Restriction Enzyme Analysis of Plasmids from Syringomycin-Producing Strains of *Pseudomonas syringae*

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ABSTRACT

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Plasmids of similar molecular mass were isolated from millet, almond, and apricot strains of *Pseudomonas syringae*. All three strains produced the phytotoxin syringomycin and contained a single plasmid with a mass of ~35 megadaltons. Restriction endonuclease digestion showed that each plasmid contained from four to thirteen fragments, depending on the plasmid and enzyme combination. Agarose gel analysis of *Eco*R1 digest showed that the three plasmids had one fragment (1.3 megadaltons) in common. The plasmids from the millet and almond strains also had 8.6- and 1.2-megadalton fragments in common, while the almond and apricot plasmids had a 1.7 megadalton fragment in common. *Hin*d III digests showed that the plasmids of the millet and almond strains had a 2.1-megadalton fragment in common. Thus, the three plasmids showed different restriction endonuclease digest patterns.

Additional key word: maize

Bacterial plasmids carry a wide variety of genetic determinants which provide additional survival potential (1,6). This additional genetic information may contribute to the bacterium's ability to produce disease (7,13,15).

Previously we reported the association of a 35-megadalton plasmid, pCG131, with production of the phytotoxin syringomycin by a strain of *Pseudomonas syringae* (isolated from millet) which causes holcus spot of maize. In addition to toxin production, resistance to bacteriocin PSC-1B and phages Psp1 and Psy4A also were plasmid-associated properties (5). Subsequently, agarose gel analyses of 13 other syringomycin-producing strains of *P. syringae* from various hosts showed the presence of one to two plasmids in six of the strains (4). Two strains, analyzed further in this paper, contained plasmids with mass similar to that of pCG131. The question arose: were the plasmids identical or related? Relatedness can be determined by restriction enzyme analysis. In this technique, DNA is digested with restriction endonucleases to generate unique fragments; these, in turn, can be fractionated according to size in agarose gels. Similar fragment patterns are evidence of relatedness(12).

In this paper, evidence is presented that plasmids of the same molecular mass from three syringomycin-producing strains of *P. syringae* from different hosts are not identical. Each of the plasmids was distinguishable on the basis of gel fragment patterns produced after digestion with the restriction enzymes *Eco*R1 and *Hin*d III.

MATERIALS AND METHODS

Bacterial strains. Bacteria used in this study are listed in Table 1.

Medium. Medium NBY (14) was prepared as described previously.

Isolation of plasmid DNA. DNA for restriction enzyme analysis was prepared from one liter of cells grown in NBY to an A_{640nm} of 0.8 (Beckman Spectronic 20 spectrophotometer, Beckman Instruments, Fullerton, CA 92632). Lysis of cells, concentration of plasmid DNA, and CsCl-ethidium bromide gradients were performed as described by Currier and Nester (3). Equilibrium density gradient centrifugation was carried out at 15 C in a Spinco Ti50 fixed-angle rotor for 48 hr at 40,000 rpm in a L3-50 Beckman ultracentrifuge. After location of the plasmid band by illumination with a UV light, plasmid DNA was removed by piercing the tube with a 1.024-mm (18-gauge) needle and removing the denser band with a syringe. The DNA was then recentrifuged to equilibrium in a second ethidium bromide-CsCl gradient. The super-coiled DNA from the second centrifugation again was collected with a syringe. Ethidium bromide was removed by several extractions with CsCl-saturated isopropanol. The DNA samples were dialyzed against TNE buffer (0.1 M Tris [hydroxymethyl] aminomethane, 0.05 M NaCl and 0.005 M ethylenediaminetetraacetic acid, pH 7.5) and an A_{260nm} was measured to determine concentration. Thirty μg/ml of yeast transfer-RNA (Miles Laboratories, Elkhart, IN 46515) was added to each of the samples to improve recovery of DNA; these were adjusted to 0.3 M potassium acetate and precipitated with two volumes of 95% ethanol. The DNA was resuspended in TNE buffer

TABLE 1. Bacterial strains of *Pseudomonas syringae* and *Escherichia coli* used for plasmid isolation

