Molecular Detection and Differentiation of *Erwinia pyrifoliae* and Host Range Analysis of the Asian Pear Pathogen

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ABSTRACT

Kim, W.-S., Jock, S., Paulin, J.-P., Rhim, S.-L., and Geider, K. 2001. Molecular detection and differentiation of *Erwinia pyrifoliae* and host range analysis of the Asian pear pathogen. Plant Dis. 85:1183-1188.

The recently described pathogen *Erwinia pyrifoliae*, isolated from Nashi pear fruit trees in Korea, resembles the fire blight pathogen *Erwinia amylovora* in some of its properties. The two pathogens were classified into different species by DNA hybridization kinetics and microbiological criteria. From the nucleotide sequences of the 16S rRNA and the internal transcribed spacer (ITS) region as well as extracellular polysaccharide (EPS)-encoding genes, polymerase chain reaction (PCR) primers were designed that specifically detect *E. pyrifoliae* but not the fire blight pathogen *Erwinia amylovora*, and these primers were also applied to identify *E. pyrifoliae* in necrotic plant material. The genomes of several strains were digested with the restriction enzyme *Spe*I, and the DNA fragments were analyzed by pulsed-field gel electrophoresis (PFGE). Three groups of patterns could be distinguished for the isolated *E. pyrifoliae* strains, all different from various *E. amylovora* strains, which produce a relatively homogeneous PFGE pattern after *Spe*I digests. Typical fire blight host plants were assayed in a growth chamber or an experimental field for their susceptibility to *E. pyrifoliae*. A strong preference was found for pear varieties, whereas apple, cotoneaster, hawthorn, or raspberry rarely produced necrotic symptoms. *E. pyrifoliae* was readily detected in samples from pear orchards in South Korea during 1995 to 1998; however, the Asian pear pathogen was not recovered in necrotic plant tissue from 1999 and 2000.

Additional keywords: Asian pear blight, disease rating

Erwinia pyrifoliae is a pathogen isolated from necrotic Asian pear fruit trees (*Pyrus pyrifolia* Nakai) in Korea (13). Symptom formation on immature pears and its colony morphology on MM2Cu agar are similar to those of *Erwinia amylovora*, the causative agent of fire blight. However, microbiological assays, negative polymerase chain reaction (PCR) using primers specific for *E. amylovora* (13), and BIOLOG/biotype 100 analysis and DNA-DNA hybridization kinetics distinguished *E. pyrifoliae* from the fire blight pathogen *E. amylovora* and from *Enterobacter pyrinus* (5,11). *E. pyrifoliae* was isolated in four consecutive years (1995 to 1998) from Korean orchards in the Chuncheon region exclusively from a diseased Nashi pear tree.

Many assays have been previously described for detection and identification of *E. amylovora* (10). The addition of 2 mM $CuSO_4$ to minimal medium further improved detection of *E. amylovora* due to an increase of extracellular polysaccharide (EPS) synthesis and to the yellow color formation of the colonies (2,17), an effect also seen with *E. pyrifoliae* (13). In contrast to other methods such as description of the colony morphology, serological assays, fatty acid profiles, and phage typing, PCR assays were more specific and sensitive (1,3). Detection of *E. amylovora* by PCR was conducted with two primer pairs derived from plasmid pEA29 (3) and the *ams* genes (encoding amylovoran synthesis) (4). Ribosomal RNA (rRNA) genes are widely used for taxonomy to classify bacteria at the levels of species, orders, or even kingdoms (14). At the species level, the rRNA operon, including a noncoding internal transcribed spacer region (ITS), has been successfully used to differentiate *E. pyrifoliae* from *E. amylovora* (11). Pulsed field gel electrophoresis (PFGE) is another powerful tool to differentiate *E. amylovora* strains (16,18; S. Jock, W.-S. Kim, V. Donat, M. M. Lopez, C. Bazzi, and K. Geider, *unpublished*). Although the PFGE patterns of *E. amylovora* isolates from Europe and the Mediterranean region are closely related, a single band can be used for distinction of strains from large regions.

