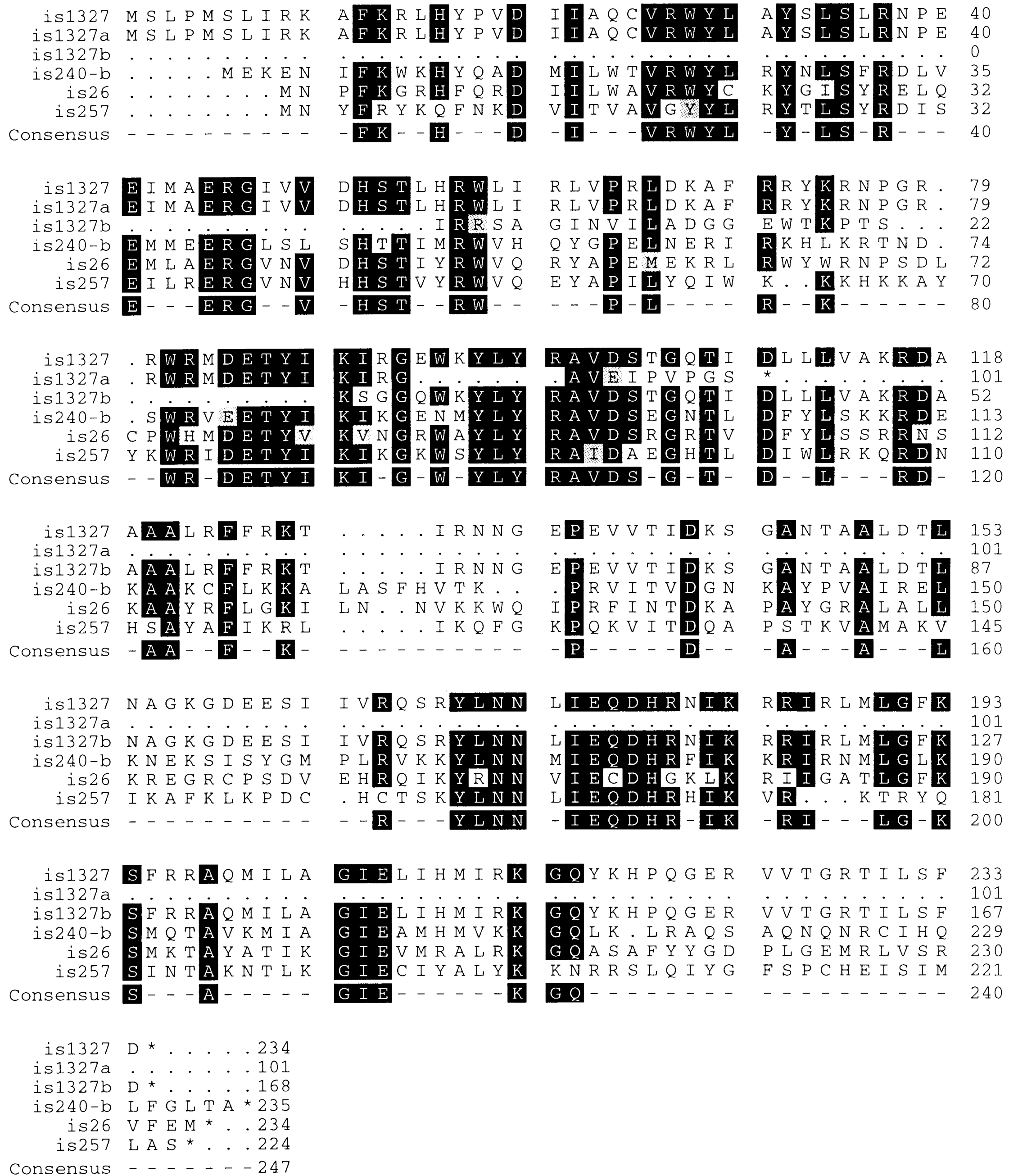

Fig. 3. Comparison of deduced translation products from the TnpA of IS*1327* with selected members of the IS*6* family. The amino acid sequences were compared using the PileUp software in a prettybox format and the Program Manual for the Wisconsin Package (Version 8, Sept. 1994, Genetics Computer Group, Madison, WI). Black background indicates identical amino acids and gray indicates similar residues. "is*1327*" is a translational product derived from nucleotide sequence of IS*1327* in which a C residue at position 328 was artificially deleted. Translation of the third and first reading frames of the DNA sequence (Fig. 2) are designated as is1327a and is1327b (capital letters in the text) corresponding to translated regions from positions 51 to 366 and 250 to 756, respectively. Accession numbers for the IS elements aligned in this figure are: IS26, X00011; IS240-b, M23740; IS257, X53951. Consensus sequence is illustrated in the bottom line.

several times using subclones derived from two different cosmid clones (pLA150 and pLA484) and by different methods, including non-ambiguous automated *Taq* polymerase sequencing. The artificially corrected version of the TnpA of IS*1327* was compared with most members of the IS*6* family elements. It exhibited the highest amino acid identity (46.2%) to the TnpA of IS*240* from *B. thuringiensis* whereas its identity to the other IS*6* elements was in the range of 38 to 42% (data not shown).

