Host Range and Molecular Phylogenies of the Soft Rot Enterobacterial Genera *Pectobacterium* and *Dickeya*

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ABSTRACT

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Pectobacterium and *Dickeya* spp. are related broad-host-range enterobacterial pathogens of angiosperms. A review of the literature shows that these genera each cause disease in species from at least 35% of angiosperm plant orders. The known host ranges of these pathogens partially overlap and, together, these two genera are pathogens of species from 50% of angiosperm plant orders. Notably, there are no reported hosts for either genus in the eudicots clade and no reported *Dickeya* hosts in the magnoliids or eurosids II clades, although *Pectobacterium* spp. are pathogens of at least one plant species in the magnoliids and at least one in each of the three eurosids II plant orders. In addition, *Dickeya* but not *Pectobacterium* spp. have been reported on a host in the rosids clade and, unlike *Pectobacterium* spp., have been reported on many Poales species. Natural disease among nonangiosperms has not been reported for either genus. Phylogenetic analyses of sequences concatenated from regions of seven housekeeping genes (*acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA*) from representatives of these genera demonstrated that *Dickeya* spp. and the related tree pathogens, the genus *Brenneria*, are more diverse than *Pectobacterium* spp. and that the *Pectobacterium* strains can be divided into at least five distinct clades, three of which contain strains from multiple host plants.

Pectobacterium spp. Waldee, 1945 (formerly *Erwinia carotovora*, Winslow et al. 1920) and *Dickeya* spp. Samson et al. 2005 (formerly *Erwinia chrysanthemi*, Burkholder et al. 1953) species are related soft rot enterobacterial pathogens with broad host ranges. These species formerly were known as the soft rot *Erwinia* spp., but several studies have shown that the soft rot enterobacteria and *E. amylovora*, the type strain of the *Erwinia* genus, are too divergent to be included in one clade; therefore, the soft rot *Erwinia* spp. were moved to two new genera as *Pectobacterium* and *Dickeya* (47,52,112,143).

Pectobacterium and *Dickeya* spp. are considered broad-host-range pathogens in part because they have been isolated from so many plant species and in part because single strains are pathogens of numerous plant species under experimental conditions. In contrast, some plant pathogens, such as *Clavibacter michiganensis*, can cause disease on only a limited number of plant species. Other pathogens, such as *Pseudomonas syringe*, as a species can cause disease on a wide range of hosts, but individual *P. syringae* strains have narrow host ranges limited to a single plant species or even varieties within a single plant species.

Exceptions to the broad-host-range nature of *Pectobacterium* spp. are the species *Pectobacterium atrospeticum* Gardan et al. 2003, which is reported almost exclusively on potato (*Solanum tuberosum* L.), and *P. betavasculorum* Gardan et al. 2003, which is reported almost exclusively on sugar beet. Genetic analysis of *P. atrosepticum* strains has shown that they are all closely related and suggests that there is limited diversity in *P. atrosepticum* compared with *Pectobacterium* spp. as a whole (11,34). Similarly, there appears to be specialization for pathogenesis of maize (*Zea mays* L.) among some *Dickeya* strains (73,99). However, the genetic basis for this apparent specialization is not yet known for either *Pectobacterium* or *Dickeya* spp.

There are no recent reviews cataloging the host range of these two genera and there has been little or no genetic characterization of soft rot enterobacterial strains from most host species. In the past, soft rot enterobacterial strains isolated from new hosts were identified, usually by biochemical reactions, as either *E. carotovora* subsp. *carotovora* or *E. chrysanthemi*, and little attempt was made to determine how strains from newly reported hosts were related to other strains of these bacterial species. Therefore, we do not know if, for example, *Pectobacterium* strains isolated from sunflower are all members of a particular genetic clade, just as all *P. atrosepticum* strains are closely related to each other. Unfortunately, obtaining cultures of the strains that have been reported is difficult or impossible; therefore, it is not possible to connect previous reports of soft rot enterobacterial pathogens to current enterobacterial taxonomy. Recently, there has been more effort to type strains isolated from various hosts. For example, Palacio-Bielsa et al. (102) found that four different *Dickeya* biovars could be isolated from potato in Spain, which allows some comparison among biovars found on potato and other hosts.

Recently, we examined the genetic diversity of *Pectobacterium* strains isolated from potato and found considerable diversity (146), similar to previous work using other methods, such as fatty acid analysis (34). This led us to hypothesize that multiple sub-

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types of *Pectobacterium* were pathogens of potato and also led us to question the relationship of *Pectobacterium* strains that cause disease in potato with *Pectobacterium* strains from other host plants.

To address this question, we obtained *Pectobacterium* and *Dickeya* strains isolated from a variety of plant species and employed multilocus phylogenetic analysis to characterize the relationship between these nominal species and subspecies. We chose to use a multilocus approach because it has been demonstrated to be more reliable than single-gene phylogenies. In addition, in at least one other system, this method was better correlated with DNA:DNA hybridization assays than phylogenies built from 16S rRNA genes (100). We also chose this method because it is inexpensive, relatively simple, and allows researchers to easily share phylogenetic data without the necessity of sharing strain collections. Unfortunately, obtaining numerous cultures from type collections is often cost prohibitive for laboratories and it is difficult to obtain permits for some bacterial pathogens. Therefore, our goal was also to determine whether multilocus phylogenetic analysis provided enough information to group strains into clades with high statistical support. This robust phylogeny served as a foundation to compare the genetic relationships of the strains with the phylogenetic distribution of hosts to complement our extensive compilation from the literature.

MATERIALS AND METHODS

Isolation of *Pectobacterium* spp. Plant samples were obtained from commercial growers, including marigold (*Tagetes erecta* L.), cabbage (*Brassica oleracea* var. *capitata* L.), pepper (*Capsicum* spp.), potato, and *Ornithogalum* spp., and during examination of wild plants growing on the edges of farms (*Arctium minus* Bernh.). *Pectobacterium* strains were isolated from plant samples using standard protocols (33) or on raffinose medium (RAF) without antibiotics (115) (Table 1). The plates were incubated at room temperature for up to 3 days and colonies that had formed pits in the CVP or that were red on the RAF medium were restreaked onto Luria-Bertani (LB) medium. If colonies with different morphologies were present on LB, bacteria were streaked from individual colonies to obtain pure cultures. Cultures then were streaked on CVP to determine whether all of the resulting colonies were pectolytic. Cultures obtained from single colonies were stored in 20% glycerol at –80°C. All strains except those from *A. minus* were tested for pathogenicity on the host species that they were isolated from and most also were tested for ability to macerate potato tubers. Although we collected several seed from *A. minus*, we were unable to get the seed to germinate and, thus, were unable to test the *A. minus* strain WPP236 for pathogenicity on *A. minus*. WPP236 was able to macerate potato tubers.

Strains that varied in host or biological phenotypes, such as the presence of a type III secretion system, carbon source utilization, or relative virulence, were included in our analysis. We focused on strains for which there was a wealth of biological information. For example, there are genome sequences available for *P. atrosepticum* SCRI 1043 and *Dickeya dadantii* 3937 (12) (J. D. Glasner, *unpublished data*), a partial genome sequence is available for *P. carotovorum* WPP14, and numerous mutant analyses have been completed with Ecc 71 (23,24,29,83). Among the 52 strains that were used in our analysis, 39 have been reported on in other studies (Table 1) and the majority are available in other culture collections.

Literature review. Because, in most cases, it is not possible to determine which species or subspecies of *Pectobacterium* or *Dickeya* were isolated from the various plant species based on the information provided in the initial reports, we summarized the host range of these two genera rather than trying to determine the host range of particular soft rot enterobacterial species. Our goal was to include in our analysis only reports that met the following criteria: (i) the isolated pathogen was clearly demonstrated to be a member of the soft rot enterobacteria by DNA analyses or phenotypic tests; (ii) Koch's postulates were completed, demonstrating that the isolated *Erwinia* strain indeed could cause disease on the host plant; and (iii) the report described naturally occurring diseases and not laboratory host range studies. One exception was the inclusion of Amusa and Baiyewu (5), which describes Pectobacterium soft rot of yam (*Dioscorea* L. spp.). Unfortunately, we were unable to obtain a copy of this report, but decided to include it in our analysis because it has been referenced by other authors.