Strain	Relevant characteristic[a]	Origin	Host
P. syringae			
HS191 (pCG131)[b]	SR^+, pCG131	D. Gross[c]	millet
B15 (pCG111)	SR^+	J. DeVay[d]	almond
5D425 (pCG112)	SR^+	D. Gross[c]	apricot
E. coli			
J53 (RSF1030)	RSF1030 (5.5×10^6 daltons)	S. Falkow[e]	...
J53 (R1*drd* 19)	R1*drd* (62×10^6 daltons)	S. Falkow[e]	...
J53 (RP1)	RP1 (39×10^6 daltons)	R. Olsen[f]	...

[a] SR^+ = syringomycin producer.
[b] Figure in parentheses indicates resident plasmid.
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[e] Department of Microbiology, University of Washington.
[f] Department of Microbiology, University of Michigan.

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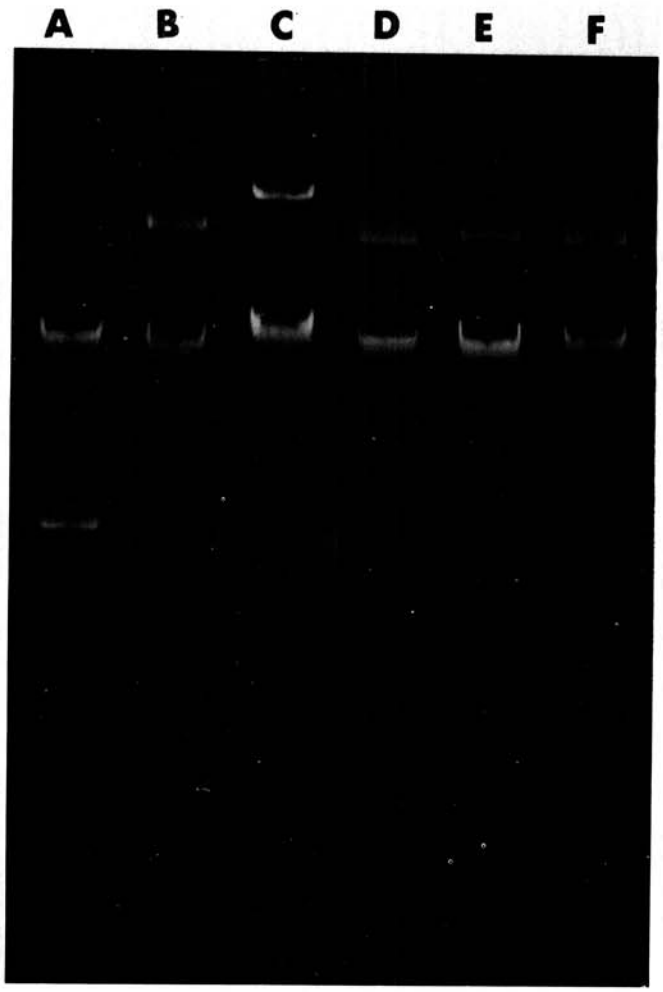

Fig. 1. Agarose gel electrophoresis of ethanol-precipitated plasmid DNA from lysates of *Escherichia coli* and *Pseudomonas syringae*. (A) *E. coli* J53 (RSF1030), 5.5×10^6 daltons. (B) *E. coli* J53 RP1, 39×10^6 daltons. (C) J53 (R1*drd*19) 62×10^6 daltons. (D) *P. syringae* HS191 (pCG131) 35×10^6 daltons. (E) *P. syringae* B15$^+$ (35×10^6 daltons) cryptic plasmid. (F) *P. syringae* 5D425 (35×10^6 daltons) cryptic plasmid. Chromosomal DNA migrates to the same position for all strains.

