Detection of New Ethylene-Producing Bacteria, *Pseudomonas syringae* pvs. *cannabina* and *sesami*, by PCR Amplification of Genes for the Ethylene-Forming Enzyme

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ABSTRACT

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Strains of *Pseudomonas syringae* (78 strains and 43 pathovars) and other strains (79) of plant and insect origin were examined for the presence of the ethylene-forming enzyme gene (*efe*) by polymerase chain reaction (PCR) assay. The sequence of the *efe* gene of *P. syringae* pv. *phaseolicola* PK2 was used to design two primer sets for amplification of the gene. In addition to *P. syringae* pv. *phaseolicola* (the "kudzu strain") and *P. syringae* pv. *glycinea*, which were efficient ethylene producers,

several strains of *P. syringae* pvs. *sesami* and *cannabina* generated PCR products of the predicted size. A DNA probe of the *efe* gene, isolated from strain PK2, hybridized to these PCR products, indicating homology to the *P. syringae* pv. *phaseolicola* *efe* gene. PCR restriction fragment length polymorphism analyses suggested that these four pathovars harbor a similar *efe* gene. Furthermore, the probe hybridized to an indigenous plasmid of *P. syringae* pv. *cannabina*, suggesting that the *efe* gene could be located on a plasmid in this pathovar, but did not hybridize to plasmids of *P. syringae* pv. *sesami* strains. *P. syringae* pvs. *sesami* and *cannabina* strains produced ethylene in King's medium B at levels similar to those of *P. syringae* pvs. *phaseolicola* and *glycinea*. Thus, two new ethylene-producing bacteria were detected by the PCR assay.

Ethylene is a plant hormone involved in regulation of numerous physiological processes. Ethylene also is produced by microorganisms, including a limited number of plant-pathogenic bacteria. Goto et al. (6) reported that "kudzu strains" of *Pseudomonas syringae* pv. *phaseolicola* produce ethylene very efficiently and are considered to be among the most efficient ethylene-producing microorganisms. Sato et al. (16) reported that all the strains of *P. syringae* pv. *glycinea* also are very efficient ethylene producers. More recently, Weingart and Volksch (18) confirmed the ethylene-producing ability of strains of *P. syringae* pvs. *phaseolicola* (kudzu strain) and *glycinea*. No other plant-pathogenic bacteria produce ethylene as efficiently, although several species, including *Ralstonia solanacearum* and *P. syringae* pv. *pisi*, produce a small amount of ethylene (3,16,17). Although the role of ethylene in pathogenicity has not yet been determined, Weingart and Volksch (18) recently showed that these strains produce ethylene in planta, indicating a potential role in pathogenicity. The study of new ethylene-producing bacteria could yield information about the role or evolution of ethylene production in plant-pathogenic bacteria.

In microorganisms, ethylene is produced either via 2-keto-4-methylthiobutyric acid, a transaminated derivative of methionine, by an NADH:Fe(III)EDTA oxidoreductase-mediated reaction, or 2-oxoglutarate, by an ethylene-forming enzyme. The latter biosynthesis process is found in *P. syringae* pvs. *phaseolicola* and *glycinea* (5,9,10). Recently, the *efe* gene of *P. syringae* pv. *phaseolicola* PK2 was cloned and sequenced by Fukuda et al. (4), who indicated that it is borne on indigenous plasmids in *P. syringae* pvs. *phaseolicola* and *glycinea* (11). These findings suggest that

horizontal transmission of the *efe* gene has occurred between bacterial strains and pathovars. Furthermore, Weingart and Volksch (18) revealed that polymerase chain reaction (PCR) amplification of a portion of the *efe* gene is a useful technique for detection of ethylene-producing bacteria.

In the current study, a PCR assay was used to examine 43 pathovars of *P. syringae* (78 strains) and other strains (79) of plant and insect origin for the presence of an *efe* gene. In addition, the possible plasmid location of *efe* genes was examined by Southern blot analysis. Finally, ethylene production by PCR-positive bacteria was verified by gas chromatography (GC) analysis.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Tables 1 and 2. The total of 157 strains includes 43 pathovars of *P. syringae*, other plant-pathogenic and epiphytic bacteria, and bacteria of insect origin. A plasmid clone, pKEFE, containing the *efe* gene (1.05 kb) from strain PK2 cloned into pKK223-3, which was supplied by K. Nagahama, was used as a DNA probe for Southern blot hybridization. To estimate the molecular size of the indigenous plasmids, the following standard plasmid markers were used: three plasmids (91, 76, and 38 kb) of *P. syringae* pv. *mori* S6801 (14) and plasmid pBPW1::Tn7 (59 kb) of *P. syringae* pv. *tabaci* BR2 (15).