DNA isolation, polymerase chain reaction amplification, and DNA sequencing. Bacterial cultures were revived from frozen stocks, streaked onto LB agar, and then inoculated into LB broth. The cultures were grown in a shaking incubator at 25°C for *P. atrosepticum* and *E. amylovora* Winslow et al. 1920 and 36°C for all other strains. DNA was extracted from overnight broth cultures as described by Staskawicz et al. (124). The precipitated DNA then was quantified using a SPECTRAmax Plus (Molecular Devices Corporation, Sunnyvale, CA), adjusted to 50 ng/μl and stored at 4°C.

The *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA* genes were chosen for multilocus sequence analysis because they are present in most enterobacteria, their products are involved in diverse aspects of bacterial metabolism, preliminary analysis suggested that there would be enough sequence diversity in these genes to allow us to reconstruct bacterial phylogenies, and these genes are not clustered in the genome. Gene homoplasy was tested using methods described by Farris (44) using PAUP* 4.0b10 (127). Portions of seven genes were amplified by polymerase chain reaction (PCR) from 53 strains (Table 1). The gene fragments were amplified and sequenced with primers designed to anneal to conserved motifs (Table 2). A 25-μl PCR mix contained the following: 9.5 μl of water; 12.5 μl of Master Mix (50 units/ml Taq DNA polymerase, 400 mM dNTP dilution mix, 3 mM $MgCl_2$, and buffer solution) (Promega Corp., Madison, WI); 0.8 mM forward and reverse primer solutions; and 1 μl of diluted DNA template. PCR amplifications were carried out in a TGradient 96 well thermocycler (Biometra, Goettinggen, Germany) with the following steps: (i) a hot start at 95°C for 3 min; (ii) 30 amplification cycles of 94°C for 0.5 min, 52°C for 0.5 min, and 72°C for 1 min; and (iii) a terminal extension phase at 72°C for 6 min.

The PCR products were purified via precipitation using the QIAquick PCR purification kit (Qiagen, Valencia, CA), the Wizard SV Gel and PCR Cleanup System (Promega Corp.), or AMPure magnetic beads (Agencourt Bioscience Co., Beverly, MA). The DNA was resuspended in 25 μl of dH_2O and quantified by spectrophotometry, and the DNA template for sequencing was prepared at a concentration of 2 ng/μl per 100 bp of PCR product length. Low-yield PCR products from problematic taxa were reamplified from Tris-acetate-EDTA gel-purified bands (QIAquick gel extraction kit; Qiagen) prior to sequencing.

Sequencing reactions were performed with 2 μl of ABI BigDye Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. CleanSeq magnetic beads (Agencourt Bioscience Co.) were used to purify the sequencing reactions following the manufacturer's instructions. Automated sequencing was conducted on an ABI3730 automated sequencer in the DNA sequence laboratory at the University of Wisconsin Biotech Center. Sequence chromatogram output files initially were aligned and edited using SeqManII (DNASTAR Inc., Madison, WI). The sequences were deposited in GenBank under accession numbers EF550599 to EF550964.

Phylogenetic analyses. Phylogenetic analyses of 53 taxa (Table 1) were conducted in PAUP* 4.0b10 using a concatenated data set of partial *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA* DNA sequences with *Yersinia pestis* van Loghem 1944 and *Y. pseudotuberculosis* Smith and Thal 1965 used as outgroup

species. The *Yersinia* sequences were obtained from GenBank (GenBank accession numbers AE017042, AL590842, AE009952, and NC_006155). The alignment for this data set was performed in MegAlign 5.08 (DNASTAR) in slow-accurate mode with a pairwise alignment gap opening penalty of 10 and a gap extension penalty of 0.1, and multiple alignment gap extension penalty of 0.2. The most suitable model of sequence evolution for each partition was determined using Modeltest 3.6 (November 2004 edition) (105,106), and corresponding parameter values were estimated in PAUP* 4.0b10. Based on this model, the corresponding likelihood parameters were estimated and then applied in PAUP* 4.0b10. Several phylogenies were produced using different methods, including uncorrected and maximum likelihood corrected neighbor joining, unweighted parsimony, and parsimony with substitutions weighted according to the instantaneous rate matrix or characters weighted according to their rescaled consistency (RC) values. Resultant phylogenies were used as starting trees for maximum likelihood estimation under the estimated likelihood parameters. Bootstrap analysis was performed using 5,000 replicates with sampling limited to parsimony-informative characters.

TABLE 1. Strains used in this study

Strain[a]	Origin	Source[b]	Other studies[c]
Brenneria spp.			
EniD312	*Juglans regia* L.	S. V. Beer	…
Equ11D3	*Quercus* spp. L.	S. V. Beer	…
EluW3L16	*Lupinus* spp. L.	S. V. Beer	18
ATCC15712	*Salix alba* L.	S. V. Beer	18
Dickeya spp.			
3937 (CFBP 3855)	*Saintpaulia ionantha* H. Wendl., France	79	112
646	*Oryza sativa* L., Japan	R. S. Dickey	…
721	*Aloe barbadensis* L., Arizona	R. S. Dickey	…
600	*Ipomoea batatas* L., Geogia	R. S. Dickey	…
586	*Philodendron* Schott, Florida	R. S. Dickey	…
678	*Harrisia* spp. Britton, California	R. S. Dickey	…
703	*Solanum tuberosum* L., Australia	R. S. Dickey	…
0862	*Zea mays* L.	A. Kelman	…
1591	*Z. mays*	A. Kelman	…
Pectobacterium spp.			
P. atrosepticum			
SCRI1043 (BAA-672)	*S. tuberosum*, Scotland	12	…
Eca6	*S. tuberosum*, British Columbia	34	34,39
Eca31	*S. tuberosum*, Wisconsin	34	34,39,42
P. betavasculorum			
Ecb1	*Beta vulgaris* L., California	R. Lewellen	…
Ecb2	*B. vulgaris*, California	R. Lewellen	…
Ecb6	*B. vulgaris*, California	R. Lewellen	…
P. brasiliensis			
BAA-417 (1692) (Ecbr212)	*S. tuberosum*, Brazil	V. Duarte	39,42,112
BAA-419 (1695) (Ecbr371)	*S. tuberosum*, Brazil	V. Duarte	39,42
Psp938	*S. tuberosum*, Brazil	V. A. Malavolta, Jr.	…
Psp939	*S. tuberosum*, Brazil	V. A. Malavolta, Jr.	…
Psp940	*S. tuberosum*, Brazil	L. O. S. Beriam	…
WPP1	*S. tuberosum*, Wisconsin, 2001	146	146
WPP17	*S. tuberosum*, Wisconsin, 2001	146	146
WPP165	*S. tuberosum*, Wisconsin, 2004	This study	…
Ec87	*S. tuberosum*, Israel	This study	…
Ec105	*Onithogalum* spp. L. stem, Israel	This study	…
Ec46	*Capsicum anuum* L., Israel	This study	…
P. carotovorum			
Ecc21	*S. tuberosum*, Netherlands	34	34,39
Ecc63	*S. tuberosum*, Netherlands	34	34,39
Ecc71	*S. tuberosum*, Netherlands	34	34,39
Ecc190	*S. tuberosum*, New York	34	34
Ecc193	*S. tuberosum*, British Columbia	34	34,39
Ecc380	*S. tuberosum*, Oregon	34	34
WPP14	*S. tuberosum*, Wisconsin, 2001	146	146
WPP220	*Tagetes patula* L., Wisconsin, 2005	This study	…
WPP221	*Tagetes patula*, Wisconsin, 2005	This study	…
WPP236	*Arctium minus* Bernh., Wisconsin, 2005	This study	…
SCRI 482 (NCPPB 3840) (CFBP 1879)	*Cichorium intybus* L., France	46	11
Ec97	*Brassica oleracea* L., Israel	This study	…
P. wasabiae			
SCRI 488	*Eutrema wasabi* Maxim.	Y. Bertheau	11
WPP19	*S. tuberosum*, Wisconsin, 2001	146	146
WPP161	*S. tuberosum*, Wisconsin, 2004	This study	…
WPP163	*S. tuberosum*, Wisconsin, 2004	This study	…
WPP168	*S. tuberosum*, Wisconsin, 2004	This study	…
WPP172	*S. tuberosum*, Wisconsin, 2004	This study	…
Pectobacterium spp.			
Ec106	*Ornithogalum* spp. bulb, Israel	This study	…

[a] Strains were assigned to species based on previously reported assays and based on the phylogeny shown in Figure 2. Alternate strain names are given in parenthesis.
[b] Cultures from R. S. Dickey's collection were provided by A. Collmer.
[c] References for other phylogenetic or diversity studies using these strains.