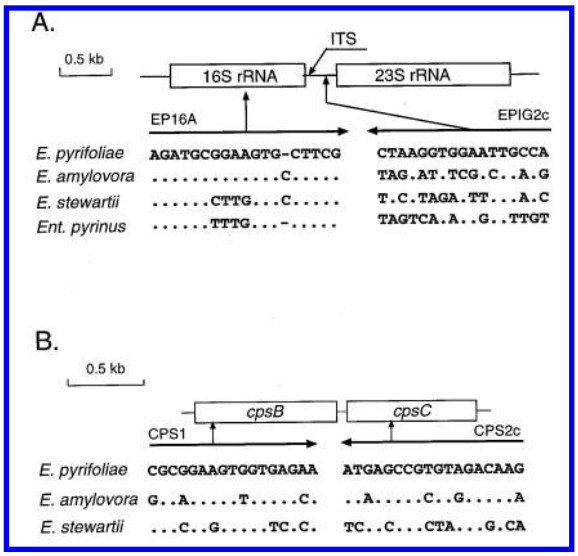

Fig. 1. A scheme for the design of primers for specific detection of *Erwinia pyrifolia* via rRNA (**A**) and *cps* (**B**) genes. Nucleotide sequences of DNA fragments covering the primer regions were aligned with a minimal allowance of gaps in the corresponding regions of other pathogens. Base pairs matching with the *E. pyrifoliae* nucleotide sequence (first line) are indicated by dots. The nucleotide sequences with accession numbers listed in Material and Methods were derived from *E. pyrifoliae* Ep1/96, *Erwinia amylovora* Ea1/79, *Erwinia stewartii* DC283, and *Enterobacter pyrinus* 90.2, respectively.

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Accepted for publication 19 July 2001.

Publication no. D-2001-0910-01R

In this study, we created two specific PCR primer pairs for specific detection of *E. pyrifoliae* and PFGE analysis to differentiate *E. pyrifoliae* strains, and we also assayed the host range of the pathogen by challenging common fire blight host plants.

MATERIALS AND METHODS

Design of primers for detection of *Erwinia pyrifoliae*. The nucleotide sequences of the 16S rRNA and the ITS RNA regions of various *Erwinia* strains were previously determined (11) and deposited in the EMBL Nucleotide Sequence Database with the accession numbers AJ009930 for *E. pyrifoliae* strain Ep1/96, AJ132969 for *E. pyrifoliae* strain Ep16/96 (ITS only), AJ010485 for *E. amylovora* strain Ea1/79, and AJ010486 for *Enterobacter pyrinus* strain 90.2. For EPS-encoding regions, the nucleotide sequence entries are AJ300463 for *cps* genes of *E. pyrifoliae* and X77921 for the *ams* region of *E. amylovora*.

The nucleotide sequences from the rRNA regions of *E. pyrifoliae*, *E. amylovora*, *Erwinia stewartii,* and *Enterobacter pyrinus* were aligned to design PCR primers specific for *E. pyrifoliae*. At position 1004 in the 16S rRNA of *E. pyrifoliae* and 1738 in the ITS of *E. pyrifoliae*, appropriate sequences were found, and primers EP16A (5′-AGATGCGGAAGTGCTTCG) and EPIG2c (5′-ACCGTTAAGGTG GAATC) were created (Fig. 1A). For the 16S rRNA, an insertion of one nucleotide after position 13 of the primer differed for *E. pyrifoliae* and *E. amylovora*, whereas primer sequences from the ITS region were largely divergent from *E. amylovora* and even more from *E. stewartii* and *Enterobacter pyrinus*.