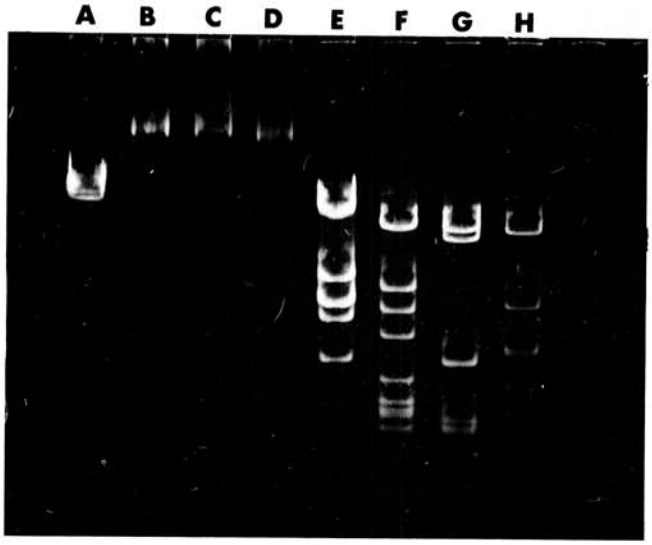

Fig. 2. Agarose gel electrophoresis of *Eco*R1 digests of λ-DNA and *Pseudomonas syringae* plasmid DNA. (A) untreated λ-DNA; (B) untreated pCG131; (C) untreated pCG111; (D) untreated pCG112; (E) *Eco*R1-digested λ-DNA; (F) *Eco*R1-digested pCG131; (G) *Eco*R1-digested pCG111; (H) *Eco*R1-digested pCG112.

and stored at −20 C.

Endonuclease digestion of plasmid DNA. λ-DNA was digested with the restriction endonucleases *Eco*R1 and *Hin*d III (Miles Laboratories, Elkhart, IN 46515) as described by Meyer et al (8). Reference λ-DNA also was purchased from Miles Laboratories. Molecular weights of the digested plasmid DNA fragments were calculated by regression analysis of the fragments. The mobility of the unknown fragments was compared to that of known *Eco*R1 and *Hin*d III λ-DNA digests (9,11).

Agarose slab gel electrophoresis. Electrophoresis was carried out in 0.7% agarose (SeaKem, ME grade; Marine Colloids, Inc., Rockland, ME 04841) dissolved in Tris-borate buffer (89 mM Tris [hydroxymethyl] methane, 2.5 mM ethylene diaminetetraacetic acid, and 89 mM boric acid) at 18 mA, 40 volts for 2.5 hr. The slab gel apparatus, dimensions, and photography of the gels were described previously (5).

RESULTS

The mobility in agarose gels of linear λ-DNA and covalently closed circular (CCC) DNA of each of the plasmids is shown in Fig. 1. Representative restriction patterns generated by *Eco*R1 for the three plasmids are shown in Fig. 2 and the estimated molecular masses for the fragments are shown in Table 2. *Eco*R1 and *Hin*d III digests of λ-DNA were included in the gels as molecular mass markers. The differences in number and mobility of the fragments (Table 2) showed the uniqueness of the plasmids. Estimates of the molecular masses of the *Eco*R1 fragments suggest that plasmids pCG131 and pCG111 have at least three fragments of common size, one of ~8.6 megadaltons, one of 1.3 megadaltons, and one of 1.2 megadaltons. Plasmid pCG112 also has the 1.3-megadalton fragment in common. Plasmids pCG131 and pCG112 have a 1.7-megadalton fragment in common.

Representative restriction patterns produced by *Hin*d III digestion of λ-DNA and the three plasmids are shown in Fig. 3. An estimate of the molecular masses of the plasmid fragments is presented in Table 2. Plasmid pCG131 and pCG111 appear to have one fragment of 2.1 megadaltons in common. The digest products of plasmid pCG112 showed four fragments, with no fragments in common with the pCG131 and pCG112.

The molecular mass sum of the fragments from either enzyme digestion totaled less than the whole plasmids. These results may be due either to a duplication of a particular region of the nucleic acid sequence or to nonidentical sequences of the same or nearly the same size which would not be discernible by electrophoresis.

TABLE 2. Approximate molecular masses of fragments of *Pseudomonas syringae* plasmids produced by endonuclease digestion.

