A Novel Phylogroup of *Pseudomonas cichorii* Identified Following an Unusual Disease Outbreak on Tomato

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ABSTRACT

Recently, in Central Florida tomato production fields, tomato foliage and fruit were observed with symptoms similar to bacterial speck. Fluorescent pseudomonads were consistently isolated and the strains were characterized by standard LOPAT tests, pathogenicity tests, and genetic characterization using 16S ribosomal RNA (rRNA) sequences and multilocus sequence analysis (MLSA) of conserved housekeeping genes. LOPAT test results indicated that the strains were likely *Pseudomonas cichorii*. These strains were pathogenic on tomato and were also pathogenic on lettuce, the host for the type strain of *P. cichorii*. Likewise, strains of *P. cichorii* isolated in Florida since the early 1980s from hosts

other than tomato, along with the type strain, were also pathogenic on tomato. Genetic characterization using 16S rRNA and MLSA confirmed that the strains were most closely related to *P. cichorii* but varied significantly from the type strain. The Florida *P. cichorii* strains formed a separate phylogenetic group along with *P. cichorii* strains isolated from tomato in Tanzania. These strains were different from the previously described morphotypes and genomovars of *P. cichorii*. Our results indicate the presence of a genetically distinct group of multihost pathogenic *P. cichorii* strains that have been present in Florida since at least the early 1980s.

Tomato is a major crop in Florida, with an ideal subtropical climate that allows for nearly yearlong production from north to south Florida. Although various pathogenic bacteria affect tomato production throughout the year, outbreaks caused by pathogenic *Pseudomonas* strains are typically favored by periods of extended rainfall and cool weather in Florida. Three diseases caused by different *Pseudomonas* spp. are reported from Florida: bacterial speck caused by *Pseudomonas syringae* pv. *tomato*, bacterial leaf blight caused by *P. viridiflava* (Jones et al. 1984), and pith necrosis caused by *P. corrugata* (Jones et al. 1983b). Foliar disease symptoms caused by bacterial speck consist of small, dark-brown, raised lesions with a yellow halo (LeBoeuf et al. 2005), whereas bacterial leaf blight symptoms consist of tissue necrosis (Jones et al. 1983b, 1984).

Tomato foliage and fruit exhibiting symptoms similar to bacterial speck were observed from 2011 and 2012. However, uncharacteristic of *P. syringae* pv. *tomato* or *P. viridiflava*, these symptoms occurred during periods of warm weather when day temperatures exceeded 29°C. Foliar symptoms consisted of irregular lesions with distinct yellow margins (Fig. 1) and fruit exhibiting pinpoint sunken lesions, distinct from those symptoms caused by *Xanthomonas perforans*, the prevalent warm-weather pathogen associated with tomato in Florida (Vallad et al. 2009). Preliminary characterization identified the strains as *P. cichorii*, considered a warm-weather pathogen able to grow under high temperature and humidity (Jones et al. 1984), a novel pathogen on tomato in Florida.

P. cichorii is reported to have a wide range (Stead et al. 2003) on hosts of high economic importance, including tomato, lettuce, and watermelon. *P. cichorii* was initially reported on endive (Swingle 1925) and later was recovered from rots on head lettuce in New York (Burkholder 1954). The bacterium is widely distributed, with recent reports from Belgium (Cottyn et al. 2009), Greece (Trantas et al. 2013), Italy (Scortichini et al. 2002), Turkey (Aysan et al. 2003), and Tanzania (Testen et al. 2015). The pathogen was previously isolated in Florida on chrysanthemum, celery, geranium, and hibiscus (Chase 1986; Engelhard et al. 1983; Jones et al. 1983a; Pohronezny et al. 1994) and, more recently, on *Stevia rebusiana* (Strayer et al. 2012) and *Duranta erecta* (Gumtow et al. 2013). Tomato was first reported as a host for *P. cichorii* in 1974 in New Zealand (Wilkie and Dye 1974). Symptoms associated with *P. cichorii* on tomato were described as being similar to late blight of tomato (Wilkie and Dye 1974) and pith-necrosis like symptoms (Testen et al. 2015). Along with tomato, plants from the Asteraceae family, including *Lactuca sativa* (lettuce), *Cichorium endivia* (endive) and *Helianthus annuus* (sunflower), are regarded as common hosts of *P. cichorii*.