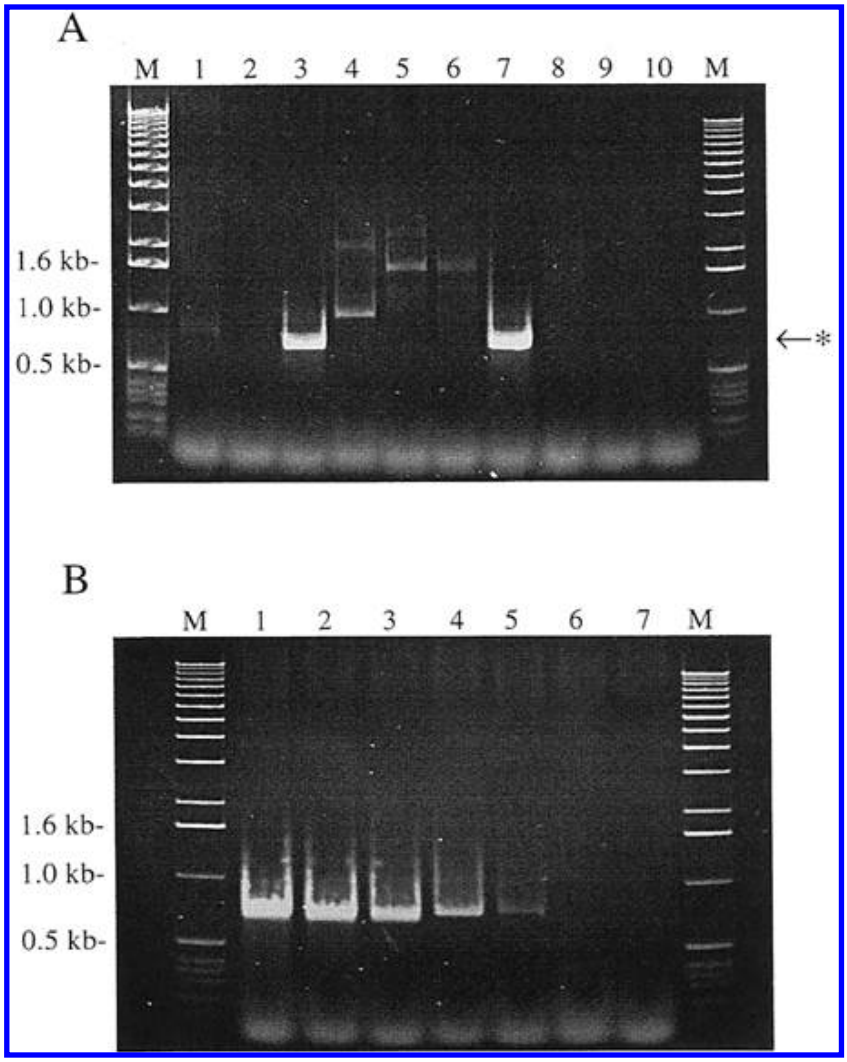

Fig. 2. A, Detection of *Erwinia pyrifoliae* with the primer pair EP16A/EPIG2c. Lane 1, *Erwinia herbicola*; 2, *Escherichia coli*; 3, *E. pyrifoliae* Ep1/96; 4, *Enterobacter pyrinus*; 5, *Erwinia stewartii*; 6, *Pseudomonas syringae*; 7, extract from a necrotic pear seedling inoculated with *E. pyrifoliae* Ep1/96; 8, *Xanthomonas campestris*; 9, *Erwinia amylovora* Ea1/79; 10, water control; ←* indicates the position of 0.73 kb, expected for *E. pyrifoliae*. **B,** Sensitivity in polymerase chain reaction (PCR) detection of *E. pyrifoliae* with rRNA primers. Cells from an overnight culture of *E. pyrifoliae* strain Ep1/96 (2×10^9 bacteria per ml) were stepwise diluted for the PCR assays. In lanes 1 to 6, aliquots with 2×10^5, 10^4, 10^3, 10^2, 10^1, and 10^0 CFU were assayed. Lane 7, water control; M, marker of 1-kb ladder DNA (sizes of bands at the left border).

To design a PCR primer pair corresponding to primers of the *ams* region of *E. amylovora* (4), DNA fragments with parts of *cpsB* and *cpsC* from the EPS encoding region of *E. pyrifoliae* were cloned and sequenced. The selected *E. pyrifoliae* primers CPS1 (5′-CGCGGAAGTGGT GAGAA) and CPS2c (5′-GAACAGATG TGCCGAGTA) differed from *E. amylovora* by four nucleotides and were even more divergent to the corresponding *cpsE/F* genes of *E. stewartii* (6) (Fig. 1B). Primer CPS1 is located at position 5714 and CPS2c at position 6935 of the *E. pyrifoliae cps* region.

PCR analysis. The PCR was done in 50 µl with 10 ng of template DNA or 1 µl of a 10^{-4} dilution of overnight grown bacterial cultures under conditions described previously (3,11) with 0.5 U of *Tth* DNA polymerase or 2 U of *Taq* DNA polymerase and 25 pmol of primers. PCR assays were carried out in an Eppendorf mastercycler 5330. The DNA was denatured for 1 min at 95°C, and amplification was done in 30 cycles applying 95°C for 15 s, 52 or 57°C for 15 s, and 72°C for 30 s. Following electrophoresis in a 0.8% agarose gel and staining with ethidium bromide, the PCR products were visualized under UV light.

Plant material was extracted by placing about 100 mg of tissue in an Eppendorf tube with 1 ml of sterile water for 30 min. Liquid was removed and up to 10 µl applied to the PCR assay or aliquots plated first on LB agar with cycloheximide (50 µg/ml, to avoid fungal growth).

PFGE analysis. PFGE analysis was done as described for *E. amylovora* (16).

Host range assays. *E. pyrifoliae* strains were inoculated in an experimental field on adult trees of a range of Rosaceous plants, among them genotypes of hawthorn (*Crataegus* sp.), apple (*Malus* sp.), and pear (*Pyrus* sp.), as well as plum (*Prunus salicina*) and raspberry (*Rubus idaeus*). Actively growing shoots were inoculated just below the apex with a syringe, infiltrating up to 0.05 ml of a 10^8 CFU/ml suspension of 24-h-old culture. Three shoots of each of three trees per genotype were inoculated. Four weeks after inoculation, the length of necrotic lesions on shoots was assessed. Response was considered positive when at least one-third of the inoculated shoots showed a necrosis of more than 2 cm in length. *E. amylovora* strains CFBP3049, a highly virulent isolate from apple (Canada), Ea1/79 from *Cotoneaster* sp. (Germany), and CFBP2151 or IL6 from *Rubus idaeus* (United States) were used in the study. Four *E. pyrifoliae* strains were applied; data presented were only for the most virulent strain, Ep1/96.

When plantlets were inoculated in a growth chamber, leaf tips of young seedlings were cut with scissors and inoculated with a toothpick dipped into

an overnight culture of an *E. amylovora* or *E. pyrifoliae* strain (5×10^9 CFU/ml). Symptoms were monitored after 2 weeks, and obvious leaf necrosis was considered to be a positive reaction.