MrBayes v. 3.0b4 (59–61) also was used with the same set of data and outgroups. Each run was composed of four chains starting from random trees, and 5,000,000 generations with trees sampled every 100 generations. The majority rule consensus tree was calculated after the removal of first 500 trees corresponding to a burn-in period (burn-in period is estimated based on log likelihood curve; data not shown).

Topologies resulting from each of the above phylogenetic analyses were compared through a one-tailed Shimodaira and Hasegawa (SH) test (118,119) implemented by PAUP* 4.0b10. Test distributions were generated using 10,000 resampling estimated log-likelihood (RELL) bootstrap replicates (48).

RESULTS AND DISCUSSION

The host range of the soft rot enterobacteria. There are reports of *Pectobacterium* or *Dickeya* hosts in one-half of angiosperm plant orders, and these host ranges overlap but are not identical. In the 1990s, A. Kelman began compiling a comprehensive list of all soft rot enterobacterial host species. The initial goal was to provide a listing of the first reliable peer-reviewed reports of *Pectobacterium* and *Dickeya* spp. causing disease on particular host species, to determine the extent that the reported host ranges of these two species overlap, and to determine the actual breadth of the host range of these bacterial plant pathogens. The identification of plant orders that lack reported hosts of soft rot enterobacteria are informative in that they describe plant groups where either no one has searched for soft rot disease or, possibly, plant families or orders have attributes that provide resistance to soft rot enterobacteria, such as high cell wall calcium content or low intercellular pH.

The taxonomy of the soft rot enterobacteria has been complicated, with many species names proposed and discarded, and it is not always clear from the reports whether *Pectobacterium*, *Dickeya*, or perhaps another soft rot enterobacterial genus is being described. Therefore, we included reports only where the genus appears to be either *Pectobacterium* or *Dickeya* and not reports that identified the bacteria only as a soft rot enterobacterial species. Like many plant pathogens, soft rot enterobacteria also have been reported on healthy plants from numerous species (90,93). These asymptomatic hosts are not included in this review.

In cases where the biochemical tests do not clearly match with either *Pectobacterium* or *Dickeya*, we used the identification provided by the authors unless subsequent reports on the same strains resulted in a new identification. One notable case of this is the stalk rot pathogen of maize, which was identified initially as a subspecies of *E. carotovora* (*Pectobacterium*) (111) at approximately the same time that the genus *Dickeya* was first described by Burkholder (19). Based on the descriptions of the bacterial strains and the later publications by some of the same authors who initially reported this maize pathogen as *E. carotovora*, it appears that this pathogen is consistently a *Dickeya* sp. and that there are no reliable reports of *Pectobacterium* causing disease on maize or any other Poales species.

One other case merits special mention. In 1942, a *Pectobacterium* sp. was reported on saguaro cacti (*Carnegiea gigantea* Britton and Rose 1908) (80), but the type culture was lost and the name has been rejected (2). Cactus pathogens later were isolated by Alcorn et al. (3) and a recent phylogenetic analysis of 16S rRNA sequences suggests that the cactus pathogen is more closely related to *Pectobacterium* than *Dickeya* spp. Eventually, *P. cacticida* Alcorn et al. 1991 may be moved to another genus; however, for this analysis, we have included it with the genus *Pectobacterium*.

In our literature review, we found that *Pectobacterium* spp. were reported as pathogens of species in 16 dicot plant families in 11 orders, and species in 11 monocot families in 6 orders (Tables 3 and 4; Fig. 1). *Dickeya* spp. were reported to cause disease in plant species in 11 dicot families in 10 plant orders, and species in 10 monocot families in 5 orders. In many cases, only one report of one species per family and order could be found. It is likely that additional species in these families and orders are soft rot enterobacterial hosts.

Although *Pectobacterium* and *Dickeya* spp. cause disease in many identical host species, their reported host ranges do not completely overlap. Of the 21 dicot and 16 monocot plant families reported as hosts for either *Pectobacterium* or *Dickeya* spp., only 6 dicot and 4 monocot families have been reported as hosts for both genera. This lack of host overlap extends in part to higher levels of classification (Fig. 1). For example, *Pectobacterium* has been reported on avocado (*Persea americana* Miller) (140), which is in the magnoliids clade (15), but there are no reports of *Dickeya* spp. causing disease in any magnoliids clade species. We could find no reports of soft rot enterobacteria causing disease under natural conditions in nonangiosperms, the basal angiosperm orders, or the eudicots clade. Because there are so few reports for some plant clades, such as the magnoliids, the partial lack of overlap in the *Pectobacterium* and *Dickeya* host ranges may be due to a paucity of reports for plant hosts in these clades.

TABLE 2. Primers used in this study

Gene	Full name	Sequence length[a]	Primer name	Primer sequence	Tm	Bases
acnA	Aconitate hydrase 1	300	acnA3F	CMA GRG TRT TRA TGC ARG AYT TTA C	54.2	25
	…	…	acnA3R	GAT CAT GGT GGT RTG SGA RTC VGT	60.2	24
gapA	Glyceraldehyde-3-phosphate dehydrogenase A	450	gapA326F	ATC TTC CTG ACC GAC GAA ACT GC	60.7	23
	…	…	gapA845R	ACG TCA TCT TCG GTG TAA CCC AG	60.2	23
icdA	Isocitrate dehydrogenase, specific for NADP+	520	icdA400F	GGT GGT ATC CGT TCT CTG AAC G	58.4	22
	…	…	icdA977R	TAG TCG CCG TTC AGG TTC ATA CA	59.5	23
mdh	Malate dehydrogenase	460	mdh86F	CCC AGC TTC CTT CAG GTT CAG A	60.1	22
	…	…	mdh628R	CTG CAT TCT GAA TAC GTT TGG TCA	57.3	24
mtlD	Mannitol-1-phosphate dehydrogenase	390	mtlD146F	GGC CGG TAA TAT CGG CCG TGG	63.5	21
	…	…	mtlD650R	CAT TCG CTG AAG GTT TCC ACC GT	61.5	23
	…	…	mtIDF	CTG YTG GAT GCI CTS AAC MGY CG	63.7	23
	…	…	mltDR	TCC ACR GCR GAA TCW ACR AAT CC	58.3	23
pgi	Glucose-6-phosphate isomerase	520	pgi815F	TGG GTC GGC GGC CGT TAC TC	65.3	20
	…	…	pgi1396R	TGC CTT CGA ATA CTT TGA ACG GC	59.5	23
	…	…	pgiF2	CTG TCY ACC AAT GCS AAA GCC G	59.9	22
	…	…	pgiR2	CAG CAG GAT GGA GTT GGT CGG	60.2	21
	…	…	pgiF	TCT YTI GGI TTT GAK AAY TTT GA	53.0	23
	…	…	pgiR	YGC CGC YGI AAA TTC IGC TTC	61.9	21
proA	Gamma-glutamylphosphate reductase	630	proAF1	CGG YAA TGC GGT GAT TCT GCG	60.4	21
	…	…	proAR1	GGG TAC TGA CCG CCA CTT C	58.0	19

[a] Only the middle portion of sequence from each amplicon was used because the ends of the sequences included the degenerate primer sequences.