Bacterial culture. For PCR assay and plasmid isolation, bacteria were grown on modified Luria-Bertani (LB) agar medium (10 g of peptone, 5 g of yeast extract, 10 g of NaCl, 15 g of agar, and 1 liter of distilled water) or modified LB broth overnight at 28°C, respectively. To detect ethylene production, the bacteria were cultured on King's medium B (KB) agar or in KB broth (7).

Plasmid DNA isolation. Plasmid DNA was extracted according to Birnboim and Doly (2) and separated by electrophoresis in Tris-

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borate buffer (0.89 M Tris-borate, 0.089 M boric acid, pH 8.0) at 8 V/cm in 0.6% agarose gel for 3 h.

PCR analysis. Two sets of primers were designed from the sequence of the *efe* gene of *P. syringae* pv. *phaseolicola* PK2 (4). To amplify the entire *efe* gene (1.05 kb), primers ETH-1 (5'-ATGAC-

TABLE 1. Detection of ethylene-forming enzyme genes (*efe*) in 43 pathovars of *Pseudomonas syringae* by polymerase chain reaction (PCR) amplification

Pathovar and strain designation(s) ^a	PCR signal ^b with a primer set		Source
	ETH-1/ ETH-2	ETH-1/ ETH-3	
<i>aceris</i> 302273 (PT)	–	–	MAFF
<i>actinidiae</i> 302091	–	–	MAFF
<i>aesculi</i> 302251 (PT)	–	–	MAFF
<i>antirrhini</i> 302252 (PT)	–	–	MAFF
<i>aptata</i> 302253 (PT)	–	–	MAFF
<i>atrofaciens</i> 302254 (PT)	–	–	MAFF
<i>atropurpurea</i> 301309	–	–	MAFF
<i>berberidis</i> 302255 (PT)	–	–	MAFF
<i>broussonetiae</i> ^c			
Koz8101(PT), 8301, 8501	–	–	K. Takahashi
<i>cannabina</i> 302256 (PT)	+	+	MAFF
<i>castaneae</i> C3, C17	–	–	K. Takahashi
<i>ciccaronei</i> 302275 (PT)	–	–	MAFF
<i>coronafaciens</i>			
302257 (PT), 301314	–	–	MAFF
<i>delphinii</i> 302261 (PT)	–	–	MAFF
<i>dysoxylis</i> 302276 (PT)	–	–	MAFF
<i>eriobotryae</i> 301062	–	–	MAFF
<i>glycinea</i> 301683, 302676,			
302683, 302694, 302754	+	+	MAFF
<i>helianthi</i> 302261 (PT)	–	–	MAFF
<i>lachrymans</i> 302278 (PT)	–	–	MAFF
<i>lapsa</i> 302263 (PT)	–	–	MAFF
<i>maculicola</i> H3-9, H3-6, H1	–	–	M. Sato
<i>mori</i> S6805, M5, Ibm	–	–	M. Sato
"halo str." SM2, SM5, Ym5-1	–	–	M. Sato
<i>morsprunorum</i> 302280 (PT)	–	–	MAFF
A54, R6, R58	–	–	K. Takanashi
<i>oryzae</i> 301529 (PT)	–	–	MAFF
<i>papulans</i> 302265 (PT)	–	–	MAFF
<i>passiflorae</i> 302266 (PT)	–	–	MAFF
<i>persicae</i> 302267 (PT)	–	–	MAFF
<i>phaseolicola</i> "kudzu str."			
301766, 302721, 302722	+	+	MAFF
KN80	+	+	K. Nishiyama
KUZ2 KUZ5 KUZ7	+	+	M. Sato
<i>phaseolicola</i> "bean str."			
301022, 301616, 301673, 311004	–	–	MAFF
<i>philadelphii</i> 302268 (PT)	–	–	MAFF
<i>pisi</i> 302269 (PT)	–	–	MAFF
<i>primulae</i> 302294 (PT)	–	–	MAFF
<i>ribicola</i> 302295 (PT)	–	–	MAFF
<i>savastanoi</i> 302283 (PT)	–	–	MAFF
<i>sesami</i> 302284 (PT)	+	+	MAFF
SUPP1472, SUPP1473	+	+	Y. Takikawa
<i>striafaciens</i> 301032	–	–	MAFF
<i>syringae</i> 302155 (PT)	–	–	MAFF
N1, R168, A17, A1, W7835	–	–	K. Takanashi
(<i>panici</i>) ^d 302281 (PT)	–	–	MAFF
(<i>japonica</i>) ^d 301072 (PT)	–	–	MAFF
<i>tabaci</i> 301075	–	–	MAFF
<i>tagetis</i> 302271 (PT)	–	–	MAFF
<i>tomato</i> 302272 (PT)	–	–	MAFF
<i>ulmi</i> 302286 (PT)	–	–	MAFF
<i>viburni</i> 302287 (PT)	–	–	MAFF