Based on phylogenetic analysis using *gyrB* and *rpoD*, *P. cichorii* is grouped within the *P. syringae* pathogen complex (Yamamoto et al. 2000) and, more recently, was placed in phylogroup 11 based on multilocus sequence analysis (MLSA) of housekeeping genes (Berge et al. 2014). A standard LOPAT assay that tests for levan production, oxidase and proteolytic activity, arginine dihydrolase utilization, and tobacco hypersensitivity is used to differentiate *P. cichorii* strains from other fluorescent pseudomonads (Lelliott et al. 1966). Within *P. cichorii*, three distinct morphotypes were defined based on colony formation and fluorescent pigmentation (Cottyn et al. 2009). Using BOX-polymerase chain reaction (PCR) and MLSA, a new genomovar of *P. cichorii* causing tomato pith necrosis was reported, indicating genetic variation among *P. cichorii* strains from diverse hosts (Trantas et al. 2013).

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The recent isolation of *P. cichorii* from tomato in Florida could be due to a recent introduction of tomato strains or, alternatively, new infection of tomato by endemic strains. The purpose of this study was to characterize the recently isolated *P. cichorii* strains from tomato in Florida using pathogenicity tests to determine host range, and conserved gene sequences to compare tomato strains with strains isolated from other hosts in Florida. In addition, we studied genetic diversity of *P. cichorii* strains isolated over a more than 30-year period and from various hosts in Florida in order to clarify

the genetic relationships with strains reported from other areas of the world.

MATERIALS AND METHODS

Bacterial strains and isolation. The bacterial strains used in this study included those recently isolated from tomato in Florida and strains isolated from various plant hosts in Florida since the early 1980s (Table 1). Based on the LOPAT tests, the tomato stains were most closely related to *P. cichorii*. Thus, the type strain of *P. cichorii*, PC22^T (= ATCC 10857^T = CFBP 2101^T = LMG 2162^T) (Jones et al. 1990) and representative *P. cichorii* strains previously reported from different hosts in Florida were used in this study. The bacterial strains were plated on nutrient agar (Difco) and on King's B medium (KB) to detect fluorescence (King et al. 1954). In total, 18 strains isolated from tomato and 7 strains isolated from seven different hosts in Florida were also characterized in this study. In addition to strains isolated in Florida, *P. cichorii* strains recently reported from tomato in Tanzania, pumpkin in Tennessee, soybean in South Korea, and potato in Japan were also included for genetic comparisons.

Pathogenicity tests. For each strain, 50 ml of bacterial suspensions prepared from 24-h cultures and adjusted to approximately 10⁸ CFU/ml were spray inoculated onto 6-week-old plants of susceptible 'Bonnie Best' tomato. The plants were sealed in plastic bags and maintained in a growth chamber at 28°C for 48 h. Given that *P. cichorii* are common pathogens of lettuce (Burkholder 1954; Mirik et al. 2011), romaine lettuce plants were also spray inoculated in the same manner. At least two plants were inoculated for each strain and the experiment was repeated. Following the appearance of disease symptoms after 48 h, bacteria were isolated

Fig. 1. Bacterial disease symptoms observed in tomato foliage caused by *Pseudomonas cichorii*.