However, there is some apparent specialization that is particularly striking. For example, there are *Pectobacterium*, but not *Dickeya*, hosts in all three of the plant orders in the eurosids II clade (Fig. 1), including cabbage (Brassicales), cotton (*Gossypium hirsutum*, Malvales), and mango (*Mangifera indica* L., Sapindales). Because these crop species are widely grown and studied, the lack of reports of *Dickeya* spp. soft rot hosts in the eurosids II clade may be significant. It is also notable that *Dickeya* spp., but not *Pectobacterium* spp., have been reported on several agriculturally important *Poales* spp., ranging from rice to maize. Because *Poales* spp. are so widely grown and studied, the lack of reports of *Pectobacterium* spp. on these hosts may be significant. Because PCR assays are now available that can distinguish between *Dickeya* and *Pectobacterium* spp. (31,68,98), it should be a straightforward matter to survey diseased plants and determine whether *Dickeya* spp. can cause disease on eurosids II species, whether *Pectobacterium* spp. can cause disease in *Poales* spp., and whether either genus can cause disease in hosts from any of the many orders of plants that have never been reported to be hosts for soft rot enterobacteria.

Phylogenetic reconstructions indicate monophyly of *Pectobacterium, Dickeya*, and *Brenneria* lineages. To date, only phylogenies built with single genes have been used to examine the relationships of the plant-pathogenic enterobacteria (18,47, 52,112) and, because these single genes do not have many informative characters, they "may not accurately reflect interspecies taxonomic relatedness" (18). In these phylogenies, the bootstrap values for branches that describe how these three genera are related generally are either not significant or were not presented.

We chose fragments of seven housekeeping genes to examine the relatedness of these three genera. Several tests were used to

TABLE 3. Host species of the genera *Pectobacterium* and *Dickeya*

Host family	Host species	References	
		Pectobacterium	*Dickeya*
Dicotyledons			
Amaranthaceae (amaranth family)	*Beta vulgaris* L.	129,133,134[a]	No report
	Spinacia oleracea L.	120	No report
Anacardiaceae (sumac family)	*Mangifera indica* Thwaites	51	No report
Apiaceae (carrot family)	*Apium graveolens* Cham.	145	No report
	Arracacia xanthorrhiza Bancr.	108	53
	Coriandrum sativum L.	21	No report
	Daucus carota L.	66,67	137
Asteraceae (daisy family)	*Arctium minus* Bernh.	This work	No report
	Chrysanthemum maximum (DC.) Parsa.	No report	88
	Chrysanthemum ×morifolium	No report	19[b]
	Cichorium intybus L.	46	77
	Dahlia sp. Cav.	No report	110
	Helianthus annuus L.	9	No report
	Lactuca sativa L.	125,144	No report
	Parthenium argentatum A. Gray	123	No report
	Tagetes patula L.	114	No report
Begoniaceae (begonia family)	*Begonia bertinii*	No report	109
Brassicaceae (mustard family)	*Eutrema wasabi* Maxim.	50	No report
	Brassica oleracea L.	10	No report
	Brassica rapa L.	139	No report
	Raphanus sativus L.	141	No report
Cactaceae (cactus family)	*Acanthocereus tetragonus* Hummelinck	3	No report
	Carnegiea gigantean Britton & Rose	3	No report
	Ferocactus wislizenii Britton & Rose	3	No report
	Opuntia ficus-indica Mill.	3	No report
	Opuntia fulgida Engelm.	3	No report
	Opuntia phaeacantha Engelm.	3	No report
	Opuntia stricta Haw.	3	No report
	Opuntia violacea Engelm.	3	No report
	Stenocereus gummosus A. C. Gibson & K. E. Horak	3	No report
	Stenocereus thurberi Buxb.	3	No report
Caryophyllaceae (pink family)	*Dianthus caryophyllus* L.	No report	78
Convolvulaceae (morning glory family)	*Ipomoea batatas* Lam.	No report	113
Crassulaceae (stone crop family)	*Kalanchoë blossfeldiana* Poelln.	43	37

(*continued on next page*)

[a] Although not the first report, the report by Thomson et al. (133) was the first to definitively demonstrate that *P. betavasculorum* (*E. carotovora* subsp. *betavasculorum*) differed from other *Pectobacterium* strains.
[b] This is the first time the name *E. chrysanthemi* was used.
[c] This report of *Pectobacterium* (*E. carotovora*) causing disease in poinsettia was made 20 years prior to the description of *Dickeya* spp. (*E. chrysanthemi*) and few details are given. Therefore, it is possible that the pathogen described here is actually a *Dickeya* sp.
[d] Only alfalfa sprouts and nonmature plants have been reported as a host.
[e] Two references are included because Spurr (122) found both *Pectobacterium* and *Dickeya* spp. on tobacco.
[f] Two types of strains were recovered. One of them appears similar to *Dickeya* spp. by biochemical tests, but did not grow at 37°C.
[g] McFadden (91) named this species *E. dieffenbachiae* because it differed from *E. chrysanthemi*. For example, it is salt tolerant and MR plus, but otherwise resembles *E. chrysanthemi* and is likely to be a *Dickeya* sp.
[h] The initial identification of the soft rot enterobacterium causing disease in pineapple was genus *Pectobacterium*, but later studies showed that this pathogen is a *Dickeya* sp.
[i] The strains causing disease in banana were described as both *Pectobacterium* and *Dickeya* spp. and few details were provided on strain characterization. Therefore, it is unclear based on these reports whether species cause disease in banana. Dickey showed in later studies that strains from banana are *Dickeya* spp.
[j] The pathogen was identified as *Pectobacterium* spp. (*E. carotovora*) in this short communication; however, the biochemical reactions reported, including a lag in acid production from lactose, growth at 40°C, indole production, MR negative, and VP positive indicate that the strain was more similar to a *Dickeya* sp.
[k] Both Sabet (111) and Kelman et al. (69) recognized that the maize pathogen differed from typical *Pectobacterium* spp. (*E. carotovora*), but did not recognize it as similar to the recently described *Dickeya* spp. (*E. chrysanthemi*). Later literature demonstrated that this pathogen is a *Dicekya* sp. (40,57).

determine whether the gene fragments were equally informative and to examine the phylogenies constructed by different algorithms. To characterize these seven gene data sets, three indicators were used, including f values and f ratio, RC index, and homoplasy index (HI) (Table 5). The various goodness-of-fit statistics suggest that the rate of evolutionary divergence of the genes used in this study is sufficiently homogenous and they are appropriate and informative to perform a reasonable phylogenetic estimation. The branching orders of *Pectobacterium*, *Dickeya*, and other major lineages are recovered in phylogenies implemented in PAUP and the Bayesian reconstructions using the TrN method (130) with a γ correction for rate variation and invariant sites, which was selected by Modeltest 3.6 as the most suitable model of sequence evolution based on hierarchical likelihood ratio tests (hLRTs). The selected model was applied using different computational algorithms implemented in PAUP* 4.0b10. A topology test (SH test) was performed on the phylogenies obtained from the uncorrected and maximum-likelihood-corrected neighbor joining, unweighted parsimony, and parsimony with substitutions weighted according to the instantaneous rate matrix or characters weighted according to their RC values (44), together with the last 50 trees produced by the Bayesian analysis. The maximum likelihood phylogeny based on the combined data set was selected to be the best tree among all the other phylogenies recovered from the different computational algorithms. The substitution and character weighted parsimony is the second-best tree, the last 50 trees produced in Bayesian are the third-best tree, and unweighted parsimony are not significantly worse than the maximum likelihood tree. However, trees based on both distance methods are significantly worse ($P < 0.05$).

