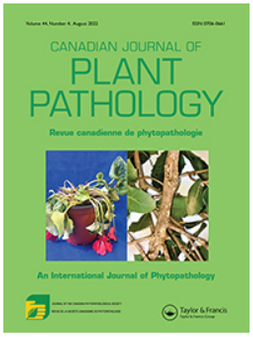

Canadian Journal of Plant Pathology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tcjp20

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To cite this article: John Alexander Rodríguez-Parra, Jenny Paola Moreno-López & Adriana González-Almario (2022) *Dickeya solani, Pectobacterium atrosepticum* and *Pseudomonas asplenii*: causal agents of bacterial soft rot in cyclamen plants (*Cyclamen persicum* Mill.) in Colombia, Canadian Journal of Plant Pathology, 44:4, 485-503, DOI: 10.1080/07060661.2022.2029943

To link to this article: https://doi.org/10.1080/07060661.2022.2029943

Published online: 03 Mar 2022.

Can. J. Plant Pathol., 2022
Vol. 44, No. 4, 485–503, https://doi.org/10.1080/07060661.2022.2029943

Bacteria and phytoplasmas / Bactéries et phytoplasmes

Dickeya solani, Pectobacterium atrosepticum and *Pseudomonas asplenii*: causal agents of bacterial soft rot in cyclamen plants (*Cyclamen persicum* Mill.) in Colombia

JOHN ALEXANDER RODRÍGUEZ-PARRA[1], JENNY PAOLA MORENO-LÓPEZ[2] AND ADRIANA GONZÁLEZ-ALMARIO[1]

[1]*Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Cra 30 # 45-03, Edificio 500, Bogotá, Colombia*
[2]*Faculty of Agricultural Sciences, Universidad de Cundinamarca, Diagonal 18 # 20-29, Fusagasugá, Colombia*

(Accepted 11 January 2022)

Abstract: Cyclamen (*Cyclamen persicum* Mill.) is an ornamental plant affected by various phytosanitary issues including bacterial soft rots, which cause crop losses of 20% worldwide. In Colombia, however, the symptoms of bacterial soft rots are not well described and the causal agents are unknown. This study aimed to characterize the bacterial soft rot disease according to its symptoms, and identify its causal agents at the species level. For this purpose, we visited nurseries in the municipality of San Antonio del Tequendama, Colombia, and selected diseased plants to describe symptoms, and to isolate and characterize pathogens using morphological, biochemical, and molecular methods. Soft rotting activity in potato tubers and pathogenicity in cyclamen plants also was evaluated. Six symptoms were found to be associated with the disease, namely: wilt, soft corm, bacterial exudates on corms, root rot, soft rot and necrotic soft rot on petioles and peduncles. Of 47 isolates, seven were pathogenic: one from the soft corms; two from petioles with soft rot; two from petioles with necrotic soft rot; and two from root rot. A multiple correspondence analysis clustered isolates in the genera *Pseudomonas, Dickeya* and *Pectobacterium* based on the morphological, biochemical, and pathogenic characteristics. A multi-locus analysis (MLSA) based on sequencing of the 16S rDNA region and housekeeping genes (*gapA, icdA, mdh, pgi, rpoB* and *rpoD*) confirmed the bacterial species *Pseudomonas asplenii, Dickeya solani* and *Pectobacterium atrosepticum* as the causal agents of cyclamen bacterial soft rot in Colombia.

Keywords: diagnoses, *Dickeya solani*, ornamentals, *Pectobacterium atrosepticum*, *Pseudomonas asplenii*