RESULTS

Detection of *E. pyrifoliae* by specific PCR primers. PCR amplification with primers EP16A and EPIG2c gave a band at 0.7 kb with *E. pyrifoliae* but not with *Erwinia herbicola*, *Escherichia coli*, *Enterobacter pyrinus*, *E. stewartii*, *Pseudomonas syringae*, *Xanthomonas campestris*, or *E. amylovora* (Fig. 2A), nor with other bacteria of the former species of *Erwinia* and other plant-associated bacteria listed in Tables 1 and 2 (data not shown). When large amounts of cells (>10^6 CFU) were applied, shadow bands above 0.7 kb were visible with *Enterobacter pyrinus* and *E. stewartii*. The sensitivity of the primers was assayed with a series of dilutions. The minimal concentration of bacterial cells to obtain a signal was approximately 20 CFU per assay (Fig. 2B). Direct detection of *E. pyrifoliae* from necrotic pear leaves gave a positive signal without any shadow bands. Healthy pear leaves were a negative control.

Primers CPS1 and CPS2c were also assayed with several bacterial species (Fig. 3A), including selected erwinias (Table 1) and other plant-associated erwinias (Table 2). The 1.2-kb PCR product was only detected with *E. pyrifoliae*. Extracts from necrotic pear leaves inoculated with *E. pyrifoliae* also gave a positive signal. Occasionally, nonspecific weak bands of different sizes were obtained with *E. herbicola*, *E. coli,* and *P. syringae*, especially at an annealing temperature of 52°C. An annealing temperature of 57°C reduced shadow bands, but also sensitivity. PCR detection of *E. pyrifoliae* was done in the range from 2×10^5 to 2 CFU, and the signal was still obtained for 200 CFU per assay using an annealing temperature of 52°C (Fig. 3B). The CPS primer pair is therefore slightly less sensitive for detection of *E. pyrifoliae* than the primers from the 16S rDNA/ITS region.

PFGE analysis of various *E. pyrifoliae* strains. Several *E. pyrifoliae* strains in our collection were assayed by PFGE analysis after digestion with the restriction enzymes *Spe*I (Fig. 4) and *Xba*I. For *Xba*I, the *E. pyrifoliae* strains were related in their band patterns (data not shown). For *Spe*I digests, the 10 strains assayed were divergent in part and could be grouped into the PFGE pattern types PtA, PtB, and PtC (Table 3), which were all different from the *Spe*I pattern of *E. amylovora* strains (Fig. 4). Based on *Xba*I digests, *E. amylovora* strains can be differentiated by the resulting DNA fragments. Although the patterns diverge often only in the size of one or two DNA fragments, many strains from Europe and the Mediterranean region could grouped into the pattern types Pt1 to Pt6 with a rare occurrence of unusual types (16,18; S. Jock, W.-S. Kim, V. Donat, M. M. Lopez, C. Bazzi, and K. Geider, *unpublished*). On the other hand, the patterns are quite homogeneous for the *Spe*I fragments of *E. amylovora*, even when the strains were derived from different geographical

Table 1. Strains used in this study[a]

Species Strain	Relevant characteristics (host, origin, year)	Reference or source
Erwinia pyrifoliae		
Ep1/96	*Pyrus pyrifolia* (Nashi pear), South Korea, 1996 (CFBP4171, DSM12162)	13
Ep4/97	*P. pyrifolia,* South Korea, 1997 (DSM12394)	This work
Ep8/95	*P. pyrifolia,* South Korea, 1995 (DSM12393)	13
Ep16/96	*P. pyrifolia,* South Korea, 1996 (CFBP4172, DSM12163)	13
Ep28/96	*P. pyrifolia,* South Korea, 1996 (CFBP4173)	13
Ep31/96	*P. pyrifolia,* South Korea, 1996 (CFBP4174)	13
Ep44/97	*P. pyrifolia,* South Korea, 1997	This work
Ep53/97	*P. pyrifolia,* South Korea, 1997	This work
Ep60/97	*P. pyrifolia,* South Korea, 1997	This work
Ep102/98	*P. pyrifolia,* South Korea, 1998	13
Erwinia amylovora		
Ea1/79	*Cotoneaster* sp., Northern Germany, 1979	8
CFBP3049	*Malus* sp., Canada	CFBP
CFBP1367	*Crataegus* sp. (hawthorn), Lille, 1972, J.P. Paulin	16
S59/5	type strain, *P. communis,* England, obtained as CFBP1232	CFBP
Ea4/82	*P. communis* cv. Le Conte, Egypt, 1982	18
Ea775	*Crataegus* sp., England, ~1959, NCPPB775	18
115.22	*Cydonia oblonga* (quince), Bulgaria, 1989	18
CFBP2151	*Rubus idaeus,* USA	CFBP
IL6	*R. idaeus,* Illinois, USA	C. Bazzi
Erwinia stewartii		
DC283	*Zea mays*, USA	7
Erwinia herbicola		
48b/90	Soybean, Jena, Germany	15
Erwinia chrysanthemi		
DC518	USA	D. Coplin
Enterobacter pyrinus		
90.2	*P. pyrifolia,* South Korea (KCTC 2520), 1990	5
Xanthomonas campestris		
XCC356	Germany	M. Nachtigall
Pseudomonas syringae		
PSB17	*P. communis,* Wädenswil, Switzerland, 1986	W. Zeller
Escherichia coli		
C600	K12 strain	Lab collection