^a (PT) = type strain of pathovar. A formal strain number from the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF), is expressed as pv. *aceris* MAFF302273.

^b + indicates PCR products of 1.05 or 0.44 kb were obtained with primer set ETH-1/ETH-2 or ETH-1/ETH-3, respectively. – indicates no PCR product was obtained.

^c A new pathovar proposed by Takahashi et al. (17).

^d Formerly used pathovars, included here in pv. *syringae*.

CAACCTACAGACT-3') and ETH-2 (5'-TCATGAGCCTGTGCGCGCG-3') were used. To amplify a 0.44-kb portion of the *efe* gene, primers ETH-1 and ETH-3 (5'-TAAGCCACCAACATCGTC-3') were used. Template DNAs were prepared by boiling a bacterial suspension (10⁸ CFU/ml in distilled water) for 5 min. In some cases, plasmid DNAs, excised from gels with a GeneClean II kit (BIO 101 Inc., La Jolla, CA) according to the manufacturer's protocol, were used as template DNAs. The standard reaction mixture (50 µl) contained 1.5 mM MgCl₂, 0.2 mM each dNTP, 1.25 units of *Taq* DNA polymerase (Promega, Madison, WI), 25 pmol each primer, 5 µl of 10× reaction buffer, and 2.5 µl of boiled bacterial suspension. Amplification included 30 cycles of denaturation (94°C for 1 min), annealing (55°C for 2 min), and extension (72°C for 3 min).

For PCR restriction fragment length polymorphism (RFLP) analysis, the PCR products were precipitated with 3 volumes of ethanol and resuspended in the appropriate restriction buffer. The purified PCR products were digested with various restriction enzymes and electrophoresed in 3.0% low melting temperature agarose gel (NuSieve agarose, FMC Bioproducts, Rockland, MD) as described above.

Southern blot analysis. The entire pKEFE plasmid or *efe* gene (a PCR product from pKEFE obtained by primer set ETH-1/ETH-2) was labeled with a digoxigenin (DIG) DNA-labeling kit from Boehringer Biochemica (Mannheim, Germany) according to the manufacturer's protocol and used as a probe for Southern blot hybridization. Southern blot analysis was performed according to standard procedures (12) with some modifications. After electrophoresis, agarose gels were soaked in several volumes of 1.5 M NaCl and 0.5 M NaOH for 30 min to denature DNA and in 0.5 M Tris-HCl (pH 8.0) and 1.5 M NaCl for 30 min to neutralize DNA.

TABLE 2. Bacterial strains of plant, insect, and other origins in which polymerase chain reaction (PCR) amplification with primer set ETH-1/ETH-2 did not detect ethylene-forming enzyme genes (*efe*) (1.05-kb product)

Strain and designation(s) ^a	Source(s)
Plant origin	
<i>Acidovorax avenae</i> K3	I. Matsuda
<i>Enterobacter cloacae</i> Mul1 ^b	M. Sato
<i>Erwinia ananas</i> 301720, 301718, 301722	MAFF
TM2	M. Sato
<i>Erwinia herbicola</i> DW1	M. Sato
<i>Erwinia herbicola</i> pv. <i>millietiae</i> 301749	MAFF
<i>Pseudomonas cichorii</i> NR7634	I. Matsuda
<i>P. fluorescens</i> LRB3W1	K. Tsuchiya
<i>P. marginalis</i> S-M	Y. Takikawa
<i>P. viridiflava</i> Ko8	M. Sato
M328, M303	K. Takahashi
<i>Burkholderia andropogonis</i> 301154	MAFF
<i>Burkholderia caryophylli</i> 13525	I. Matsuda
<i>Burkholderia gladioli</i> pv. <i>gladioli</i> NR8810	I. Matsuda
<i>Burkholderia plantarii</i> 301723	MAFF
<i>Ralstonia solanacearum</i> 301520, 301522	MAFF
<i>Xanthomonas campestris</i> pv. <i>campestris</i> 301151	MAFF
pv. <i>citri</i> 301079	MAFF
pv. <i>oryzae</i> 301225	MAFF
Insect origin	
50 strains	M. Sato, K. Watanabe
Other origins	
<i>Alcaligenes faecalis</i> 1474	JCM
<i>Bacillus cereus</i> 2152	JCM
<i>Bacillus pumilus</i> 2508	JCM
<i>Bacillus subtilis</i> 1465	JCM
SAT1	M. Sato
<i>Clostridium butyricum</i> 1391	JCM