Inverted repeats (IRs) identified at positions 1 to 17 and 796 to 812 (Fig. 2) confined IS*1327* to 812 bp, which is a common size for IS*6* elements. The central nucleotide in the IRs did not match. Comparison of the IRs corresponding to the IS*6* elements listed in Figure 3 demonstrated that 8 to 10 of the nucleotides were conserved among these elements (data not shown). It is noteworthy that the sequence flanking the IRs lacked direct repeats that are characteristic of target site duplication after transposition in several IS*6* elements (Mollet et al. 1983; Polzin and Shimizu-Kadota 1987). Other IS*6* elements such as IS*6100* and IS*240* lack direct repeats in their flanking sequences (Kato et al. 1994; Delecluse et al. 1989). The possible mechanisms for the latter phenomena are discussed by Delecluse et al. (1989).

As indicated earlier, preliminary results exhibited ambiguous hybridization patterns, obtained when probes containing the *etz* and its downstream regions were used (e.g., 5.5-kb *Eco*RI fragment, Fig. 1). The localization of IS*1327* downstream to *etz* suggested that this IS element may be responsible for the observed ambiguity in hybridization analyses. Therefore, it was essential to identify additional copies of IS*1327* and determine their distribution in the *E. herbicola* genome.

Dot blot hybridization analysis of total genomic DNA using an IS-specific probe (Fig. 1) showed that six pathogenic but not eight nonpathogenic strains listed in Table 1 hybridized with IS*1327* (results not shown). Southern blot analysis