[a] CFBP, Collection Française des Bactéries Phytopathogènes; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; KCTC, Korean Collection of Type Cultures. In recent nomenclature, *Erwinia stewartii* is *Pantoea stewartii* subsp. *stewartii*, and *Erwinia herbicola* has been named *Pantoea agglomerans*.

Table 2. Properties of *Erwinia* strains on minimal medium with copper ions and in polymerase chain reaction (PCR) assays with *Erwinia pyrifoliae*–primers[a]

Erwinia	Strain	MM2Cu	MM1Cu	ITS primers	CPS primers
mallotivora	CFBP2503	–	–	–	–
quercina[B]	CFBP3617	–	–	–	–
rubrifaciens[B]	CFBP3619	–	–	–	–
psidii	CFBP3627	–	–	–	–
cacticida[P]	CFBP3628	–	–	–	–
salicis[B]	CFBP802	–	–	–	–
tracheiphila	CFBP2355	–	–	–	–
cypripedii[P]	CFBP3613	(+)/nm	(+)/nm	–	–
persicinus	CFBP3622	+/mc, yl	+/mc, yl	–	–
rhapontici	CFBP3618	+/nm	+/nm	–	–
nigrifluens	CFBP3616	(+)	–	–	–
pyrifoliae	Ep1/96	+/mc, (yl)	–	+	+
amylovora	Ea1/79	+/mc, yl	–	–	–

[a] +, growth or positive PCR signal; –, no growth or no PCR signal; mc, mucoid; nm, nonmucoid; yl, yellow colonies; (yl), yellowish. Some *Erwinia* species have been recently renamed: [B], *Brenneria*; [P], *Pectobacterium*. ITS primers = EP16A, EIG2c; CPS primers = CPS1, CPS2c.