^a Strains of insect origin include *Erwinia herbicola*, *Erwinia ananas*, *Pseudomonas syringae*, *Serratia marcescens*, *Enterobacter cloacae*, *Micrococcus* sp., *Staphylococcus* sp., and other unidentified bacteria. A formal strain number from the Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF), or the Japan Collection of Microorganisms (JCM) is expressed as *Xanthomonas campestris* pv. *oryzae* MAFF301225 or *Bacillus cereus* JCM2152.

^b Isolated from mulberry.

DNAs were transferred to nylon membrane filters from gels by capillary blotting. Filters were baked at high temperature (120°C for 1 h). The baked filters were prehybridized in 20 ml of prehybridization fluid (4× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0], 50% formamide, 0.1% *N*-lauroylsarcosine sodium salt solution, 0.2% sodium dodecyl sulfate [SDS] solution, and 2% blocking reagent [Boehringer Biochemical]) and warmed to 43°C for 4 h. DIG-labeled DNA (10 µl) was added and hybridized overnight at 43°C. After hybridization, the filters were washed once with 2× SSC and 0.1% SDS at room temperature for 30 min and three times with 0.1× SSC and 0.1% SDS at 65°C for 20 min. Bands were detected by an enzyme-catalyzed chemiluminescence reaction with a DIG luminescent detection kit from Boehringer Biochemica according to the manufacturer's protocol.

Ethylene production. To detect ethylene production, bacteria were inoculated onto an agar slant of KB (10 ml) in a test tube (15 mm, 32-ml capacity) or in KB broth (20 ml) in a 100-ml flask. The tubes were sealed with a rubber serum cap and incubated with slow shaking (liquid medium) or without shaking (agar medium) at 25°C for 20 or 48 h. A gas sample was withdrawn through the rubber seal with a gas-tight syringe and analyzed with a Hewlett Packard 5890-series II GC equipped with a GC-alumina capillary column (50 m × 0.53 mm inside diameter; Hewlett-Packard Co., Palo Alto, CA), 0.25-µm thick film (J & W Scientific, Folsom, CA), and a hydrogen flame ionization detector.

RESULTS

PCR detection. Five strains of *P. syringae* pv. *glycinea* (MAFF301683, 302676, 302683, 302694, and 302754) and seven

strains of *P. syringae* pv. *phaseolicola* (kudzu strain) (MAFF301766, 302721, 302722, KN80, KUZ2, KUZ5, and KUZ7), which are efficient ethylene producers (6,16), were assayed by PCR with two sets of primers designed to amplify regions of the *efe* gene. All of the strains produced a band of the predicted size (1.05 kb with ETH-1/ETH-2 or 0.44 kb with ETH-1/ETH-3), indicating these primer sets are suitable for detecting *efe* genes. A total of 157 strains, including 43 pathovars of *P. syringae*, were assayed. Using primer set ETH-1/ETH-2, only *P. syringae* pvs. *cannabina* and *sesami* produced a PCR product of the same size as that of *P. syringae* pvs. *glycinea* and *phaseolicola* (Fig. 1). In contrast, the other pathovars did not produce any PCR products. These positive strains were examined further with primer set ETH-1/ETH-3. All of the PCR-positive (ETH-1/ETH-2) pathovars produced a 0.44-kb band with ETH-1/ETH-3, unlike the PCR-negative strains (Table 1). Moreover, other bacterial strains, including those of insect and plant origin, did not produce any PCR products amplified by the primer set (ETH-1/ETH-2), as shown in Table 2. The *efe* probe hybridized with these PCR products, indicating amplified DNAs contain an *efe* gene (Fig. 1).