TABLE 1. List of *Pseudomonas cichorii* strains used for this study^a

Strain ^b	Host ^c	Year	Location	16S	<i>gapI</i>	<i>gltA</i>	<i>gyrB</i>	<i>rpoD</i>	<i>cts</i>	Source
GEV417	Tomato	2011	Florida	2	2	2	2	2	2	This study
GEV1093	Tomato	2012	Florida	3	3	3	3	3	NS	This study
GEV1105	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1112	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1124	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1127	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1129	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1141	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1144	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1149	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1151	Tomato	2012	Florida	NS	3	3	3	3	3	This study
GEV1154	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1158	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1164	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1169	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1171	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1173	Tomato	2012	Florida	NS	3	3	3	3	NS	This study
GEV1178	Tomato	2012	Florida	3	3	3	3	3	NS	This study
PC2	Geranium	1985	Florida	5	6	8	9	11	6	This study
PC4	Chrys	1984	Florida	3	5	5	5	5	NS	This study
PC22 ^T	Endive	1929	Germany	1	1	1	1	1	1	This study
PC24	Hibiscus	1984	Florida	4	4	4	4	4	NS	This study
PC25	Basil	1986	Florida	2	2	3	7	9	NS	This study
PC27	<i>F. pan</i>	1985	Florida	2	5	6	6	6	4	This study
PC37	<i>F. lyr</i>	1985	Florida	2	5	7	8	8	5	This study
PC49	CT	1995	Florida	2	5	7	8	10	NS	This study
PPST 50936	Stevia	2012	Florida	4	NS	NS	7	7	NS	Strayer et al. 2012
<i>Pseudomonas</i> sp. StFLB209	Potato	NA	Japan	6	6	9	10	12	6	Morohoshi et al. 2014
<i>P. cichorii</i> JBC1	Soybean	2008	South Korea	1	1	1	1	1	1	Ramkumar et al. 2015
<i>P. cichorii</i> TN-E4	Pumpkin	2014	Tennessee	7	NS	NS	11	13	NS	Newberry et al. 2016
<i>P. cichorii</i> 14-WTREC	Pumpkin	2014	Tennessee	8	NS	NS	11	13	NS	Newberry et al. 2016
Mab1A	Tomato	2014	Tanzania	4	2	10	8	14	NS	This study
Msu3A	Tomato	2014	Tanzania	9	7	11	12	15	NS	This study

^a Numbers represent the allele type of a gene present in each strain. The type strain of *P. cichorii*, PC22, was used as the reference allele. NS = genes not sequenced.

^b Superscript ^T indicates type strain.

^c Abbreviations: Chrys = chrysanthemum, *F. pan* = *Ficus pandurata*, *F. lyr* = *F. lyrata*, and CT = crown of thorns.

from lesions on KB to confirm that the symptoms were associated with the strain used for inoculation.

PCR amplification and gene sequencing. Bacterial strains were grown overnight at 28°C on nutrient agar. The 16S ribosomal RNA (rRNA) gene was sequenced for three representative strains isolated from tomato along with *P. cichorii* strains isolated from other hosts for comparison with the reference strains (Marchesi et al. 1998). Multiple copies of the 16S rRNA gene were detected in Florida tomato strains that resulted in sequencing errors. Therefore, the PCR products were cloned prior to sequencing, as described below. The PCR products from strains with multiple copies of 16S rRNA gene were purified using a Qiagen PCR purification kit (Qiagen N.V.). Single copies for each of the amplified products were then cloned into *Escherichia coli* using the pGEM-T plasmid vector (Promega Corp.). Colonies of *E. coli* carrying a single copy of 16S rRNA sequence from each individual *P. cichorii* strain were amplified using specific primers. Two clones for each strain were sequenced to obtain a consensus sequence.

Based on preliminary results from 16S rRNA genes, four housekeeping genes—*gapA*, *gyrB*, *gltA*, and *rpoD*—from all *P. cichorii* strains isolated in Florida were amplified (Hwang et al. 2005). The resulting PCR products were Sanger sequenced at the Interdisciplinary Center for Biotechnology Research at University of Florida. Along with *P. cichorii* isolated in Florida, four housekeeping genes from two *P. cichorii* strains recently reported from tomato in Tanzania were sequenced at the Ohio State University. Berge et al. (2014) defined *Pseudomonas* phylogroups based on *cts* gene sequences using a threshold of 4.0% for phylogroup and 1.8% for clade delineation. Representative *P. cichorii* strains from Florida were sequenced for the *cts* gene using primers described by Sarkar and Guttman (2004).