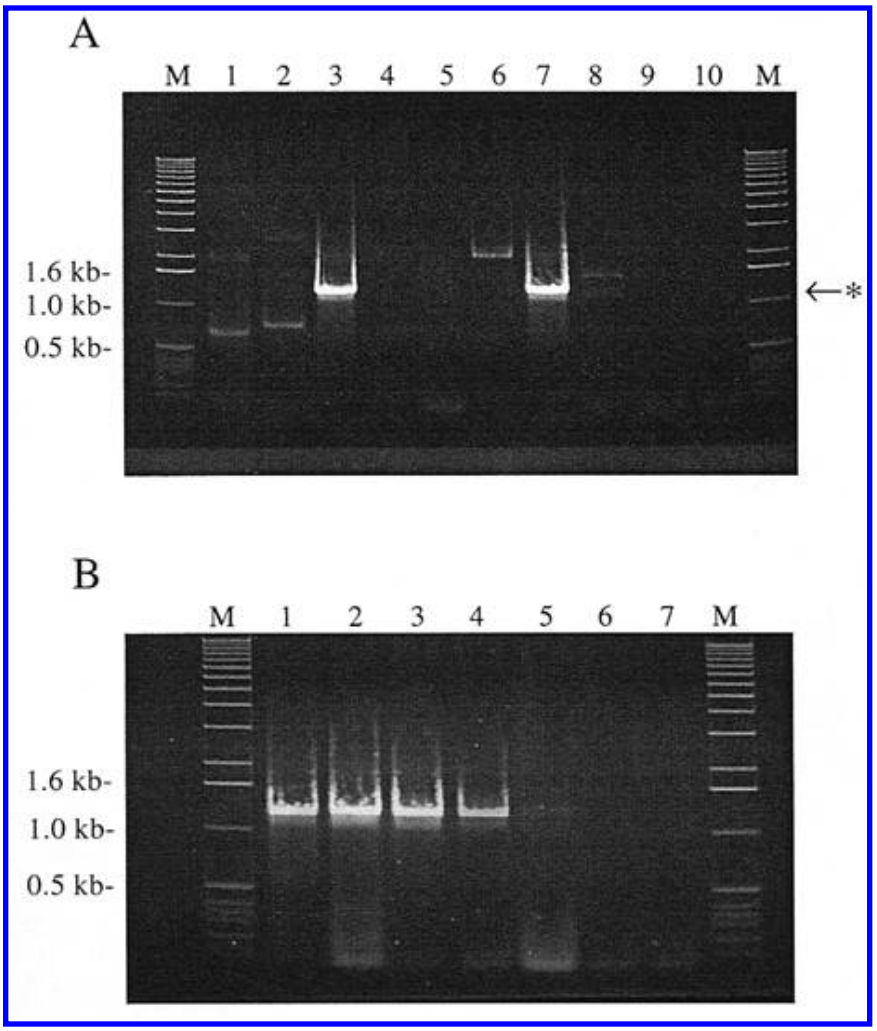

Fig. 3. A, Detection of *Erwinia pyrifoliae* with primers CPS1/CPS2c. Lane 1, *Erwinia herbicola*; 2, *Escherichia coli*; 3, *E. pyrifoliae* Ep1/96; 4, *Enterobacter pyrinus*; 5, *Erwinia stewartii*; 6, *Pseudomonas syringae*; 7, Extract from a necrotic pear seedling inoculated with *E. pyrifoliae*; 8, *Xanthomonas campestris*; 9, *Erwinia amylovora* Ea1/79; 10, water control; ←* indicates the position of 1.2 kb, expected for *E. pyrifoliae* Ep1/96. **B,** Sensitivity of detection of *E. pyrifoliae* with CPS primers. Cells from an overnight culture of *E. pyrifoliae* Ep1/96 (2×10^9 bacteria per ml) were diluted in steps of 10. In lanes 1 to 6, aliquots with 2×10^5, 10^4, 10^3, 10^2, 10^1, and 10^0 CFU were assayed. Lane 7: water control; M, marker of 1-kb ladder DNA (sizes of bands at the left border). The annealing temperature was 52°C.

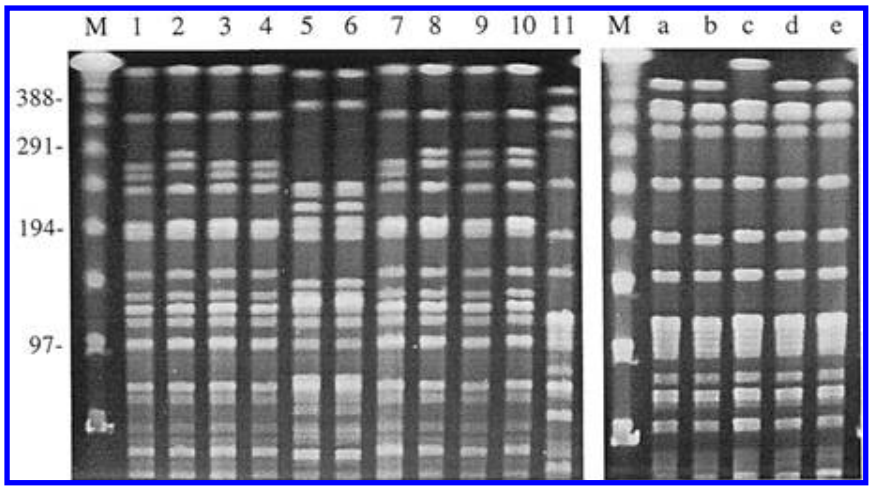

Fig. 4. Pulsed-field gel electrophoresis (PFGE) analysis of various *Erwinia pyrifoliae* and *Erwinia amylovora* strains. The genomic DNA was digested with *Spe*I for all lanes. (left) Lane: 1, Ep1/96; 2, Ep4/97; 3, Ep8/95; 4, Ep16/96; 5, Ep28/96; 6, Ep31/96; 7, Ep102/98; 8, Ep44/97; 9, Ep53/97; 10, Ep60/97 (all *E. pyrifoliae*); 11, Ea1/79 (*E. amylovora*). (right) Lane a, S59/5 (classified with an *Xba*I digest as pattern type Pt1); b, Ea4/82 (Pt2); c, CFBP1367 (Pt3); d, Ea775 (Pt4); e, 115.22 (Pt5). Conditions for PFGE were: 21 h, 5 V/cm; 129 mA; the ramping time was 1 s to 25 s. M, DNA marker of multimeric λ genomes (sizes in kb at the left border).

regions and various times of isolation (Fig. 4, lane 11 and lanes a to e). Only Pt3-strains show a large *Spe*I-fragment at 800 MDa, a band missing from most other *E. amylovora* strains. For *E. pyrifoliae*, the pattern types PtA and PtB were closely related to each other and differed only in the position of one band. The strains grouped into PtA were isolated in 1995, 1996, and 1998 in orchards near Chuncheon in South Korea. This indicates a conservation of the pattern during at least 3 years. Pattern type PtC is represented in strains from 1996 and is quite diverged from the two other pattern types. The data show a pronounced tendency of *E. pyrifoliae* to occur in distinguishable strains within a small area of their isolation. Explanations for the RFLPs could be a high tendency for genome changes or the occurrence of different strains independently evolved in this area.