Plasmid	Enzyme	Number of fragments	Calculated molecular masses (megadaltons)[a]
pCG131	*Eco*R1	13[b]	8.61, 4.15, 3.40, 2.55, 1.74, 1.50, 1.40, 1.30, 1.19, 0.76, 0.64, 0.51, 0.44
pCG111	*Eco*R1	6	8.61, 7.89, 2.02, 1.30, 1.19, 0.81
pCG112	*Eco*R1	8	8.24, 3.51, 2.32, 1.73, 1.65, 1.33, 1.14, 1.09
pCG131	*Hin*d III	8	5.94, 5.36, 5.18, 4.28, 4.03, 3.43, 2.13, 1.16
pCG111	*Hin*d III	8	8.16, 5.73, 4.15, 3.05, 2.69, 2.10, 1.83, 1.00
pCG112	*Hin*d III	5	6.87, 5.01, 3.22, 2.90, 2.50

[a] Molecular masses were calculated by comparison with phage λ-DNA fragments produced by *Eco*R1 and *Hin*d III; those of the *Eco*R1 fragments were, in megadaltons, 13.7, 4.74, 3.74, 3.48, 3.02, and 2.13 (11). The 3.74- and 3.48-megadalton fragments were not resolved in our gels. *Hin*d III fragments were: 15.0, 6.4, 4.3, 2.9, 1.6, and 1.4 (9). Correlation coefficients of known values for λ-DNA fragments were compared with those obtained for fragments on our gels. For *Eco*R1, the coefficient was 99.98%; for *Hin*d III, 99.62%.

[b] Only eleven fragments can be seen clearly; the negative shows an additional two fragments.

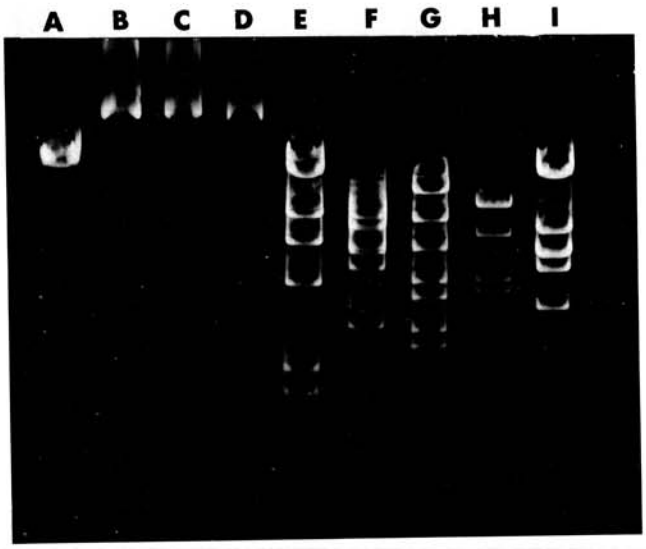

Fig. 3. Agarose gel electrophoresis of *Hind* III digests of λ-DNA and *Pseudomonas syringae* plasmid DNA. (A) untreated λ-DNA; (B) untreated pCG131; (C) untreated pCG111; (D) untreated pCG112; (E) *Hind* III-digested λ-DNA; (F) *Hind* III-digested pCG131; (G) *Hind* III-digested pCG111; (H) *Hind* III-digested pCG112; (I) *Eco*R1-treated λ-DNA.

DISCUSSION

This study shows that the plasmids of approximately the same molecular mass from three syringomycin-producing strains of *P. syringae* isolated from different hosts could be distinguished by restriction endonucleases. Some crown gall plasmids of approximately the same molecular mass show restriction endonuclease digest similarities or differences that are correlated to some degree with metabolic function and DNA homology (10). Even plasmids which show 85% homology by heteroduplex analysis may produce restriction patterns with few bands in common (12). Therefore, the heterogeneity seen in plasmid patterns could be due to small changes in DNA sequence which would change the recognition sites for the enzyme.

The sum of the fragments from the three plasmids totaled less than the molecular mass of the whole plasmid; this problem might be resolved by fluorophotometric scanning of gels to determine if fragments were present in equimolar amounts. In a crown gall plasmid, several SMA I digest fragments were estimated to be in multiple form (2). In addition, some fragments generated by digestion may be too small to be retained by the gels.

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