Symbiosis, 8 (1990) 21–31
Balaban, Philadelphia/Rehovot

Repeated Sequences in *Pseudomonas syringae* pv. *phaseolicola*; Distribution and Possible Function as Insertion Sequences

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Received October 25, 1989; Accepted November 29, 1989

Abstract

The distribution of the repeated sequences RS-I, RS-II and RS-III in different pathovars of *Pseudomonas syringae* was determined. The presence of the different repeated sequences correlates roughly with the division into pathovars. The nucleotide sequence of a fragment that has been defined as the prototype of RS-I was determined. The sequenced fragment showed partial homology to a DNA fragment from *P. syringae* pv. *phaseolicola* that was found transposed into the *sacB*-gene of the broad host range cosmid pUCD800.

Keywords: *Pseudomonas syringae*, repeated sequence, insertion sequence, distribution

1. Introduction

Repeated sequences (RS) and insertion sequence elements (IS elements) have been observed in a number of different prokaryotic organisms, including both Gram-negative and Gram-positive eubacteria (for a review see Berg and Howe, 1989), as well as in the archaebacteria (Sapienza et al., 1982; Fishman et al.,

0334-5114/90 /$03.00

1985). Some IS elements have been shown to mediate insertion and deletion mutations, replicon fusions, and activation of gene expression (Comai and Kosuge, 1983; Gay et al., 1985; Scordilis et al., 1987; Kearney et al., 1988; Tomasek et al., 1989; Haugland et al., 1989; Bartlett and Silverman, 1989). Repeated sequences have also been implicated as sites for homologous recombination that lead to the production of deletions (Kaluza et al., 1985), gene duplications and translocations (Negoro et al., 1983), as well as to the integration of plasmids into the bacterial chromosome and subsequent formation of excision plasmids (Barsomian and Lessie, 1986; Szabo and Mills, 1984b; Poplawsky and Mills, 1987; Ehrenshaft et al., submitted).

RS and IS elements also have the potential to mediate translocation of genetic material between replicons, and in combination with conjugation, also between bacterial strains. This phenomenon has been observed with the IS-elements of the F-plasmid of *Escherichia coli* (reviewed by Willets and Skurray, 1986), and has recently been suggested to occur in *Pseudomonas cepacia* (Haugland et al., 1989). Cryptic plasmids found in numerous strains of phytopathogenic bacteria (Ulaganathan and Mahadevan, 1985) often carry multiple copies of repeated sequences (Barsomian and Lessie, 1986; Quant and Mills, 1984) that can function as IS-elements (Yamada et al., 1986).

P. syringae pv. *phaseolicola*, the causal agent of halo blight of bean (*Phaseolus vulgaris* L.), carries repeated sequences in the indigenous plasmic pMMC7105, as well as in the chromosome. A repeated sequence, RS-I, has been detected only in pMMC7105 and not in the host chromosome, whereas two other repeated sequences, RS-II and RS-III have been found both in the plasmid and the chromosome (Szabo and Mills, 1984b). RS-II has been shown to promote integration of the plasmid into the chromosome through homologous recombination between copies of RS-II in the plasmid and the chromosome (Ehrenshaft, 1986), whereas all three appear to be sites at which homologous recombination occurs during the formation of excision plasmids, which occur in various sizes (Szabo and Mills, 1984b; Poplawsky and Mills, 1987; Ehrenshaft et al., submitted). Some of the plasmids resulting from imprecise excision contain only sequences from pMMC7105, whereas others contain chromosomal and plasmid sequences (Szabo and Mills, 1984a).

2. Materials and Methods

Bacterial strains, plasmids and growth media

P. syringae pv. *phaseolicola* LR700 carries the cryptic plasmid pMMC7105 (formerly pMC7105; Curiale and Mills, 1982). Strain LR781 is a rifampicin resistant derivative of LR700. *E. coli* HB101 (Maniatis et al., 1982) was used as the host for cloning fragments containing the repeated sequences RS-I, RS-II, and RS-III into pBR322, for use as molecular probes to determine their distribution. Plasmid pOSU2103 contains a 543 base pair *Bgl*II-*Bam*HI fragment from pMMC7105 that contains RS-I, which is the prototype for this repeated sequence. Plasmid pOSU2480 carries a 480 base pair *SstI* fragment internal to RS-II (Ehrenshaft, 1986). Plasmid pOSU0010 carries a 1.9 kb *Sal*I-*Bam*HI fragment from *Bam*HI-10 of pMMC7105 that has homology with RS-III (Mills et al., 1987). The broad host-range cosmid pUCD800 was used as an entrapment device for transposable elements as described by Gay et al. (1985).