The relationships of *Pectobacterium, Dickeya*, and *Brenneria* lineages and their relatives were recovered with high resolution

TABLE 3. (*continued from preceding page*)

Host family	Host species	References	
		Pectobacterium	*Dickeya*
Cucurbitaceae (cucumber family)	*Cucurbita maxima* Duchesne	114	No report
	Cucurbita pepo L.	121	No report
	Cucumis sativus L.	114	No report
Euphorbiaceae (spurge family)	*Euphorbia pulcherrima* Willd. ex Klotzsch	25[e]	55
	Manihot esculenta Crantz	30	No report
Fabaceae (pea family)	*Medicago sativa* L.	No report	104[d]
Geraniaceae (geranium family)	*Pelargonium capitatum* L.	No report	107
Gesneriaceae (African violet family)	*Saintpaulia ionantha* Wendl.	No report	79
Lauraceae (laurel family)	*Persea americana* Mill.	140)	No report
Malvaceae (mallow family)	*Gossypium hirsutum* L.	27,62	No report
Moraceae (mulberry family)	*Morus* spp. L.	128	No report
Myrsinaceae (myrsine family)	*Cyclamen* spp. L.	103	103
Primulaceae (primrose family)	*Primula* spp. L.	89	No report
Rosaceae (rose family)	*Pyrus communis* L.	21	No report
Solanaceae (nightshade family)	*Capsicum annuum* L.	142	No report
	Solanum lycopersicum L.	87	4
	Nicotiana tabacum L.	56,122[e]	122
	Solanum melongena L.	No report	70
	Solanum tuberosum L.	6	131[f]
Monocotyledons			
Agavaceae (agave family)	*Agave tequilana* F. A. C. Weber	64	No report
Alliaceae (onion family)	*Allium cepa* L.	116	No report
Araceae (arum family)	*Aglaonema pictum* Kunth	No report	92
	Aechmea fasciata Baker	No report	28
	Dieffenbachia spp. Schott	26	91,97[g]
	Philodendron selloum K. Koch	No report	94
	Scindapsus aureus Engl.	71	No report
	Syngonium podophyllum Schott	No report	72,75
	Xanthosoma sagittifolia Schott	No report	20
	Zantedeschia aethiopica Spreng	138	76
	Zantedeschia elliottiana Burtt Davy	8	No report
	Zantedeschia rehmannii Engl.	7	No report
Arecaceae (palm family)	*Phoenix dactylifera* L.	No report	1
Asphodelaceae (aloe family)	*Aloe arborescens* Mill.	10	No report
	Aloe vera Burm. F.	No report	35,65
Bromeliaceae (bromeliad family)	*Ananas comosus* Merr.	No report	81,132[h]
Dioscoreaceae (yam family)	*Dioscorea* L. spp.	5	No report
Hyacinthaceae (hyacinth family)	*Ornithogalum* spp. L.	No report	This work
Iridaceae (iris family)	*Iris* ×*germanica*	58,74	74
Lilaceae (lily family)	*Tulipa* spp. L.	14	No report
Musaceae (banana family)	*Musa paradisiaca* L.	No report	36,117,126[i]
Orchidaceae (orchid family)	*Cattleya* spp. Lindl.	82	No report
	Cymbidium spp. Sw.	86	No report
	Phalaenopsis spp. Blume	86	65
Pandanaceae (screw pine family)	*Pandanus conoideus* Lam.	135	No report
Poaceae (grass family)	*Brachiaria* spp. Griseb	No report	13
	Oryza sativa L.	No report	49
	Saccharum officinarum L.	No report	38[j]
	Sorghum bicolor Moench	No report	63
	Zea mays L.	No report	57,69,111[k]
Ruscaceae (lily of the valley family)	*Dracaena sanderiana* Mast.	22	No report
Strelitziaceae (bird of paradise family)	*Strelitzia reginae* Banks	41	No report
Zingiberaceae (ginger family)	*Elettaria cardamomum* Maton	No report	136

and strong support in both maximum likelihood (Fig. 2) and Bayesian majority-rule consensus topologies (Fig. 3). *Dickeya*, *Brenneria*, and *Pectobacterium* spp. each form strongly supported monophyletic groups, and together they form a 100% bootstrap value-supported supergroup, which is a sister to *Yersinia* spp., which were used as outgroups. *Brenneria* and *Dickeya* spp. are the most closely related to each other; together, they constitute a group with much deeper-branching lineages than the group of *Pectobacterium* spp. This suggests that the *Brenneria* and *Dickeya* spp. represent a more ancient divergence in the evolution of soft rot and non-soft rot enterobacteria. A more comprehensive understanding of these two lineages will require analysis with a much larger strain collection.

The incongruence of our phylogenies and the trees from other studies in inferring the evolutionary relationships of soft-rotting *Pectobacterium* and *Dickeya* lineages and non-soft-rotting *Brenneria* lineages. Our phylogenies also support the emergence of a nonpectolytic necrogenic group from a-soft rot-associated lineage. It confirms the close relationship of *Brenneria* spp. and these soft rot enterobacteria found by others (47), although our well-supported clustering of *Brenneria* and *Dickeya* spp. is at odds with Samson et al. (112), which suggests that the *Dickeya* spp. are more closely related to *Pectobacterium* spp. and form a supergroup that is sister to *Brenneria* spp. Furthermore, when Brown et al. (18) examined the phylogenetic divergence of necrogenic members of *Erwinia* and *Brenneria*, their data suggest that *Brenneria* clades are disrupted taxonomically by several clades of soft-rotting *Pectobacterium* and *Dickeya* lineages. However, both the Brown et al. (18) and Samson et al. (112) analyses lacked strong statistical support for many of the branches in their phylogenies.

The incongruence of our data with previous studies also could be caused by one or more of the following major factors in tree inferences. A disadvantage of our study is that some of the clades (e.g., *P. wasabiae* Gardan et al. 2003) are underrepresented relative to other neighboring clades because we intensively sampled *P. carotovorum* Gardan et al. 2003. However, there are several advantages of our study. (i) Our strains came from a variety of plant hosts and geographical locations, and our phylogeny has the highest number of strains used in soft rot phylogenetic analysis. (ii) We used concatenated molecular markers from seven genes. The multilocus phylogenetic assessment provides a substantial amount of phylogenetically informative characters, which mitigates the shortcomings of single-gene phylogenetic inference. The previous phylogenetic studies almost exclusively relied on 16S rRNA gene phylogenies. Although 16S rDNA gene sequences have several advantages, including being effectively a single-copy gene, highly conserved, and easy to amplify, these advantages are partly offset by its other properties. The 16S rDNA is relatively short and contains fewer phylogenetically informative characters, and some of the variable sites tend to get saturated (45,54,101). (iii) We used statistics-based algorithms which have been proven to be much more reliable than distance-based algorithms that were used in other studies. (iv) We used model-based phylogenetic analysis in this study, whereas the other studies did not. Model selection recently is considered to be a critical step in phylogenetic analysis in order to find the most suitable model of sequence evolution; it corrects for multiple substitutions at sites and is especially important for anciently divergent sequences (45,101). (v) Furthermore, phylogenies selected by the topology test are in agreement with each other, demonstrating consistency in the reconstructions, and bootstrap values and posterior probabilities indicate strong confidence in the results. Both consistency and confidence yield a robust and much more reliable phylogeny. (vi) Finally, we used sequences from members of the closely related genus *Yersinia* as outgroups, which maximizes the likelihood of observing the most informative polarities and inferring the most recent common ancestor in our phylogenetic trees. Other studies use either more distantly related outgroups (e.g., *Bacillus* spp. [15] or *Serratia*, *Enterobacter*, *Erwinia*, and *Pantoea* spp. [36]), or do not use outgroups at all (112).

***Pectobacterium* spp. are divided into at least five clades.** Within the genus *Pectobacterium*, there are five major clades designated I, II, III, IV, and V, which differs from previous studies. Hauben and co-workers (52) and other researchers (47) suggested that there are five subspecies or species-level clades of *Pectobacterium* (*E. carotovorum*): *atrosepticum*, *betavasculorum* Gardan 2003, *carotovorum*, *odoriferum* Gardan et al. 2003, and *wasabiae*. These analyses did not include clade I strains (*E. carotovora* subsp. *brasiliensis* Duart et al. 2004). Our reconstructed phylogenies agree that *P. atrosepticum*, *P. betavasculorum*, and *P. wasabiae* do form individual clades and place the *brasiliensis* strains in an individual clade.