Phylogenetic analysis. The resulting sequences from individual strains were compared with sequences available from the National Center for Biotechnology Information (NCBI) and Plant Associated and Environmental Microbe Database (PAMDB) to find the closest relatives. Based on sequence similarity, sequences of closely related *P. cichorii* and *P. syringae* strains were retrieved from the databases for phylogenetic analyses. The sequences for respective genes were aligned individually using MUSCLE (Edgar 2004) in MEGA v. 6 software (Tamura et al. 2013). Sequences from the four housekeeping genes were concatenated to generate a combined phylogenetic tree. A maximum-likelihood phylogenetic tree was generated using the general time-reversible model with γ -distributed invariant sites within MEGA with 1,000 bootstrap

samples. Phylogenetic analyses of 16S rRNA and housekeeping gene sequences were carried out using the same model in MEGA.

Multiple MLSA schemes are available for characterizing *Pseudomonas* spp. Although different housekeeping genes are used for characterizing *Pseudomonas* spp. in various MLSA schemes (Hwang et al. 2005; Mulet et al. 2010; Trantas et al. 2013), *gyrB* and *rpoD* genes are included in more than one MLSA scheme. In addition, Yamamoto et al. (2000) reported that significant phylogenetic information on *Pseudomonas* spp. can be retrieved using *gyrB* and *rpoD* gene sequences. Thus, along with the MLSA using four housekeeping genes, *Pseudomonas* strains were phylogenetically analyzed using only the concatenated sequences of *gyrB* and *rpoD* genes.

Pseudomonas phylogroups were determined by phylogenetic comparison of *cts* gene sequences for representative *P. cichorii* strains in Florida with sequences available from the Morris multilocus sequence typing scheme in PAMDB (Morris et al. 2010). The genetic distance between *P. cichorii* strains isolated from Florida to representatives of *Pseudomonas* phylogroups and clades was calculated using Kimura-2 parameter model, with γ correction of 1 using MEGA.

GenBank accession numbers. Genes sequenced during this study were submitted to the NCBI database. The accession numbers for each gene sequenced during this study are *gapA* = KX673643 to KX673668, KY172992, and KY172993; *gltA* = KX673669 to KX673694, KY172994, and KY172995; *gyrB* = KX673695 to KX673720, KY172996, and KY172997; *rpoD* = KX673721 to KX673746, KY172998, and KY172999; *cts* = KY940267 to KY940272; and 16S = KX670410 to KX670420.

RESULTS

Isolation and phenotypic characterization. Bacteria were isolated from symptomatic tissue, which was similar but not identical to symptoms observed for bacterial speck disease caused by *P. syringae* pv. *tomato* (Fig. 1). Unlike bacterial speck, the symptoms included irregular leaf lesions with distinct yellow margins along with fruit exhibiting sunken, pinpoint necrotic lesions. Interestingly, these symptoms were observed when temperatures in central Florida were in excess of 29°C, unfavorable for the development of bacterial speck caused by *P. syringae* pv. *tomato*. All bacterial strains isolated from tomato were fluorescent on KB. The strains were negative for levan production, arginine dihydrolase activity, and pectolytic activity but positive for oxidase activity and caused a hypersensitive reaction when infiltrated into tobacco leaves. The LOPAT results indicated that the strains are *P. cichorii*.

Pathogenicity test. All Florida tomato strains were pathogenic on tomato and produced symptoms similar to natural infection (Fig. 2A). Furthermore, Florida strains isolated from tomato were also pathogenic on lettuce, producing disease symptoms 48 h post-inoculation (Fig. 2B). Disease incidence was observed in 100% of the inoculated plants. Typical *P. cichorii* was reisolated from symptomatic tissue. Interestingly, the *P. cichorii* type strain PC22^T was also pathogenic on tomato. Among the other *P. cichorii* strains from Florida, PC2, PC24, PC25, PC37, PC49, and PPST 50936, isolated from geranium, hibiscus, basil, *Ficus lyrata*, crown of thorns, and stevia, respectively, were also pathogenic on tomato (Supplementary Fig. S1). However, PC4 and PC27 isolated from chrysanthemum and *F. pandurata* produced no obvious symptoms on tomato.