Table 1. Bacterial strains, cosmids, and plasmids used in this study

Strain/Plasmid	Relevant characteristics[a]	Reference or source
Escherchia. coli		
DH10B	F′, *mcr*A Δ-(*mrr hsd*RMS-*mcr*BC), ϕ80d*lacZ*ÆM15, Δ*lac*X74, *deo*R, *rec*A1, *ara*D139, Δ(*ara, leu*)7697, *gal*U,*gal*K, l-, rpsL, *end*A1, *nup*G	GIBCO BRL (Gaithersburg, MD)
Erwinia. herbicola		
Eh824-1	Wild-type pathogenic strain, Rif^r, serotype I	Manulis et al. 1991
EhPD713	Wild-type pathogenic strain, serotype I	J. Jense
Eh3-1a	Wild-type pathogenic strain, serotype I	S. Manulis
Eh441	Wild-type pathogenic strain, serotype I	Manulis et al. 1991
Eh350-1	Wild-type pathogenic strain, serotype II	Manulis et al. 1991
Eh1188	Wild-type, beet pathovar	Burr et al. 1991
Eh3-1	Nonpathogenic strain, derived from Eh3-1a	Manulis et al. 1991
Eh717-4	Nonpathogenic strain, serotype I	Manulis et al. 1991
Eh23-9	Nonpathogenic strain, serotype I	Manulis et al. 1991
Eh24-8	Nonpathogenic strain, serotype I	Manulis et al. 1991
Eh27-3	Nonpathogenic strain, serotype I	Manulis et al. 1991
Eh163-5	Nonpathogenic strain, serotype II	Manulis et al. 1991
Eh163-6	Nonpathogenic strain, serotype II	Manulis et al. 1991
Eh112y	Nonpathogenic strain, serotype I	S. Beer
Eh3-106	Km^r, Rif^r, Eh824-1::Tn*5*, non-pathogenic, Tn*5* in the chromosome, a deletion of 40 to 50 kb in pPATH, Δ(*iaa*M, *iaa*H, pre-*etz*, *etz*)	Lichter et al. 1995b
Eh85	Spec^r, Rif^r, Eh824-1::Tn*3*, non pathogenic, deletion of 30 to 50 kb in the pPATH following mutagenesis of the cosmid clone pLA258, Δ(*iaa*M, *iaa*H, pre-*etz*, *etz*)	This study
Cosmids		
pLAFR3	Tc^r, broad host range vector *IncP-1 rlx* RK2$^+$ *lac*Za Tra$^-$ Mob$^+$ cos	Staskawicz et al. 1987
pLA150	Tc^r, a 28-kb cosmid clone of pPATH in pLAFR3	Lichter et al. 1995b
pLA484	Tc^r, a 9-kb cosmid clone of pPATH in pLAFR3	Lichter et al. 1995b
pLA258	Tc^r, cosmid clone derivative of pPATH used as a source for the 10-kb *Eco*RI fragment probe	This study
Plasmids		
pBluescript	Amp^r, subcloning and sequencing vector, (pBS)	Stratagene (La Jolla, CA)
pRK2073	Spec^r, Strep^r, helper plasmid for triparental matings	Ditta et al. 1980
pTn*3*-Spice	Amp^r, Spec^r, clone containing the *ina*Z reporter gene in Tn*3*	Lindgren et al. 1989
pMBL-17	Amp^r, Km^r, pMBL-S3.7(10), Km^r in the *Eco*RI site of the vector	Lichter et al. 1995a
pMBL-RI5.5	Amp^r, a 5.5-kb EcoRI fragment from pLA484 cloned in pBS	Lichter et al. 1995b
pMBK-Sac2.6	Amp^r. a 2.6-kb *Sac*I-*Bam*HI derivative of pMBL-R5.5	This study
pMBL-R.73	Amp^r , 0.73-kb *Eco*RI fragment from pMBL-S3.7, in pBS	Lichter et al. 1995a
pMBL-4	Amp^r, a 2.2-kb *Eco*RI-*Sma*I fragment from pMBL-S3.7 cloned to pBS *Eco*RI-*Eco*RV digest	Lichter et al. 1995a
pMBV-d(#N)	Amp^r, nested deletion derivatives of pMBL-4 (N;3, 2, 34, 7, 1, 35)	This study
pMBL-2B	Amp^r, a 0.7-kb EcoRI-*Kpn*I fragment subcloned from pMBL-4	Lichter et al. 1995a
pMBL-23B	Amp^r, a 0.6-kb *Eco*RI-*Alu*I cloned from pMBL-2B	Lichter et al. 1995a
pMBL-T4-1	Amp^r, a 1.3-kb *Eco*RI-*Nco*I fragment from pMBL-4 by deletion of a *Hin*dIII-*Nco*I fragment	This study
pMBL-T4-2	Amp^r, a 1.1-kb *Eco*RI- *Nde*I fragment from pMBL-T4-1, deletion of *Nde*I-*Xho*I fragment	This study
pMBL-T4-3	Amp^r, a 0.9-kb *Eco*RI-*Bst*EII fragment from pMBL-T4-1, deletion of *Bst*EII-*Xho*I fragment	This study
pMBL-T4-4	Amp^r, a 0.8-kb *Eco*RI-*Hinc*II fragment from pMBL-T4-1, deletion of a *Hinc*II fragment	This study