The type strain for *E. carotovora* subsp. *brasiliensis*, 1692, is in clade I and, until now, this subspecies has been reported only in Brazil (39). We found that strains from both the United States and Israel were also in clade I. Over the past decades, there has been controversy over whether *P. carotovorum*, or only *P. atrosepticum*, can cause potato blackleg, which is defined as a stem necrosis extending up from a seed potato piece (95,96). Perhaps some of the confusion is due to researchers not realizing that four different clades of *Pectobacterium* were infecting potato. Ongoing genome analyses in several laboratories should shed light on which bacterial genes are required for blackleg symptoms and the distribution of these genes among *Pectobacterium* strains.

Our phylogeny places *P. carotovorum* subsp. *odoriferum* SCRI482 with other *P. carotovorum* subsp. *carotovorum* inside clade II. However, our results do not support maintaining this

TABLE 4. Number of plant orders in each clade reported as hosts of *Pectobacterium* or *Dickeya* spp.

Orders	*Pectobacterium* spp.	*Dickeya* spp.	Neither
Dicots			
Magnoliids	1/4 (*Laurales*)	0/4	3/4 (*Canellales*, *Magnoliales*, *Piperales*)
Eudicots	0/2	0/2	2/2 (*Proteales*, *Ranunculales*)
Core Eudicots	2/4 (*Saxifragales*, *Caryophyllales*)	2/4 (*Saxifragales*, *Caryophyllales*)	2/4 (*Gunnerales*, *Santalales*)
Rosids	0/3	1/3 (*Geraniales*)	2/3 (*Crossosomatales*, *Myrtales)*
Eurosids I	3/7 (*Malpighiales*, *Rosales*, *Cucurbitales*)	3/7 (*Malpighiales*, *Fabales*, *Cucurbitales*)	3/7 (*Celastrales*, *Oxalidales*, *Fagales*)
Eurosids II	1/3 (*Brassicales*, *Malvales*, *Sapindales*)	0/3	0/3
Asterids	1/2 (*Ericales*)	1/2 (*Ericales*)	1/2 (*Cornales*)
Euasterids I	1/4 (*Solanales*)	1/4 (*Solanales*)	3/4 (*Garryales*, *Gentianales*, *Lamiales*)
Euasterids II	2/4 (*Apiales*, *Asterales*)	2/4 (*Apiales*, *Asterales*)	2/4 (*Aquifoliales*, *Dipsacales*)
Monocots			
Base monocots	5/6 (*Alismatales*, *Asparagales*, *Dioscoreales*, *Liliales*, *Pandanales*)	2/6 (*Alismatales*, *Asparagales*)	1/6 (*Acorales*)
Commelinids	1/4 (*Zingiberales*)	3/4 (*Arecales*, *Poales*, *Zingiberales*)	1/4 (*Commelinales*)
Total dicots	11/33 = 33%	10/33 = 30%	18/33 = 55%
Total monocots	6/10 = 60%	5/10 = 50%	2/10 = 20%
Total families	16/43 = 37%	15/43 = 35%	21/43 = 47%

subspecies designation because the level of divergence of SCRI482 from other clade II strains is similar to or less than that seen within other clades, such as the *P. atrosepticum* and *P. wasabiae* clades. Conspicuously, our data indicate that the strains that had been classified as *E. carotovora* subsp. *carotovora* based on biochemical tests (146) do not form a monophyletic group. Rather, these strains, which are designated by WPP names, are spread among clades I, II, and III.

The clade III strains include *P. wasabiae* SCRI 488 and several strains from potato tubers. Curiously, based on PCR and DNA hybridization results, nearly all of the clade III strains, including WPP161, WPP163, WPP168, WPP172, and SCRI 488, lack the type III secretion system (T3SS) (H.-S. Kim, *unpublished data*). The T3SS is a protein secretion system found in many gram-negative pathogens where it is used to inject virulence proteins directly into host cells. Once inside, the virulence proteins

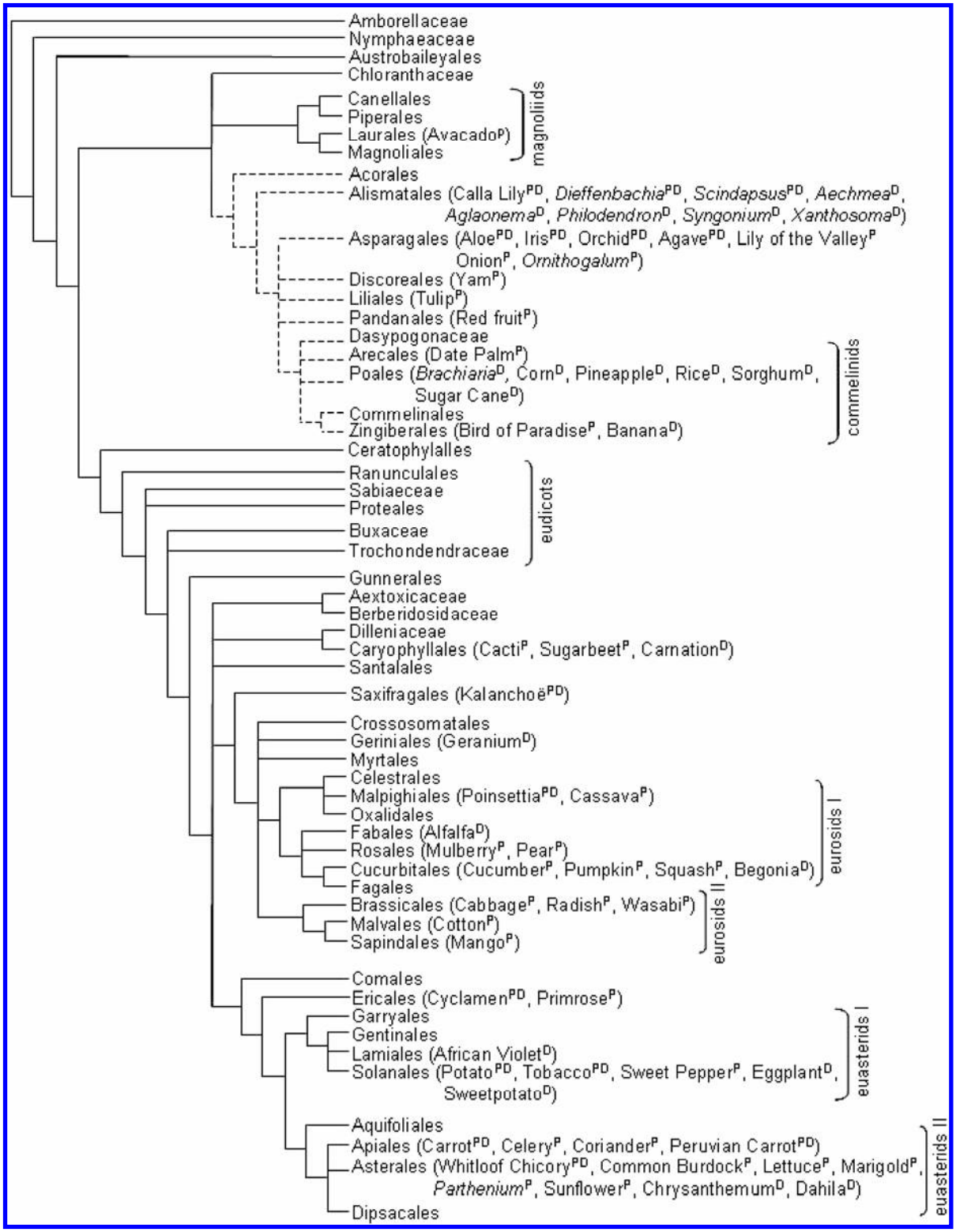

Fig. 1. Phylogenetic tree of angiosperms adapted from Bremer et al. (15) showing the reported hosts for *Pectobacterium* and *Dickeya* spp. The dotted lines denote monocot families. *Pectobacterium* and *Dickeya* hosts are indicated by a superscript "P" or "D," respectively. Plant clades are indicated with brackets on the right side of the tree.