Résumé: Le cyclamen (*Cyclamen persicum* Mill.) est une plante ornementale qui est sujette à divers problèmes phytosanitaires, y compris les pourritures molles bactériennes qui occasionnent des pertes de 20% mondialement. En Colombie, toutefois, les symptômes de ces pourritures molles ne sont pas bien décrits et les agents causaux sont inconnus. Cette étude vise à caractériser cette maladie en fonction de ses symptômes et à identifier les agents causaux au niveau de l'espèce. À cette fin, nous avons visité des pépinières dans la municipalité de San Antonio del Tequendama, en Colombie, et avons sélectionné des plants infectés pour en décrire les symptômes ainsi que pour isoler et caractériser les agents pathogènes à l'aide de méthodes morphologiques, biochimiques et moléculaires. L'activité de la pourriture molle dans les tubercules de pomme de terre et la pathogénicité chez le cyclamen ont également été évaluées. Six symptômes ont été associés à la maladie, en l'occurrence la flétrissure, le corme mou, les exsudats bactériens sur les cormes, le pourridié, la pourriture molle et la pourriture molle nécrotique sur les pétioles et les pédoncules. De 47 isolats, 7 étaient pathogènes: un issu des cormes mous, deux de la pourriture molle des pétioles, deux de la pourriture molle nécrotique des pétioles et deux du pourridié. Une analyse de correspondance multiple a regroupé les isolats dans les genres *Pseudomonas, Dickeya* et *Pectobacterium*, et ce, en se basant sur les caractéristiques morphologiques, biochimiques et pathogènes. Une analyse de séquences multilocus (MLSA), basée sur le séquençage de la région de l'ADNr 16S et des gènes constitutifs

Correspondence to: John Alexander Rodríguez-Parra. E-mail: joharodriguezpar@unal.edu.co

Published online 03 Mar 2022

(*gapA, icdA, mdh, pgi, rpoB* et *rpoD*), a confirmé les espèces bactériennes *Pseudomonas asplenii, Dickeya solani* et *Pectobacterium atrosepticum* en tant qu'agents causaux de la pourriture molle bactérienne chez le cyclamen en Colombie.

Mots clés: Diagnostics, *Dickeya solani*, *Pectobacterium atrosepticum*, plantes ornementales, *Pseudomonas asplenii*

Introduction

Cyclamen (*Cyclamen persicum* Mill.) is an ornamental pot plant of economic importance in Germany and The Netherlands, although it is mainly commercialized in the United States and Japan (Takamura 2007; Jalali et al. 2012). In Colombia, cyclamen is one of the most marketable ornamental pot plants at nurseries, where the plant nursery business involves more than 3000 producers and 35 000 families in rural areas (Colviveros 2019), under a peasant economy model (Gorillo 2016). Colombian production of cyclamen plants is concentrated in the municipality of San Antonio del Tequendama, where bacterial soft rot disease is responsible for losses of up to 50% (Vivero Cinco Sentidos SAS and Plantas y Plantas de Colombia SAS, personal communication). However, there are no reports on the causal agents of this disease in Colombia. In other countries, bacterial soft rot of cyclamen accounts for losses of 20% (Chandrashekar and Diriwaechter 1983; Romero and Rivera 2005) and is caused by enterobacteria and by the genus *Pseudomonas* (Elmer and Daughtrey 2018).

Bacterial soft rot is a disease that may occur in succulent organs such as fruits, tubers, stems and bulbs of vegetable or ornamental plants. It is caused by multiple genera of Gram-negative and Gram-positive bacteria, with *Dickeya* and *Pectobacterium* being the most studied, followed by species of *Pseudomonas, Bacillus* and *Clostridium*. Bacterial soft rot symptoms include water-soaked spots that turn wet and foul-smelling; although these symptoms can occur over a wide temperature range, the worst decay is observed between 20°C and 27°C, particularly when oxygen is limited (Charkowski 2018). Plant pathogenic bacteria have evolved different strategies to promote infection and disease development. *Pectobacterium* secretes plant cell wall-degrading enzymes (PCWDEs) and small virulence proteins, such as Nip and Svx, by different secretion systems, such as Type II (T2SS), Type III (T3SS) and Type VI (T6SS) (Wang et al. 2021). *Pseudomonas* directly injects a variety of T3SS effectors and produces phytohormones, phytotoxins, and extracellular polysaccharides to allow it to colonize and overcome plant defence responses (Carrión et al. 2014; Shao et al. 2021).

Phytopathogenic bacteria of genus *Erwinia*, including *E. carotovora* (*Pectobacterium carotovorum), E. herbicola* (*Pantoea agglomerans), E. chrysanthemi* (*Dickeya* spp.) and *E. rhapontici*, along with species of *Pseudomonas*, have been identified as independent causal agents of bacterial soft rot in cyclamen plants, but not as a bacterial complex (Butcher 1934; Beaumont 1953; Amani 1967; Nicolas and Aggery 1937; Panagopoulos and Psallidas 1970; Lemattre 1973; Chandrashekar and Diriwaechter 1983; Carta 1993; Romero and Rivera 2005; Elmer and Daughtrey 2018). However, these bacteria have shown a wide biochemical diversity, which hinders their identification (Brenner and Farmer 2005; Octavia and Lan 2014), even when using molecular diagnosis methods based on the 16S rDNA region, which are not meant for differentiation at the species and subspecies levels (Adeolu et al. 2016). Therefore, this study aimed to describe the bacterial soft rot disease in cyclamen according to its symptoms, and to identify its causal bacterial agents at the species level, through pathogenic, morphological, physiological, biochemical, and molecular characterization of isolates from nursery grown cyclamen plants in the municipality of San Antonio del Tequendama, Colombia.