[a] Antibiotics were used in the following concentrations (μg per ml): Ampicillin (Amp) 100; Kanamycin (Km) 50; Spectinomycin (Spec) 50; and Rifampicin (Rif) 100.

of the pathogenic strains (Eh1188; Eh824-1) and the nonpathogenic strains (Eh3-1; Eh717-4) confirmed that IS*1327* was exclusively present in pathogenic strains (Fig. 4, lanes a to d, respectively). One of the two nonpathogenic strains analyzed (Eh3-1) was obtained by spontaneous loss of the pPATH from Eh3-1a, suggesting that IS*1327* may be specific to pPATH.

Southern analysis of *Eco*RI-*Hin*dIII digested genomic DNA revealed five hybridization bands in Eh824-1 (Fig. 4, lane b). An identical pattern was obtained for other *E. herbicola* pv. *gypsophilae* strains analyzed (Eh3-1a; EhPD713; Eh350 and Eh441, results not shown). A beet pathovar (Eh1188) of *E. herbicola*, which also incites galls on gypsophila (Burr et al. 1991), exhibited a completely different pattern of four hybridization bands (Fig. 4, lane a). *Sma*I digestion of Eh824-1 plasmids indicated the presence of four hybridizing bands: two bands at 2.6 and 3.7 kb and two high molecular weight bands (Fig. 4, lane f). Several fragments detected in the Southern blot exhibited stronger intensity than other similar-sized fragments (i.e., the 3-kb fragment in lane a; the 6.1-kb fragment in lane b; the 3.7-kb and the higher molecular weight fragments in lane f). These fragments may contain more than one IS copy assuming that all copies of the element are intact.

Two nonpathogenic deletion mutants of pPATH were analyzed by Southern hybridization to study their IS*1327* content. Eh3-106 is a derivative of Eh824-1 that was previously found to have a deletion of approximately 50 kb in pPATH. This deletion included the genes for IAA and cytokinin biosynthesis (Lichter et al. 1995b). Eh85 is an additional nonpathogenic mutant with a deletion in the pPATH that was obtained following Tn*3* mutagenesis (Lindgren et al. 1989) of a cosmid clone (pLA258) derived from pPATH of Eh824-1 (Lichter et al. 1995b). Eh85 possess a deletion of pPATH in a similar but not identical region to the deletion of Eh3-106 (results not shown). The IS*1327* probe hybridized with two and three bands corresponding to DNA of Eh85 and Eh3-106, respectively (Fig. 4, lanes e, g, and h). Both Eh3-106 and Eh85 lacked several hybridization bands compared with plasmid digests of the wild-type strain including the 6.1-kb fragment of pLA150 containing the *etz* and its adjacent IS*1327* element (Fig. 1). Eh3-106 contained a new 10- to 11-kb *Sma*I fragment that could arise from either the deletion event or from active transposition. The hybridization pattern obtained for Eh3-106 and Eh85 indicated that the missing IS*1327* copies were part of the deleted portion of pPATH. Our previous results demonstrated that the deleted pPATH of Eh3-106 did not hybridize with the other plasmids or the chromosomal fraction of this strain (Lichter et al. 1995b). Since this plasmid hybridized with IS*1327* (results not shown), it appears that IS*1327* is exclusively present on pPATH in strain Eh824-1.