Luria-Bertani (LB) broth was used as the growth medium for *E. coli* (Maniatis et al., 1982). *P. syringae* pv. *phaseolicola* was grown at 28°C in MaNY medium as described earlier (Curiale and Mills, 1982), and solid media which contained 1.5% agar. Colonies resistant to sucrose were selected in MaNY agar plates supplemented with 5% sucrose. Kings medium B was used to test for fluorescent pigment production by *P. syringae* pv. *phaseolicola* (King et al., 1954). Ampicillin (150 mg/ml), kanamycin (50 mg/ml) and rifampicin (100 mg/ml) were added to the media when required.

Triparental mating

Plasmids were transferred between strains by triparental mating as previously described (Poplawsky and Mills, 1987), using plasmid pRK2013 in *E. coli* HB101 as the conjugation helper plasmid (Ditta et al., 1980).

DNA manipulations and sequencing

Plasmids were isolated from *E. coli* by an alkaline lysis method (Maniatis et al., 1982), and from *Pseudomonas* as described previously (Curiale and Mills, 1977). Restriction enzymes and T4 ligase were used according to manufacturers' recommendations. Whole cell DNA and plasmid DNA preparations were digested with restriction enzymes and analyzed in agarose gels using standard laboratory procedures (Maniatis, 1982). DNA fragments separated by gel electrophoresis in low melting point agarose were excised and isolated from the agarose by two rounds of freezing and thawing, followed by phenol-chloroform extractions and ethanol precipitation in the presence of 2M NH_4Ac. The fragments were suspended in water and either radiolabelled for use as DNA probes, or ligated into cloning vectors for restriction analysis and sequencing. DNA fragments separated by agarose gel electrophoresis were transferred to nylon hybridization filters by capillary blotting (Southern, 1975). Probe DNA was labeled by nick translation using [α-^{32}P]dCTP, and the hybridization reactions were carried out as described previously (Ehrenshaft et al., submitted).

DNA fragments were subcloned into pUC18 and pUC19 for restriction analysis and into M13 mp18 and mp19 for sequencing (Yanisch-Perron et al., 1985). The nucleotide sequences were determined by the Sanger dideoxy method (Sanger, 1977) using Sequenase (United States Biochemical) and [^{35}S]dATP (New England Nuclear). The nucleotide sequences were analyzed with the PC/Gene program package (Genofit SA).

3. Results

Distribution of RS elements

Total DNA from a variety of *P. syringae* pathovars was isolated, digested with *Hin*dIII and *Eco*RI in separate reactions and separated in an agarose gel. A Southern blot of the gel was hybridized in separate experiments with the plasmids containing RS-I, RS-II and RS-III (Table 1). The three repeated sequences were found in 4 of 5 strains of pv. *phaseolicola*, in pv. *adzakicola*, and in one strain of pv. *tomato*. None was found in pv. *pisi*, and it is interesting that, with the exception of RS-III, they were not found in strains of pv. *syringae* that attack bean. Moreover, some strains had only one or two of the repeated sequences, but not all three.