TABLE 5. Informativeness indicators for the sequenced gene fragments[a]

Data set	No. of taxa	No. of characters	Parsimony-informative characters	No. of constant characters	RC index	f value	f ratio	HI
acnA	53	288	132	146	0.61	9,920	0.85	0.32
gapA	53	330	103	307	0.39	13,340	0.68	0.52
icdA	53	499	143	332	0.42	15,950	0.49	0.49
mdh	53	440	167	247	0.43	9,980	0.31	0.48
mtlD	53	386	177	179	0.50	14,240	0.44	0.42
pgi	51	501	173	300	0.42	19,580	0.51	0.50
proA	51	628	320	290	0.35	29,070	0.52	0.55

[a] Rescaled consistency (RC), f value, f ratio, and homoplasy index (HI) were implemented in PAUP* 4.0b10 according to Farris (44).

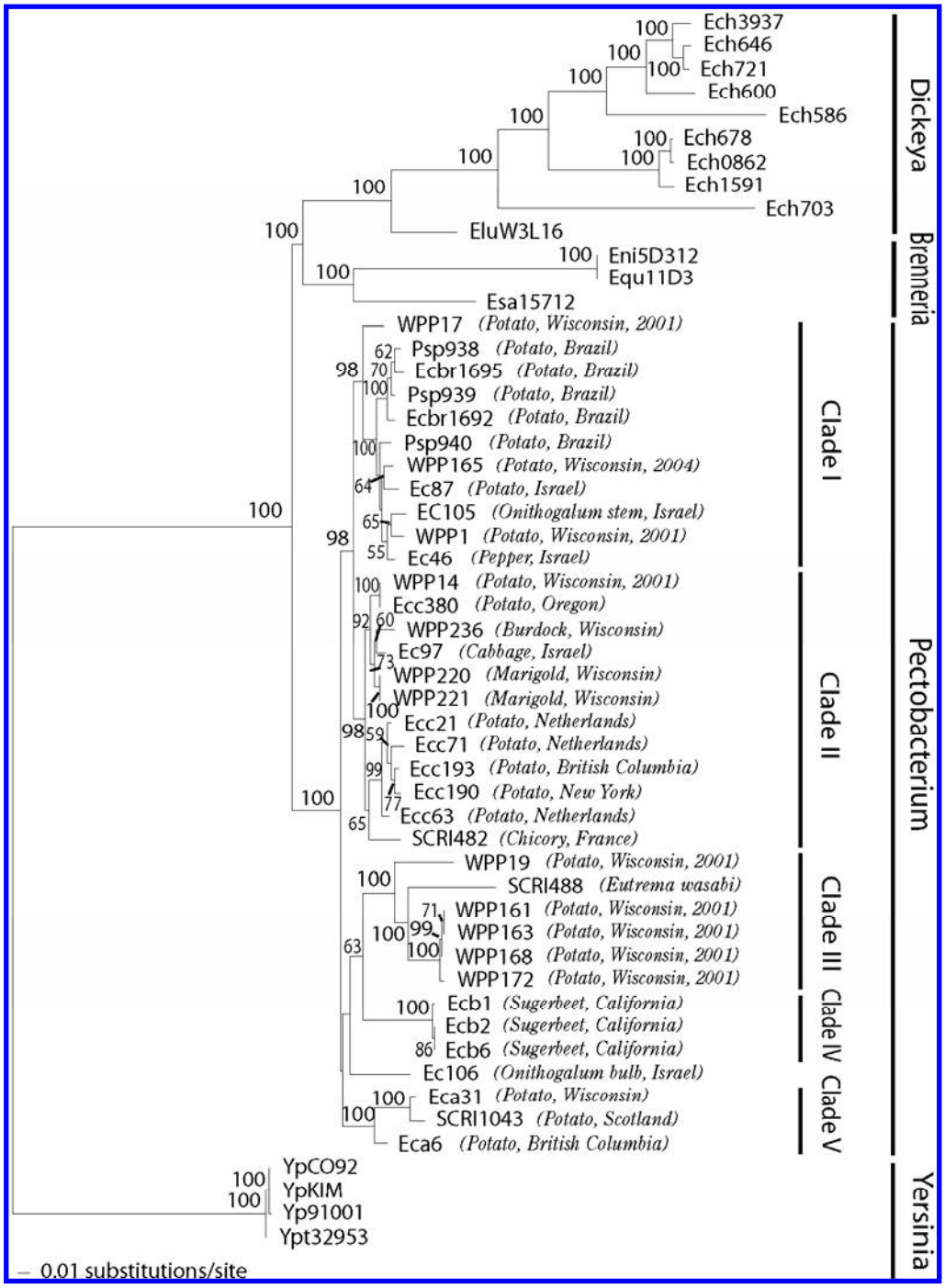

Fig. 2. Maximum likelihood tree (–LnL = 39,410.96) of 53 *Enterobacteriaceae* taxa using concatenated gene portions of *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA*. The number at each node is bootstrap support-based 5,000 replicates using heuristic research based on parsimony with substitutions weighted according to the instantaneous rate matrix. Bootstrap supports <50% were not shown. The names of *Pectobacterium* clades I–V and *Enterobacteriaceae* groups of *Dickeya, Brenneria*, *Pectobacterium*, and *Yersinia* spp. are marked on vertical lines. The host species of *Pectobacterium* were added inside parenthesis in italics next to the names of the isolated strains.

suppress host cell defenses. WPP19, which is at the base of clade III, has an atypical T3SS (146). In 2001, we isolated an atypical *Pectobacterium* strain from potato, WPP17, and found that it lacked the T3SS (146). However, WPP17 is not in clade III, suggesting that multiple losses of the T3SS have occurred. So far, T3SS-minus strains have been isolated only from potato and wasabi (*Eutrema wasabi* Maxim.); thus, we cannot determine whether the T3SS is required for *Pectobacterium* spp. to cause disease in other hosts. Experiments are ongoing to determine whether clade III strains use mechanisms analogous to the T3SS to suppress plant defenses or, alternatively, if clade III strains are unable to suppress plant defense responses.

There is an orphan taxon, Ec106, which was isolated from an *Ornithogalum* bulb, located between clades IV and V, which does not group with any other *Pectobacterium* spp. Another strain, Ec105, isolated from the stem of the same plant species, grouped firmly with other *Pectobacterium* spp. inside clade I. There are likely additional members of the "Ec106 clade" among the soft