Genetic characterization. Results from MLSA and 16S rRNA sequences were in agreement with LOPAT characterization and confirmed the strains as *P. cichorii*. The *Pseudomonas* strains shared high sequence similarity with housekeeping genes (Fig. 3; Supplementary Fig. S2) and the 16S rRNA gene (Fig. 4) of the type strain *P. cichorii* PC22^T. However, strains from Florida formed a distinct cluster even though they originated from multiple hosts. The 16S and housekeeping gene sequences from the strains isolated from tomato

Fig. 2. Disease symptoms on tomato and lettuce hosts (48 h postinoculation) inoculated with representative *Pseudomonas cichorii* strains isolated from Florida along with the type strain on **A**, tomato and **B**, lettuce. Representative strains were (i) GEV417, (ii) GEV1127, and (iii) *P. cichorii* PC22.

were identical to each other, except GEV417. The tomato strain sequences varied from the type strain *P. cichorii* PC22^T in *gapI*, *gltA*, *gyrB*, and *rpoD* by a total of 106 of 2,043 nucleotides. Sequences from the unique tomato strain GEV417 differed by a total of 20 nucleotides from the other tomato strains. The *P. cichorii* strains isolated from hosts other than tomato were within the same phylogenetic group as

the other *P. cichorii* strains from tomato (Fig. 3). The sequence types of genes for each strain used in this study are listed in Table 1. The nontomato strains from Florida carried multiple different allelic forms of housekeeping genes compared with tomato *P. cichorii* strains (Fig. 3). Only PC25 shared 100% sequence identity with tomato strains in *gapI* and *gltA* (Table 1). Strain PC25, isolated from basil, was distinct

Fig. 3. Maximum-likelihood phylogeny of *Pseudomonas* strains isolated from Florida using concatenated sequences of *gyrB* and *rpoD* genes. Hosts of isolation for strains isolated from Florida, Tennessee, and Tanzania are provided next to the strain name: T = tomato, P = pumpkin, B = basil, FP = *Ficus pandurata*, FLY = *F. lyrata*, CT = crown of thorns, S = stevia, C = chrysanthemum, H = hibiscus, and G = geranium. Bootstrap values for each branch are expressed as percentage and scale bar represents substitutions per site.

various *Pseudomonas* strains characterized using different MLSA schemes (Fig. 3). The *P. cichorii* strains from Florida formed a separate phylogenetic group compared with the three previously described morphotypes and genomovars of *P. cichorii* strains, including the type strain PC22^T. Two *P. cichorii* strains isolated from pumpkin in Tennessee also clustered with the Florida *P. cichorii* strains along with the strains isolated from tomato in Tanzania.

Phylogenetic analysis of 16S rRNA sequences produced results similar to those observed using the housekeeping genes (Fig. 4). Two representative tomato strains differed by 4 nucleotides in the 16S gene from the *P. cichorii* type strain PC22^T. Strain GEV417 showed a single nucleotide difference from the other Florida tomato strains. Florida strain PC4 isolated from chrysanthemum in 1984

Sequences of *gyrB* and *rpoD* genes from Florida *P. cichorii* strains were concatenated and phylogenetically compared with

had a 16S rRNA sequence identical to the two Florida tomato strains. Florida strains PC25, PC27, PC37, and PC49 isolated in the 1980s and 90s from basil, *F. pandurata*, *F. lyrata*, and *Euphorbia milii*, respectively, had 16S sequences identical to the GEV417 strain from tomato. *P. cichorii* strain PC24 isolated from hibiscus had a 16S rRNA sequence identical to *P. cichorii* strain 50936 previously reported from *S. rebaudiana* in Florida and Mab1A (accession KP295477) isolated in Tanzania. The 16S rRNA sequences from two representative strains of *P. cichorii* isolated from pumpkin in Tennessee (14-WTREC and TN-E4) and an additional strain isolated from tomato in Tanzania (Msu3A) were available in NCBI (accessions KU174188, KU174189, and KP295476) (Newberry et al. 2016; Testen et al. 2015). Phylogenetic analysis using 16S rRNA sequences clustered these strains together but distinct from the type strain (Fig. 4).