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                  10          20          30          40          50
                  |           |           |           |           |
      BglII
    1 AGATCTGTCG TTTCATCCAT CAGCCTTGCG TCGGCGCTGG ATGTGGAATG

   51 TACGCCAGTC CCTGCTCAGT CAGTGGGAGC ACACCACCGT CCCACCTGAA
            PstI
  101 AACGCTCATC TGCAGAGCGA AAATGACTGG CGTCACCTTG TGCTCAACGC
                                                    XmaIII
  151 CGGTGGTCAG CACTGGCACA TCCACTTGTC GAAGAAGACG AAAAACGGCC
                                                        AvaI
  201 GAAAGACCGT CAATTACCTG GGCCGCTACC TGAAAAAACC GCCCATCTCG

  251 GGCAGTTGTC TGGCGCATTA CACCAACGGG GCCACGTTGA GCTTCACCTA

  301 CCTGGATCAC CGCACACAGA CCTATCAGCA GGAAACGCTG AGCCAGGCCG

  351 ACATGCTTTT CCGGGTGGTG CAGCACATCC CGGAGAAGCA CTTTCGGATG
                          BalI
  401 ATCCGGTATT TTGGATTTCT GGCCAACCGC GTCTGTGGCC GACAGCTACC
      SmaI
  451 CCGGGTGTAT GAGGCCGTAC GCATGGAAAG GCGTGGCAAA GCGCAAAAAC
                                                  BamHII
  501 TGTATTTTGC GCAGATGAGC AAAGCGTTCT TGCATCGGGA TCC
```

Figure 1. Nucleotide sequence of the *Bam*HI-*Bgl*II fragment from pMMC7105 of *Pseudomonas syringae* pv. *phaseolicola* that contains the repeated sequence RS-I.

Table 1. Distribution of repeated sequences, RS-I, RS-II and RS-III in pathovars of *Pseudomonas syringae*

	Host plant	Homology to: RS-I	RS-II	RS-III
Pseudomonas syringae pv.				
adzakicola	Adzaki bean	+	+	+
coronafaciens	Oat	–	–	–
glycinea	Soybean	+	+	+
morsprunorum LR920	Stone fruits	–	+	ND
phaseolicola LR700	Bean	+	+	+
phaseolicola PP601	Bean	+	+	+
phaseolicola PP631	Bean	+	+	+
phaseolicola PP652	Bean	+	+	+
phaseolicola 9	Bean	–	–	+
philadelphii B	Mock orange	–	–	ND
philadelphii D	Mock orange	+	–	+
pisi	Pea	–	–	–
striafaciens	Oats	–	+	+
syringae R32	Bean	–	–	–
syringae J900	Bean	–	–	–
syringae Y30	Bean	–	–	–
syringae 11/81	Bean	–	–	+
syringae 84-43	Bean	–	–	–
syringae	Wheat	–	–	–
tabaci	Tobacco	+	–	+
tomato 8	Tomato	–	–	–
tomato 5796-36	Tomato	+	+	+
Pseudomonas marginalis LR900	Lettuce	–	–	–

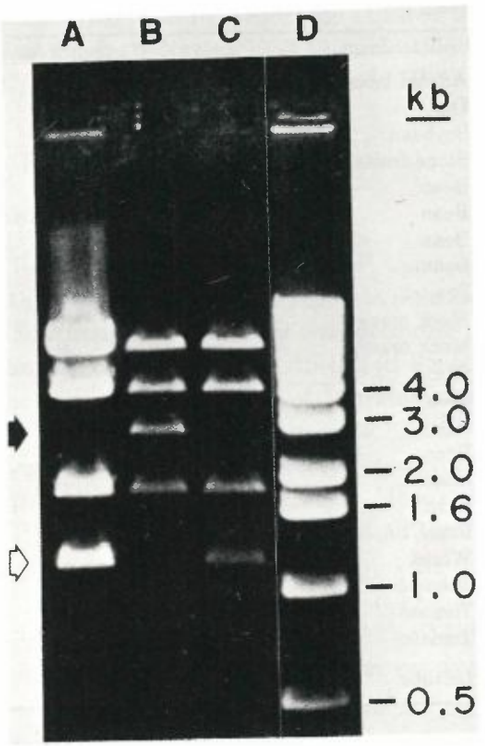
\+ hybridization signal
– no hybridization signal
ND not determined. See Materials and Methods for probes used in these analyses

Nucleotide sequence of RS-I

Plasmid pOSU2103 carries a *Bam*HI-*Bgl*II fragment subcloned from *Bam*HI fragment 8 of pMMC7105 that was previously designated the prototype for RS-I because it was initially shown to have homology with 6 of the 19 *Bam*HI fragments of pMMC7105 (Szabo and Mills, 1984b). The size of this fragment was determined to be 543 bp and it has no features or sequences indicative of known insertion sequence elements (Fig. 1).

Generation of sucrose-resistant insertion mutants

The broad host range cosmid pUCD800 was transferred from *E. coli* HB101 into *P. syringae* pv. *phaseolicola* strain LR781 by triparental mating, and a



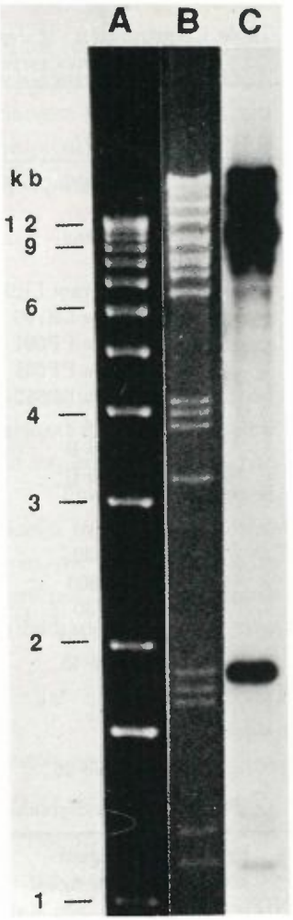