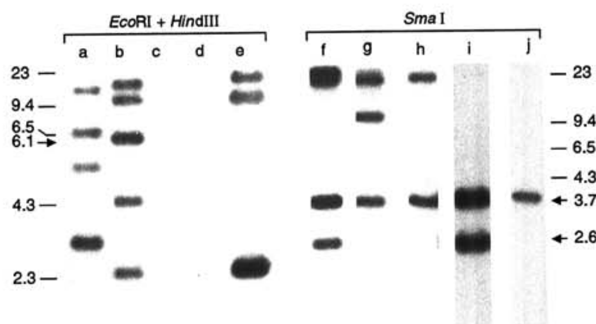

Fig. 4. Distribution of IS*1327* in *Erwinia herbicola*. Southern blot hybridization analysis of the DNA from *E. herbicola* was conducted by using the IS*1327*-specific probe (Fig. 1). The template for digoxigenin (DIG)-labeled probe (Boehringer GmbH, Mannheim, Germany) corresponding to IS*1327* was isolated and prepared by digesting pMBL-T4-1 with *Kpn*I located in the upstream region of IS*1327* and *Xho*I of the multi-cloning site of pBluescript to yield a fragment of 0.62 kb. The genomic DNA (prepared according to Ausubel et al. 1989) in lanes a through e was digested with *Eco*RI and *Hin*dIII, and plasmid DNA (prepared according to Kado and Liu 1981) in lanes f through j was digested with *Sma*I. **A,** Eh1188 (beet pathovar); **B,** and **F,** Eh824-1 (gypsophila pathovar); **C,** Eh3-1 and **D,** Eh717-4 (nonpathogenic strains); **E,** and **G,** Eh3-106; **H,** Eh85 (the latter two strains are deletion derivatives of pPATH); **I,** pLA150 in *Escherichia coli*; **J,** Eh824-1 hybridized with a DIG-labeled *etz*-specific probe prepared from pMBL-R.73 digested with *Eco*RI (Lichter et al. 1995a). The location of size markers from λ–*Hin*dIII DNA digest is illustrated at the left and right of the figure. Fragments of the sizes 6.1, 2.6, and 3.7 kb (indicates by arrows) were assigned to pLA150.

The linkage between the cytokinin biosynthesis gene (*etz*) and IS*1327*-L on one hand, and the multiplicity of the IS element on the other hand, raised the possibility that *etz* is linked to additional IS copies. To resolve this question, the *Sma*I blot (Fig. 4, lanes f, j) that was hybridized with the IS*1327* probe was stripped and re-hybridized with an *etz*-specific probe composed of the *Eco*RI fragment of 0.73 kb (Lichter et al. 1995a). Results obtained showed that this probe hybridized only with the 3.7-kb *Sma*I fragment of Eh824-1 (Fig. 4, lane j) and pLA150 (results not shown). Thus, Eh824-1 contained a doublet of 3.7-kb *Sma*I bands, one of which harbors the *etz* and its adjacent IS*1327* copy and an additional fragment possessing another copy of the IS element. In addition, this *etz*-specific probe hybridized only with the 6.1-kb *Eco*RI-*Hin*dIII fragment marked in Figure 4, lane b (results not shown). It was therefore demonstrated that *etz* had a single copy in the plasmids of *E. herbicola* pv. *gypsophilae,* in spite of its proximity to the multi-copy IS*1327* element.

According to the *Sma*I digestions, the minimal number of IS*1327* copies on Eh824-1 was five. Assuming that the higher-molecular-weight band that had a stronger hybridization intensity contained two IS copies, the total number of the IS elements would be six. This conclusion is in accordance with *Eco*RI-*Hin*dIII digestion, which had demonstrated the presence of five distinct hybridization bands. However, as will be demonstrated below, the 6.1-kb fragment that corresponds to the central region of pLA150 was shown to contain two copies of IS*1327*. Since neither of the above-mentioned restriction enzymes contains sites in the sequence-characterized IS*1327* element, these results indicate six copies of IS*1327* are present in *E. herbicola* pv. *gypsophilae*. The beet pathovar of *E. herbicola* contained only four fragments hybridizing with IS*1327*, one of which had stronger hybridization intensity.