Phylogenetic analysis of the *cts* gene produced similar phylogenetic groupings of the *P. cichorii* strains isolated from Florida. Interestingly, *P. cichorii* strain 83-1, isolated in Arizona from an unknown plant host (Cottyn et al. 2010), had a *cts* sequence identical to tomato strain GEV1151, isolated in Florida (Supplementary Fig. S3). Genetic distance calculated among *cts* sequences showed significant differences among the strains (Supplementary Table S1). Strains isolated from Florida along with *P. cichorii* 83-1 and IBSBF 1274 had a genetic distance of 0 to 2% and varied from the type strain PC22^T by 4.5 to 5.6%. Additionally, PC2 strain isolated from geranium was genetically different from other *P. cichorii* strains by more than 10% based on the *cts* gene sequence only but was identical to *Pseudomonas* sp. strain StFLB209.

DISCUSSION

The bacterial strains isolated from symptomatic tomato in Florida were confirmed as *P. cichorii* based on standard LOPAT assay and phylogenetic analyses of housekeeping genes and 16S rRNA sequences. Three different morphotypes and a distinct genomovar pathogenic on tomato are reported within *P. cichorii* (Cottyn et al. 2009; Trantas et al. 2013). However, our study indicates that the *P. cichorii* strains from Florida along with the strains isolated from tomato in Tanzania form a genetic subgroup distinct from the type strain and other reported *P. cichorii* strains. Phylogenetic analyses using housekeeping genes provided substantial evidence that the population in Florida represents a distinct phylogroup of *P. cichorii*.

The tomato *P. cichorii* strains form a distinct cluster based on MLSA and 16S rRNA sequences. Two allele types of the 16S rRNA sequence were detected among tomato strains. One allele was identical to the 16S rRNA of chrysanthemum strain PC4, while only tomato strain GEV417 carried the second 16S rRNA allele type. Interestingly, this second 16S rRNA allele type was detected in other *P. cichorii* strains isolated from hosts such as basil, crown of thorns, hibiscus, and two *Ficus* spp. Both 16S rRNA allele types varied from the type strain (Table 1). Additionally, *P. cichorii* strain PC2 isolated from geranium was similar to a distant group of *Pseudomonas* spp. isolated from the potato phyllosphere based on 16S rRNA sequences.

Similar results were also observed with the housekeeping gene sequences. Based on housekeeping gene sequences, two haplotypes were observed among the tomato-pathogenic strains of *P. cichorii*. The strain GEV417 was isolated in 2011 and other tomato strains were isolated in 2012. Because these strains were isolated in different years, the sequence variation between these strains suggests that there were multiple shifts by *P. cichorii* to tomato in Florida. In addition to the Florida strains, representative strains of *P. cichorii* isolated from pumpkin in Tennessee and tomato in Tanzania also clustered with the Florida strains based on 16S rRNA and the housekeeping genes *gyrB* and *rpoD*.

The *P. cichorii* strains isolated in Florida clustered together in the same phylogenetic group, except for the PC2 strain isolated from geranium. Based on sequence comparisons using 16S rRNA and four housekeeping genes, PC2 was found to be similar to *Pseudomonas* sp. StFLB209, isolated from potato in Japan. Whole-

genome sequence analysis showed that strain StFLB209 from potato is closely related to *P. cichorii* strain JBC1 isolated from soybean in Korea (Morohoshi et al. 2014; Someya et al. 2009). The sequence variations within the Florida *P. cichorii* strains may be due to their associations with different hosts.

Based on the currently established phylogroup definition for the *P. syringae* complex, *P. cichorii* is grouped as phylogroup 11 (Berge et al. 2014). The study described *P. cichorii* phylogroups based on *cts* gene sequence but included only three representative *P. cichorii* strains and did not include the type strain. In this study, we focused on *P. cichorii* strains isolated from various hosts in Florida along with the type strain. The results from phylogenetic and genetic distance analysis confirmed the possibility of three independent phylogroups within *P. cichorii* based on current standards. One phylogroup would encompass two strains, including the type strain PC22^T and CFBP 4407, isolated from lettuce. The second phylogroup would encompass *P. cichorii* strains isolated from Florida, except PC2, along with *P. cichorii* 83-1 isolated in Arizona. Meanwhile, PC2 along with *Pseudomonas* sp. strain StFLB209 could represent a potential third phylogroup of *P. cichorii*. The results further indicate the strong possibility that the *P. cichorii* strains present in Florida represent a new phylogroup.