RFLP analysis. Strains of *P. syringae* pvs. *cannabina* MAFF302256, *sesami* MAFF302284, *glycinea* MAFF301683, and *phaseolicola* KUZ2 were used for PCR-RFLP analysis. PCR products from these strains were digested with restriction enzymes, such as *Hha*I, *Eco*RV, *Msp*I, and *Hae*III. As shown in Figure 2, similar RFLP patterns were obtained in all the samples, suggesting the *efe* genes of the four *P. syringae* pathovars are closely related.

Plasmid profiles and Southern blot analysis. Plasmid profiles of one strain of *P. syringae* pv. *cannabina* and three strains of *P. syringae* pv. *sesami* were compared with those of *P. syringae* pvs. *glycinea* and *phaseolicola* (kudzu strain). Numerous indigenous plasmids were detected in all of these strains (Fig. 3). The *efe* probe hybridized to only one plasmid in *P. syringae* pv. *cannabina* (the largest plasmid in Fig. 3, lane a), as well as to plasmids in *P. syringae* pvs. *glycinea* and *phaseolicola* (≈105- and ≈68-kb plasmids in Figure 3, lanes e and f, respectively) but did not hybridize to any plasmids in the *P. syringae* pv. *sesami* strains. These findings suggest that *P. syringae* pv. *cannabina* MAFF302256 harbors an "ethylene plasmid," whereas the *efe* gene is located on the chromosome in the three *P. syringae* pv. *sesami* strains used in this study. The molecular size of the ethylene plasmid of *P. syringae*

Fig. 1. A, Polymerase chain reaction (PCR) products from *Pseudomonas syringae* pathovars and *Ralstonia solanacearum* with primer set ETH-1/ETH-2 and B, Southern blot hybridization to the PCR products probed with the *P. syringae* pv. *phaseolicola* *efe* gene. Lane M, molecular size markers (λ DNA digested with *Hind*III and *Eco*RI); lane a, *P. syringae* pv. *sesami* strain MAFF302284; lane b, *P. syringae* pv. *sesami* strain SUPP1472; lane c, *P. syringae* pv. *sesami* strain MAFF302256; lane d, *P. syringae* pv. *cannabina* strain MAFF302256; lane e, *P. syringae* pv. *glycinea* strain MAFF301683; lane f, *P. syringae* pv. *glycinea* strain MAFF302676; lane g, *P. syringae* pv. *phaseolicola* strain KUZ2; lane h, *P. syringae* pv. *phaseolicola* strain MAFF301766; lane i, *P. syringae* pv. *phaseolicola* strain MAFF301022; lane j, *R. solanacearum* strain MAFF301520; lane k, *P. syringae* pv. *mori* strain S6801; and lane l, *Escherichia coli* strain JM109 (pKEFE).

Fig. 2. Restriction fragment length polymorphism analysis of polymerase chain reaction products from *Pseudomonas syringae* pvs. *sesami*, *cannabina*, *glycinea*, and *phaseolicola*, using restriction enzymes A, *Hha*I, B, *Eco*RV, C, *Msp*I, and D, *Hae*III. Lane M, molecular size markers (ϕ X174 DNA digested with *Hinf*I); lane a, *P. syringae* pv. *sesami* strain MAFF302284; lane b, *P. syringae* pv. *cannabina* strain MAFF302256; lane c, *P. syringae* pv. *glycinea* strain MAFF301683; and lane d, *P. syringae* pv. *phaseolicola* strain KUZ2.

pv. *cannabina* was estimated to be ≈ 110 kb and was designated pETH1 (Fig. 3). pETH1 DNA was excised from agarose gel and used as a PCR template. This generated a 1.05-kb PCR product with primer set ETH-1/ETH-2, indicating this plasmid carries an *efe* gene.

Ethylene production. Three strains of *P. syringae* pv. *sesami* and one strain of *P. syringae* pv. *cannabina* were examined for ethylene production by GC analysis. All of the PCR-positive strains produced ethylene, whereas the PCR-negative strains did not. The amount of ethylene produced by each strain was determined relative to that produced by *P. syringae* pv. *glycinea* strain MAFF301683. Under conditions similar to those used here (20-h-old shake cultures in KB broth), Sato et al. (16) reported that this strain produced 69.2×10^{-9} nl per cell per h. *P. syringae* pvs. *sesami* and *cannabina* produced essentially the same amount of ethylene as strain MAFF301683. Relative amounts of ethylene, expressed as a ratio of the unknown to the standard strain, were as follows: *P. syringae* pv. *sesami* strains MAFF302284, 1.0; SUPP1472, 0.9; SUPP1473, 0.7; *P. syringae* pv. *cannabina* strain MAFF302256, 1.2; and *P. syringae* pv. *phaseolicola* (kudzu strain) MAFF302721, 1.1.