The *Sma*I DNA digestion of pLA150 contained two hybridization bands (2.6 and 3.7 kb, respectively; Fig. 4, lane i) corresponding in size to those of Eh824-1, suggesting the presence of two IS elements on this cosmid. As indicated earlier, the first copy was located on the 3.7-kb *Sma*I fragment adjacent to the *etz* whereas a second copy was located farther downstream. The second copy of IS*1327* was confined to a

1.5-kb *Sma*I-*Bam*HI fragment (Fig. 1). Confirmation of the location and orientation of the additional IS*1327* copy on pLA150 was obtained by amplification of the element in pMBK-Sac2.6, using polymerase chain reaction (Fig. 1). Primer 625 generated from the sequence of IS*1327* (5′-TGTCGTTGATCATTCCAC-3′; corresponding to positions 194 to 211 in the sequence) was used together with the universal primers T-3 and T-7 of pBluescript. A 1.3-kb amplification product was obtained with 625-T3 primers combination indicating a direct orientation between the two copies. The two IS copies were separated by 2.8 kb of intervening DNA sequence. Primer 625 was also used for partial sequencing of IS*1327*-R from pMBK-Sac2.6. Results indicated that the two IS*1327* copies were identical over 216 bases (positions 231 to 447 in Figure 2) except a T residue that may replace a C residue that is present in IS*1327*-L at position 379. The apparent identity between the two ISs suggests that the shift in reading frames between the similar sequences is not unique to IS*1327*-L and may be a characteristic of IS*1327*.

Northern blot hybridization analysis was performed on RNA from Eh3-106, Eh824-1, Eh824-1/pMBL-17, and pMBL-17 in *Escherichia coli* using the [^{32}P]-labeled IS*1327* DNA probe.

pMBL-17 contained IS*1327*-L on a 3.7-kb *Sma*I fragment as well as the *etz* locus (Lichter et al. 1995a). Results in Figure 5 showed the occurrence of a 0.75-kb transcript for the *E. herbicola* strains. Eh824-1, with or without pMBL-17, contained an additional transcript of approximately 0.64 kb. Eh824-1/pMBL-17 contained additional bands (e.g., 1.3 kb) and its hybridization intensity was much stronger than that of the other strains. Interestingly, no hybridization was observed with RNA of *E. coli* harboring pMBL-17. The foregoing results indicated a transcriptional activity of IS*1327* in *E. herbicola* as well as the specific transcription of IS*1327*-L. The transcript size of 0.75 kb corresponds to the size of the IS*1327* TnpA, which is 0.72 kb.

It has been previously demonstrated that pathogenicity of *E. herbicola* pv. *gypsophilae* is governed by the presence of pPATH (Manulis et al. 1991) that harbor genes encoding virulence determinants (Clark et al. 1993; Lichter et al. 1995a). Several lines of evidence support the notion that IS*1327* is present only on pPATH: (i) IS*1327* was identified in pathogenic but not nonpathogenic strains that lack pPATH; (ii) a DNA probe containing IS*1327* did not hybridize to the chromosomal fraction or to plasmids other than pPATH in the pathogenic strain Eh824-1; and (iii) two IS*1327* copies were characterized in a cosmid derivative (pLA150) of pPATH and several copies (three or four) were missing in deletion derivatives of this plasmid.