Assays for the host range of *E. pyrifoliae*. Several fire blight host plants were challenged with pathogenic strains of *E. pyrifoliae* and *E. amylovora,* including rubus variants (CFBP2151 and IL6). In field assays, the *E. amylovora* fruit tree strain CFBP3049 was positive for most pear and apple cultivars, hawthorn (one exception), and cotoneaster, but only for one of the four *Prunus salicina* cultivars tested and negative for the raspberry cultivars (Table 4). In growth chamber experiments, seedlings from open pollinated apple (Golden Delicious), European pear (*Pyrus communis* cv. Bartlett), Asian pear (*P. pyrifolia*), and cotoneaster (*Cotoneaster pannosus*) were inoculated at the tips of leaves, which were cut off with scissors. The *E. pyrifoliae* strain Ep1/96 produced necrosis only on leaves of Asian and European pear seedlings, but not on apple seedlings (Table 4). In the experimental orchard, pathogen suspensions were injected into shoot tips. Strain Ep1/96 produced good symptoms on most pear cultivars. Strains Ep16/96, Ep28/96, and Ep31/96 were also positive, but less virulent. To a low degree, the four *E. pyrifoliae* strains produced weak symptoms on some apple cultivars, such as Ep1/96 on Idared, but never on hawthorn, cotoneaster, raspberry, or plum (Table 4). The host range of *E. pyrifoliae* is thus largely restricted to pear, with little impact on apple and none for other host plants such as hawthorn or cotoneaster. The data were confirmed by inoculation of *C. pannosus*, which is highly susceptible for fire blight. *E. amylovora* strain Ea1/79 readily produced necrotic symptoms in contrast to *E. pyrifoliae* strain Ep1/96 and the rubus strain IL6.

Detection of *E. pyrifoliae* by PCR assays in necrotic plant tissue including field studies. Besides leaves from inoculated Nashi pear seedlings grown in a growth chamber, necrotic tissues (branches, leaves, small pear fruits) from

diseased Nashi pear trees of an orchard near Chuncheon (South Korea) were extracted in 1997–98, and positive signals were obtained with primer pairs EP16A/EPIG2c and CPS1/CPS2c. In addition, the pathogen was also isolated on agar plates, and *E. pyrifoliae* was confirmed by PCR assays. In a sample with intermediate to strong PCR signals, about 1,000 CFU/g of plant material was recovered.

Several attempts were made to isolate *E. pyrifoliae* from Korean Nashi pear orchards in 1999 and 2000. In three orchards near Chuncheon in South Korea, samples of necrotic tissue were taken from 150 Nashi pear fruit trees in the late growth season 1998. The orchards had been treated before with chemicals containing copper hydroxide and streptomycin. Dilutions of the water extracts from leaves or bark were plated on LB-agar and white colonies transferred on MM2Cu agar. Cells of yellowish mucoid colonies were screened with primers CPS1 and CPS2 (from the *cps* region) and with EP16A and EPIG2c (from the 16S rRNA/ITS region) (Fig. 1), and no correct amplification product was obtained. With primers CPS1 and CPS2c, a false band above 1.2 kb was visible for some of these strains, and a *Hae*III digest produced DNA fragments different from 653, 273, 210, and 86 bp expected and confirmed for the PCR product of *E. pyrifoliae.*

DISCUSSION

E. pyrifoliae, the causative agent of Asian pear blight, was isolated from orchards in the region of Chuncheon in South Korea (13). The occurrence of the disease in other Korean regions with growth of Nashi pears has not been reported. After sanitary measurements in presumably affected orchards, Asian pear blight has not been recently detected again, even at sites of its original isolation. To facilitate screening for *E. pyrifoliae*, two PCR primer pairs were designed, based on the 16S rRNA/ITS region and the *cps* region corresponding to the *ams* region of *E. amylovora* (4). The primer pair from the *cps* genes of *E. pyrifoliae* can also detect *Erwinia* strains from Japan (12), but not *E. amylovora*, the causative agent of fire blight, which has not been described to occur in Korea. The rRNA primers for detection of *E. pyrifoliae* were only positive for a part of the Japanese *Erwinia* strains assayed (12).

Differences in the genomes of *E. pyrifoliae* and *E. amylovora* were further confirmed by PFGE analysis. Digestion of a large number of *E. amylovora* strains from different regions and times of isolation indicated a high conservation of PFGE patterns (16), especially for digests with restriction enzyme *Spe*I (Fig. 4, right panel). Digests with *Xba*I produced typical patterns which allowed their grouping for large regions of the Mediterranean area and of Europe (16,18; S. Jock, W.-S. Kim, V. Donat, M. M. Lopez, C. Bazzi, and K. Geider, unpublished). Spontaneous changes of PFGE patterns of *E. amylovora* strains apparently occur during long times of their natural propagation and distribution. *E. amylovora* strains of PFGE pattern type Pt2, obtained from an *Xba*I digest, were isolated in Egypt, where fire blight appeared first in the 1960s and then spread to Israel, Turkey, Greece, the Balkans, and to Hungary, where the disease was first detected in 1996. In contrast, *E. pyrifoliae* has been isolated in a narrow area within South Korea. All *E. pyrifoliae* strains of 1997 were isolated from a sample derived from one diseased Nashi pear tree, so a