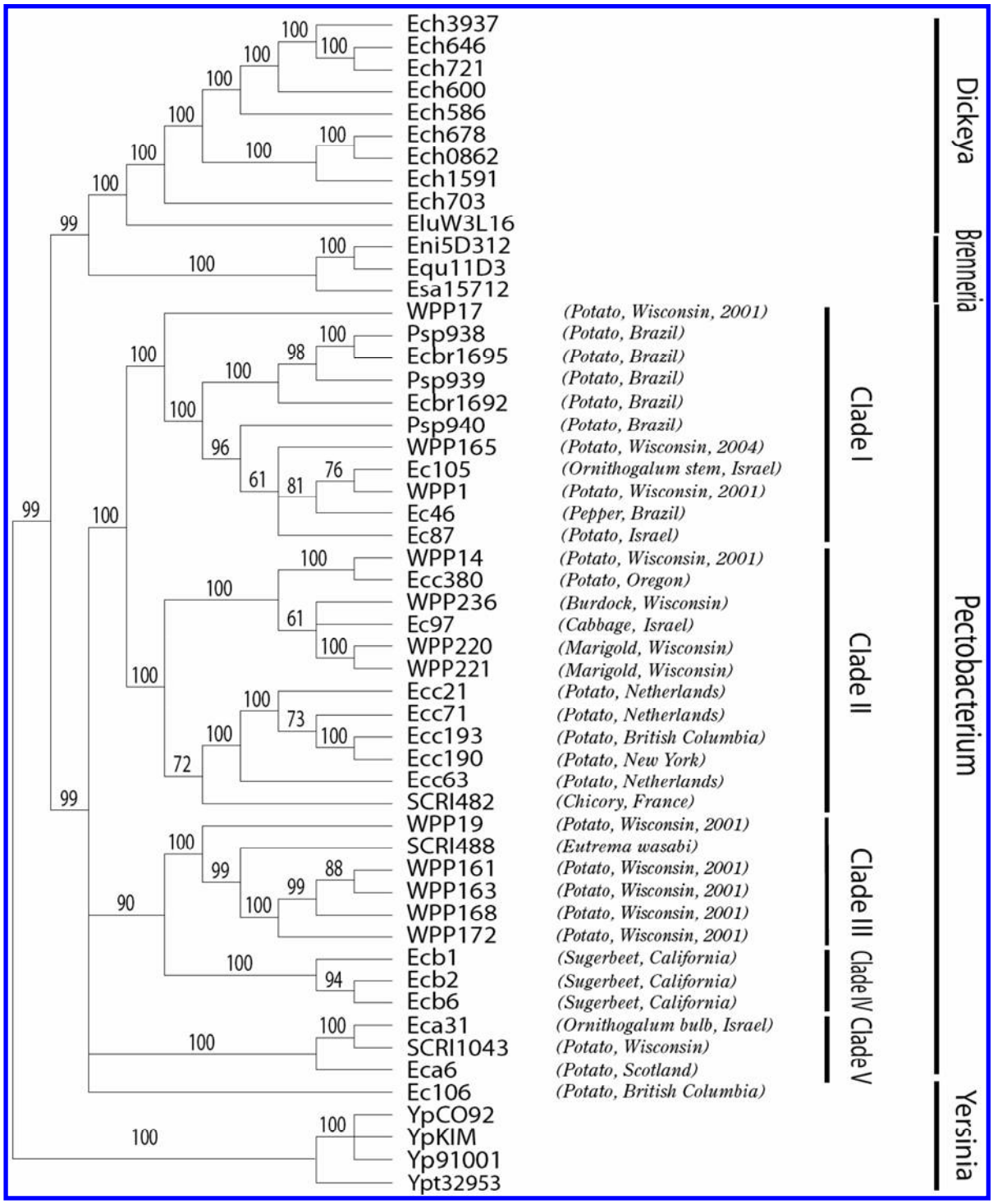

Fig. 3. Bayesian phylogeny of 53 *Enterobacteriaceae* taxa using concatenated gene portions of *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA*. Each run contained four chains starting from random trees, and 5,000,000 generations with trees sampled at every 100 generations. The majority rule consensus tree was calculated after removal of the first 500 trees that were saved during burn-in period. The number at each node is posterior probabilities Bayesian produced. The names of *Pectobacterium* clades I–V and *Enterobacteriaceae* groups of *Dickeya, Brenneria, Pectobacterium,* and *Yersinia* spp. are marked on vertical lines. The host species of the *Pectobacterium* strains are in parentheses in italics next to the strain designations.

rot enterobacteria that remain undetected because our taxon sampling was not comprehensive. A more thorough sampling of monocot strains is likely to reveal additional members of this clade. Clades IV and V comprise two *Pectobacterium* spp. with narrow host ranges, with *P. betavasculorum* infecting mainly sugar beet and *P. atrosepticum* infecting potato.

Pectobacterium spp. have been divided into multiple serogroups (32,34) and representatives of several of these serogroups were included in our data set. *P. carotovorum* serogroups 3 (Ecc71) and 29 (Ecc380), which are commonly found on diseased potato (84), and serogroups 9 (Ecc63) and 11 (Ecc193), which have been reported to be more common on symptomless potato (32), all were placed in clade II. Two *P. atrosepticum* serogroups, 1 (Eca31) and 18 (Eca6), also were included in our data set. The phylogenetic distance between the two *P. atrosepticum* serogroups is similar to that between some of the *P. carotovorum* serogroups, suggesting that there has been a similar amount of divergence among clade II and clade V strains.

Multiple *Pectobacterium* lineages are broad-host-range pathogens. We found that strains isolated from the same host can be located in different clades. For example, *Pectobacterium* spp. causing disease in potato are distributed in four clades and do not form a monophyletic group. We also found that strains isolated from different plant hosts can be grouped together paraphyletically, which suggests that the *Pectobacterium* clades I, II, and III have broad host ranges. For example, strains within clade II were isolated from potato (WPP14), marigold (WPP220 and WPP221), cabbage (RS3/91), burdock (WPP236), and chicory (SCRI 482). Noticeably, some potato strains collected in different years and in widely different locations are grouped closely with each other, whereas potato strains obtained from a single field may fall into multiple clades. For example, within the well-supported clade I, the potato strains are closely related to each other, although these strains were collected at different locations, including Wisconsin, Brazil, and Israel. Similarly, strains from clade II also have worldwide distribution, originating in the United States, the Netherlands, Israel, British Columbia, and France. Compared with other clades of *Pectobacterium*, clade I and II have much shallower branches, which suggest that their descendents are more recently evolved lineages. In addition, the *Dickeya* spp. have deep branches compared with the *Pectobacterium* spp. Therefore, our data do not support a correlation between clade diversity and host range breadth.

Our analyses show that the genera *Dickeya* and *Pectobacterium* contain broad-host-range pathogens that, together, cause disease in half of the angiosperm plant orders, and the lineages of *Dickeya* spp. have evolved over a longer time than the *Pectobacterium* spp. There are some host range limitations associated with subgroups of both genera. For example, two clades of *Pectobacterium*, *P. atrosepticum*, and *P. betavasculorum*, have been reported on only a limited number of plant species, and *Pectobacterium* spp., unlike *Dickeya* spp., have not yet been reported to cause disease in grain species.

In contrast, *Brenneria* spp. have a more limited host range, causing disease only in woody plants in the eurosids I clade, including the Fagales trees oak (*Quercus* spp. L.), walnut (*Juglans* spp. L.), and Malpighiales tree willow (*Salix* spp. L.). Strain EluW3L16, which is placed at the base of the *Dickeya* clade, also was isolated from a eurosids I clade host, *Lupinus* spp. L., which is in the Fabales. Our data support the emergence of the necrogenic genus *Brenneria* from the soft rot enterobacteria. Like the genus *Dickeya*, the genus *Brenneria* consists of a more ancient group than the genus *Pectobacterium*. This suggests that the progenitor of the genus *Brenneria* may have gained the abilities to cause disease in tree species, none of which have been reported as hosts for species of the genera *Pectobacterium* or *Dickeya*; and, perhaps subsequently, the genus *Brenneria* lost genes encoding pectinases. More insight awaits further information on which bacterial virulence genes are required to cause disease in trees, possibly through a genome-wide comparison of *Brenneria* spp. and *Erwinia amylovora*, which may have evolved independently as necrogenic tree pathogens.

Our analysis divides the genus *Pectobacterium* into five clades, three of which include strains isolated from multiple hosts. One of the strains, Ec106, was not placed into any clade; thus, there are likely to be additional *Pectobacterium* clades. Our analysis supports the hypothesis that the ancestor of the genus *Pectobacterium* was a broad-host-range pathogen and demonstrates that strains isolated from the same host plant, whether it is potato or *Ornithogalum* spp., are not necessarily closely related. The apparently narrow host ranges of *P. atrosepticum* and *P. betavasculorum* suggests that these species have acquired genes that limit their host range or have lost genes required to cause disease in a diverse range of host plants.

The concatenated data set used for this analysis provides robust insight into evolution of the soft rot enterobacteria and a strongly supported framework into which newly isolated *Pectobacterium*, *Dickeya*, or *Brenneria* strains can be placed using technologies available to most microbiology laboratories. Because the seven genes chosen for this analysis are present in nearly all enterobacteria, this data set also can be used as a basis for determining the relationships of other enterobacterial genera to these plant pathogens and to each other. Traditionally, DNA hybridization assays have been an important tool in designating new species and these assays played an important role in earlier characterization of soft rot enterobacteria (16,17). However, with the advent of new sequencing technologies (85), we predict that full genome sequence comparisons will become a standard method for defining bacterial species. Multilocus phylogenetic analysis is likely to remain an important tool because it is relatively rapid and inexpensive and, as demonstrated here and elsewhere (100), it provides enough informative characters for robust phylogenies.

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