DISCUSSION

PCR amplification with primers containing sequences of the *efe* gene of *P. syringae* pv. *phaseolicola* PK2 (4) was a useful technique to detect ethylene-producing bacteria among a number of bacteria. PCR-positive strains produced ethylene very efficiently. PCR detection of the *efe* gene was first described by Weingart and Volksch (18). They found that all of the strains of *P. syringae* pvs. *phaseolicola* (kudzu strain) and *glycinea* that produced ethylene efficiently were PCR-positive. However, they could not detect any other new ethylene-producing bacteria among a large number of other strains. These findings suggest that the *efe* gene may be distributed in a very limited number of bacteria. Likewise, we showed that most of the bacterial strains in this study did not harbor an *efe* gene. However, we detected two new ethylene-producing *P. syringae* pathovars: *cannabina* and *sesami*. Interestingly, Weingart and Volksch (18) reported that a strain of *P. syringae* pv. *cannabina* did not produce ethylene. These findings suggest that *P. syringae* pv. *cannabina* strains may differ in ethylene-producing ability.

The same RFLP patterns were obtained for the *efe* gene in *P. syringae* pvs. *cannabina*, *sesami*, *phaseolicola*, and *glycinea*. In addition, the *P. syringae* pv. *phaseolicola* *efe* probe hybridized to PCR products from the other pathovars. Therefore, the *efe* genes amplified from these strains may be closely related to each other.

Nagahama et al. (11) reported that the *efe* gene was encoded by indigenous plasmids in *P. syringae* pvs. *phaseolicola* and *glycinea*. In the current study, we found that all the strains of *P. syringae* pvs. *glycinea* and *phaseolicola* tested harbored indigenous plasmids containing the *efe* gene, supporting the results of Nagahama et al. (11). Our data also indicate that *P. syringae* pv. *cannabina* may harbor an ethylene plasmid. The ethylene plasmids from the three pathovars varied in molecular size, which is not surprising because a similar size variation has been reported in the "coronatine plasmids" in several pathovars of *P. syringae* (1,13,19). It is assumed that these ethylene plasmids may have been modified by cointegration or recombination with other plasmids. Interestingly, these pathovars, which harbor ethylene plasmids, can infect plants belonging to the family Leguminosae. *P. syringae* pv. *cannabina* infects *Phaseolus vulgaris* and *Vicia sativa*, as well as *Cannabis sativa* of the family Moraceae (8). This observation suggests that the ethylene plasmid may move via conjugation among strains of *P. syringae* that colonized Leguminosae plants in the past. Subsequently, the plasmid genes became established in strains of each pathovar. It is likely that ethylene production is advantageous for survival on Leguminosae plants. This assumption is supported by Weingart and Volksch (18), who concluded that ethylene production in planta may play an important role in the pathogenicity of *P. syringae*. In contrast, no strains of *P. syringae* pv. *sesami* appear to carry an autonomous ethylene plasmid, and the *efe* gene appears to be chromosomal (we could not detect any plasmids that hybridized to the *efe* probe). Strains of *P. syringae* pv. *sesami* are pathogenic to *Sesamum indicum* (sesame) in the family Pedaliaceae but not the family Leguminosae. The origin of the *efe* gene in *P. syringae* pathovars is still obscure. The gene may have been acquired from other microbial or plant genomes. As a result, *P. syringae* pv. *sesami* strains as well as other ethylene-producing pathovars may be useful in investigating the evolution of the *efe* gene and ethylene plasmids.

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Fig. 3. A, Plasmid profiles of *Pseudomonas syringae* pathovars and B, Southern blot hybridization to plasmids with the *P. syringae* pv. *phaseolicola* *efe* probe. Lane a, *P. syringae* pv. *cannabina* strain MAFF302256; lane b, *P. syringae* pv. *sesami* strain MAFF302284; lane c, *P. syringae* pv. *sesami* strain SUPP1472; lane d, *P. syringae* pv. *sesami* strain SUPP1473; lane e, *P. syringae* pv. *glycinea* strain MAFF302676; lane f, *P. syringae* pv. *phaseolicola* strain MAFF301766; and lane M, molecular size markers (indigenous plasmids of *P. syringae* pv. *mori* strain S6801). The plasmid pBPW1::Tn7 (59 kb) also was used as a marker but is not shown. In Southern blot hybridization, strain S6801 plasmids were used as a negative control.

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