Table 3. Classification of *Erwinia pyrifoliae* strains according to their pulsed-field gel electrophoresis (PFGE) pattern after an *Spe*I digest

PFGE pattern	Strain
PtA	Ep1/96
	Ep8/95
	Ep16/96
	Ep102/98
PtB	Ep4/97
	Ep44/97
	Ep53/97
	Ep60/97
PtC	Ep28/96
	Ep31/96

Table 4. Disease rating of fire blight host plants after inoculation with *Erwinia pyrifoliae* and *Erwinia amylovora* strains

Plant species Cultivar	*E. amylovora*	*E. pyrifoliae*	*E. amylovora*
	CFBP3049[$a]	Ep1/96[$]	CFBP2151 (Rubus)[$]
Cotoneaster sp.			
Skogolm	+[b]	–	ND
Crataegus			
folia lavallei	+	–	ND
grignonensis	–	–	ND
oxyacantha paulii	+	–	ND
oxyacantha punicea	+	–	ND
seedling 27.61	+	–	ND
seedling 28.23	+	–	ND
tanacetifolia	+	–	ND
turkestanica	+	–	ND
Malus domestica			
Golden Delicious	+	–	ND
Idared	+	+	ND
Prairie fire	+	–	ND
Prima	+	–	ND
Repinaldo Picon	+	–	ND
Xuanina	+	–	ND
hupehensis	+	–	ND
Prunus salicina			
Angelo	–	–	ND
Fortune	+	–	ND
Friard	–	–	ND
hybrid (INRA)	–	–	ND
Pyrus communis			
4703/78	+	+	ND
Conférence	+	–	ND
Harrow Gold	+	–	ND
Harrow Sweet	+	+	ND
HW614	+	–	ND
HW619	+	–	ND
hybrid (INRA)	+	+	ND
Old Home	+	+	ND
US 65.063.13	+	+	ND
Rubus idaeus			
Heritage	–	–	+
Malling Promise	–	–	+
Wawi	–	–	+
	Ea1/79*[c]	Ep1/96*	IL6 (Rubus)*
Cotoneaster pannosus	+	–	–
Malus domestica			
Golden Delicious	+	–	–
Pyrus communis			
Bartlett	+	+	ND
P. pyrifolia	+	+	ND

[a] $, shoot inoculations in experimental orchard. Inoculations with *E. pyrifoliae* strains Ep16/96, Ep28/96, and Ep31/96 were done in parallel with Ep1/96 with similar results.
[b] + or –, with or without fire blight symptoms; ND, not assayed.
[c] *, leaf inoculations in a growth chamber on plants from open pollinated seeds.

unique PFGE pattern can be expected. On the other hand, the population of *E. pyrifoliae* in this plant tissue was not homogeneous, because part of the isolates were HR-negative with the same PFGE-pattern as the HR-positive isolates (S. Jock, W.-S. Kim, and K. Geider, *unpublished*). For isolates from different samples, the PFGE pattern of an *Spe*I digest was quite diverse, and the isolated strains could be grouped into three pattern types. There are no data on how long Asian pear blight could have existed in Korea before its first described isolation in 1995. Divergent PFGE patterns could thus reflect slow independent evolution of strains within that area or introduction from other regions or just a higher tendency of *E. pyrifoliae* for genome changes than those observed for *E. amylovora*. Most recently, the pear pathogen has not been isolated in Korea. For isolation attempts during the late growth season, other bacteria could have replaced *E. pyrifoliae* in the affected plant tissue, or weak necrotic symptoms could have been caused by other microorganisms. The pathogen also could not be isolated in early growth seasons from Nashi pear fruit trees nor from necrotic tissue of apple trees (cv. Fuji). Accordingly, recent phytosanitary measurements in possible affected orchards could have reduced or even eliminated *E. pyrifoliae* in the region of Chuncheon in South Korea.

The host range of *E. pyrifoliae* is apparently limited to pears, especially Asian pears (*Pyrus pyrifolia*), but it can also infect European pears. Apple cultivars can occasionally produce slight symptoms. Clear symptoms were not observed for several fire blight host plants, although among more than 200 species (9), others might exist that are susceptible to *E. pyrifoliae*. For some plants such as apricots, cherry, or even strawberries, *E. amylovora* can be an opportunistic pathogen (16) without an ability to persist in those plants. It is possible that more plants than the described hosts can contribute to the spread of fire blight. Asian pear blight may also be able to infect more plants than Nashi pears and could also persist in plants without obvious symptom formation. Specific primers for sensitive detection of *E. pyrifoliae* are therefore convenient tools, not only to identify Asian pear blight on diseased Nashi pear trees, but also to trace the pathogen in asymptomatic plant tissue.

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