Figure 2. Agarose gel of plasmid pUCD800 and a derivative containing an insertion from the genome of *Pseudomonas syringae* pv. *phaseolicola* strain LR781. Lane A, pUCD800; Lane B pUCD800 isolated from a sucrose-resistant cell with an insertion; Lane C, pUCD800 isolated from a sucrose-sensitive cell; Lane D, DNA size standards. Preparations were digested with *Hin*dIII and *Eco*RI. The arrows indicate the fragment that has increased in size from 1.1 kb (open arrow) to 2.6 kb (closed arrow).

Figure 3. Restricted plasmid pMMC7105 separated by agarose gelelectrophoresis. A, DNA size standard; B, the plasmid digested with *Bam*HI; C, autoradiogram of a blot of lane B probed with a *Dra*I fragment containing the pUCD800-insert (lane B in Fig. 2).

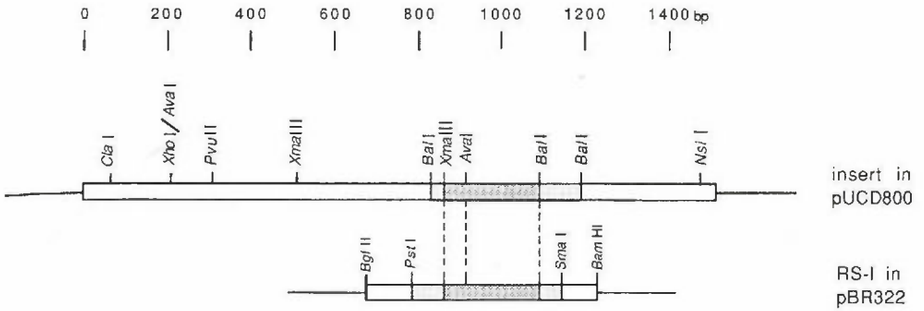


Figure 4. Partial restriction map of the insert DNA in pUCD800 and a homologous region in RS-I. The homologous area is indicated by dark shadowing and the area to the first restriction site that differs is indicated by light shadowing. The lower limit of homology is 226 bp and the upper limit is 300 bp.

fluorescent, kanamycin- and rifampicin-resistant, sucrose-sensitive strain was used for entrapping transposable elements. A culture was grown to saturation in LB supplemented with kanamycin, and 0.1 ml of the culture was plated onto LB plates supplemented with kanamycin and 5% sucrose. The plates were incubated at 30°C and sucrose-resistant colonies appeared after 4 or 5 days at a frequency of approximatcly 10^{-7}. The DNA from six sucroseresistant colonies was extracted and used to transform HB101 to facilitate the analysis of the putative mutated pUCD800 plasmid, and to eliminate confusion inherent in analyzing fragments in the presence of the indigenous plasmid pMMC7105. The *E. coli* transconjugants were tested for acquired resistance to sucrose, and pUCD800 was isolated from selected colonies. One of the colonies contained a pUCD800 derivative that had an inserted sequence in the *sacB* gene. The internal *Eco*RI *Hin*dIII fragment had increased in size by approximately 1.5 kb (Fig. 2). The insert with flanking *sacB* DNA was subcloned as a *Dra*I fragment into pUC18 radiolabelled and used to probe a Southern blot of *Bam*HI-digested pMMC7105 DNA (Fig. 3). The probe hybridized to the same six fragments as were previously shown to hybridize to RS-I (Szabo and Mills, 1984b). The vector pUCD800 shows no homology to pMMC7105 (not shown).

A restriction map of the insert in pUCD800 was constructed. By comparing the restriction map to the RS-I sequence the area of homology could be localized to a 226 bp *Xma*III – *Bal*I fragment (Fig. 4). The *Bal*I site next to the *Xma*III site in the pUCD800 insert is not present in the RS-I sequence and the *Sma*I site in RS-I is absent in the insert giving an upper limit to the homologous area of 300 bp. Thus it appears that only an internal portion of the 543 bp *Bgl*II-*Bam*HI fragment that contains the repeated sequence RS-I has homology to the sequence that inserted into pUCD800.

4. Discussion