The multiplicity and plasmid-specificity of IS*1327* to pPATH is comparable to that of other IS*6* elements such as IS*240* and ISS*1* (Delecluse et al. 1989; Polzin and Shimizu-Kadota 1987) . The dispersal of IS*1327* on pPATH may serve as a target site for recombinations leading to deletions as well as to other rearrangements. The apparent specificity of IS*1327* to pPATH could result from its inability to transpose to other plasmids or to the chromosomal DNA. Alternatively, IS*1327* may have been actively distributed on pPATH prior to its acquisition by *E. herbicola,* in which it became nonactive. At present we do not have evidence to support a current transposition of IS*1327* although IS*1327*-L as well as other copies are transcribed and their transcripts may accommodate the TnpA size.

The lack of a complete ORF corresponding to the TnpA could be the reason that this IS element is nonactive in *E. herbicola.* The fact that IS*1327*-R was identical to IS*1327*-L in the region analyzed, including the frameshift site, may be examined in the light of two hypotheses: (i) that the two IS elements were duplicated by a nontranspositional event, or (ii) that IS*1327* can transpose following mutation suppression by a process such as translational frameshifting. This process has been identified in several retrotransposons, retroviruses, a few cellular genes, and bacterial insertion sequences from the IS*3* superfamily and IS*1* (Chandler and Fayet 1993; Engelberg and Schoulaker 1994) .

Detailed research is required to determine if the polymorphism in fragment size and copy number of IS*1327* between the gypsophila and beet pathovars of *E. herbicola* (Fig. 4) could result from active transposition after segregation of the pathovars or plasmids rearrangements. Nevertheless, the presence of IS*1327* in both strains as well as the apparent conservation of the phytohormone gene cluster (Lichter et al. 1995a) supports the common origin of the pPATH in both pathovars.

IS*1327* could have been instrumental in horizontal gene transfer of virulence determinants to and from *E. herbicola.* The transfer of the IAA biosynthesis genes in *P. syringae* pv. *savastanoi* and *A. tumefaciens* was assumed to be mediated

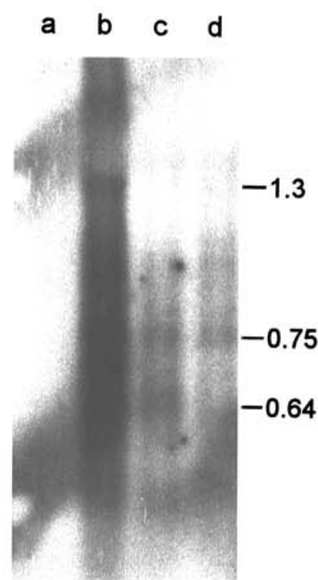

Fig. 5. Northern blot hybridization analysis of IS*1327*. RNA was extracted from bacterial cultures grown for 10 h and hybridized with the IS*1327*-specific probe (Fig. 1). RNA was extracted from: **A,** *Escherichia. coli*/pMBL-17; **B,** Eh824-1/pMBL-17; **C,** Eh824-1; **D,** Eh3-106. RNA isolation and Northern hybridization were performed as previously described (Lichter et al. 1995a). An IS*1327*-specific probe was composed of the same fragment used for Southern hybridizations and the probe (100 ng) was labeled by means of the Prime-a-Gene random primer kit (Promega, Madison, WI) and [α^{32}P]dATP (Rotem, Dimona, Israel). A 0.73-kb *Eco*RI fragment containing portions of the pre-*etz* and *etz* in pBluescript was used to generate a 0.8-kb in vitro transcription product, which was used as a size marker (Lichter et al. 1995a). This marker together with the ribosomal RNAs visualized in the ethidium bromide–stained gel were used to estimate the size of the transcripts.

by IS*51* and its homologous IS*868* element (Yamada et al. 1986; Paulus et al. 1991). The fact that IS*1327*-L was located adjacent to the *etz* could imply that a different IS element was responsible for horizontal transfer of the phytohormone biosynthesis gene cluster in *E. herbicola*. Characterization of IS*1327* that is linked to virulence determinants is vital for understanding the genomic structure of the pPATH as well as for elucidating the evolutionary processes leading to the creation of a pathogenic bacterium from an epiphyte.

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