The strains of *P. cichorii* isolated over the last 30 years in Florida clustered together in the same group. Thus, it seems that the *P. cichorii* strains recently isolated from tomato in Florida are representatives of *P. cichorii* already present in Florida that are pathogenic on tomato under favorable environmental conditions and host availability. Although strains of *P. cichorii* have been reported from various hosts in Florida since the early 1980s (Chase 1986), they were not genetically characterized until recently. *P. cichorii* was previously reported to cause pith necrosis disease of tomato in Greece, New Zealand, and Turkey (Mirik et al. 2011; Trantas et al. 2013; Wilkie and Dye 1974) but sequence information for these strains is not available.

In Florida, *P. cichorii* caused foliar symptoms on tomato, whereas a wide range of symptoms varying from pith necrosis to leaf blight have previously been reported for *P. cichorii* infection in tomato (Testen et al. 2015; Wilkie and Dye 1974). Angular lesions with distinct yellow margins were observed during *P. cichorii* infections in tomato. Pathogenicity results show that *P. cichorii* strains isolated from Florida were pathogenic on more than one host. The *P. cichorii* strains isolated from tomato in Florida were pathogenic on lettuce. Likewise, *P. cichorii* strains isolated from various hosts in Florida and the type strain PC22^T were also pathogenic on tomato. The ability of *P. cichorii* to cause disease on multiple hosts suggests that the pathogen can spread to other economically important hosts.

These strains span collections from the early 1980s until 2012. With the exception of PC2, Florida *P. cichorii* strains are phylogenetically similar and their ability to cross-infect hosts suggests that the same phylogenetic group of *P. cichorii* has been affecting multiple hosts within Florida for over 30 years. In addition to Florida, this distinct phylogenetic population of *P. cichorii* encompasses strains from Tennessee and Tanzania. The similarities suggest that phylogenetically similar *P. cichorii* strains could be widespread in various hosts and environmental niches but further identification and characterization using additional representative *Pseudomonas* strains would be necessary.

The ability of *P. cichorii* strains to infect multiple hosts may cause significant losses to economically important crops in Florida and other areas. In addition to various isolations made in Florida, the reported frequency of diseases caused by *P. cichorii* has increased substantially over the last decade from different geographical locations and from economically important hosts, including tomato (Mirik et al. 2011; Myung et al. 2013; Newberry et al. 2016). Although environmental conditions for *P. cichorii* are favorable in Florida from midspring to early fall, diseases caused by *Pseudomonas* spp. in tomato are relatively rare compared with the regular outbreaks caused by *Xanthomonas* spp. during the same period. No

major disease outbreak linked to *Pseudomonas* spp. has occurred in tomato in Florida since 2012. However, the pathogen has a wide host range and the increasing spread of *P. cichorii* may pose a potential threat to agricultural industries in Florida. McCarter et al. (1983) reported that *Pseudomonas* strains could spread through seed and weed hosts. The presence of volunteer hosts in Florida fields may have increased the pathogen's potential to spread to different hosts, including tomato.

In conclusion, a distinct phylogenetic group of *P. cichorii* was observed in Florida and a phylogenetic subgroup specific to tomato was observed among *P. cichorii* strains isolated from Florida. Strains from Tennessee and Tanzania were similar to the *P. cichorii* phylogenetic group from Florida. Considering the genetic variations within Florida strains, the *P. cichorii* strains already present in various hosts in Florida may have transferred to tomato multiple times. The presence of tomato fields in close proximity to other natural hosts of *P. cichorii* and favorable environmental conditions in Florida may lead to isolated outbreaks. Conducive weather conditions and the presence of multiple hosts throughout the year in Florida are ideal for disease development and the survival of this pathogen.

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