The repeated sequence RS-I shows no features or sequences indicative of its being an IS-element, and moreover, it appears to be too small to constitute a complete element. The partial homology between RS-I and the element that inserted into the *sacB* gene suggests that RS-I is a subfragment of a larger unit that is transposable. Confirmation of the pUCD800 insert as an IS-element awaits sequencing of the insert containing fragment. Whether most of the other sites in pMMC7105 that have homology to RS-I represent the whole transposable element or merely subfragments of it is not known. However, the restriction pattern of some of these regions within pMMC7105 appear to differ somewhat from sequences both in RS-I and the inserted DNA in the *sacB* gene (unpublished data).

The genetic rearrangements mediated by repeated sequences and IS-elements in different species of *Pseudomonas* can lead to the modulation of expression of genes that affect the fitness of the bacterium (Kearney et al., 1988; Tomasek et al., 1989; Haugland et al., 1989; Bartlett and Silverman, 1989; Yamada et al., 1986).

Some repeated sequences in the various pathovars of *P. syringae* are found only on indigenous cryptic plasmids that might simply function as reservoirs for RS and IS elements. Interestingly the element which has inserted into pUCD800 has in the strain LR700 been detected only in pMMC7105 and not in the bacterial host chromosome (Szabo and Mills, 1984b).

Although RS-I, RS-II and RS-III are linked to pMMC7105, the presence of only one or two of these sequences in some strains (Table 1) suggests that if they were initially linked to a plasmid, they have become spatially separated by various recombination mechanisms during evolution. Conversely, they could have become linked to a plasmid (e.g. pMMC7105) by transposition. If in future studies, specific repeated sequences are found to be uniquely present in one or a few related pathovars of *P. syringae*, they could prove useful as diagnostic probes for the identification of the pathovars. The limited distribution of a given RS element among pathovars might reflect a close taxonomic relationship. On the other hand, if they are predominantly carried on conjugative plasmids or plasmids that are mobilizable, more distantly related strains could harbor similar repeated sequence DNA. The prerequisite for their wide distribution might then only be that the plasmids are able to replicate in the new host, or that the elements on a transient plasmid are capable of transposition into the host cell chromosome or its indigenous plasmids.

Acknowledgements

This work was supported in part by Science and Education Administration (USDA) grant number 85-CRCR-1-1771 from the Competitive Research Grants Office and grant number MB8315689 from the National Science Foundation awarded to D.M. and by the Oregon State University Agricultural Experiment Station from which this is technical paper no. 9093. M.R. was supported by a long term fellowship from the European Molecular Biology Association, and by a grant from the Ministry of Agriculture and Forestry of Finland. We thank Ulla Lehtinen, MSc for critical reading of the manuscript.

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