Materials and methods

Plant material for characterization of the disease and for bacterial isolation

In 2018 and 2019, four nurseries located in San Antonio del Tequendama, Colombia, were visited, where cyclamen plants (*Cyclamen persicum* Mill.) were found with symptoms associated with the bacterial soft rot disease, including wilt, chlorotic leaves, and soft rot on petioles, peduncles, roots, and corms. The nurseries were maintained at an average temperature of 28°C, maximum 32°C and minimum 15°C, with RH above 80%.

Description of symptoms and disease progression

Of the 45 cyclamen plants collected from the four nurseries, 12 showed different symptoms associated with the bacterial soft rot disease. Of those 12 plants, a detailed description of the development of symptoms and the presence of signs, such as bacterial exudates, was documented. Each plant was placed under humid chamber conditions for 48 h, and disease progression was monitored for 15 days.

Isolation and morphological characterization of the bacterial agents associated with bacterial soft rot symptoms in cyclamen plants

To isolate the bacterial agents associated with the disease, 20 symptomatic and five apparently healthy plants were sampled from the nurseries. From the affected plants, bacterial isolates were recovered from petioles, corms and roots. From the healthy plants, the isolates were obtained from corms. The diagnosis process was carried out at the Plant Health Laboratory of the Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogotá.

In peduncles and roots, 1-cm-long pieces from the disease progression zone were cut and disinfected with 70% ethanol for 30 seconds and 2% sodium hypochlorite (NaClO) for 30 seconds, followed by two rinses with distilled water for 2 min (Ortiz et al. 2011). The disinfected pieces were macerated, and serial dilutions were carried out (to a maximum of three dilutions); the dilutions were cultured in Petri dishes with nutrient agar medium (NA) to obtain colonies. For corms, in turn, cross-sections were made, non-disinfected inner tissue explants were macerated with distilled water, and the isolation process was carried out in the same way as for the disinfected pieces of peduncles and roots. Afterwards, bacterial colonies were grouped according to their morphology, which included appearance, colour, shape, edge, size, surface, and elevation. Under the microscope, the shape and type of cell wall was determined by Gram staining, and colonies underwent a 3% potassium hydroxide solubility test (KOH test). These colonies were cultured on NA via depletion to obtain pure cultures. Finally, the isolates were preserved at −80°C in nutrient broth with 20% glycerol.

Soft-rotting activity test on potato and cyclamen petioles

One isolate from each morphology group was selected to continue with the diagnosis. Pure bacterial cultures, grown on NA medium and incubated at 28°C for 48 h, were used for the soft rotting activity test on potato and cyclamen petioles. For the test, potato tubers (*Solanum tuberosum*) were disinfected by immersion in 10% NaClO for 15 min, followed by a rinse with distilled water, and were then dried out in a laminar flow cabinet for 20 min. Afterwards, they were cut into slices and a cavity was made in the centre of each slice with a hole-punch, where they were inoculated with 25 µL of a bacterial suspension of each isolate at a concentration of 1×10^8 CFU mL^{-1}. As a rot-causing positive control strain, an isolate of *Pectobacterium brasiliense* (provided by the Plant Health Laboratory of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá) from potato plants (*S. tuberosum*) with basal stem soft rot symptoms was used; sterile distilled water was included as a negative control. Finally, the treatments were incubated at 28°C for 48 h with three replicates each. Soft rotting activity on potato was evaluated through visual inspection of the inoculated zones (Torres et al. 2017).

Soft rotting activity on cyclamen petioles was evaluated in 10-month-old standard-type plants from a red cultivar. Fifteen bacterial isolates, which previously had shown soft rotting activity on potato slices, were examined. The inoculation of petioles was carried out using a 1 mL syringe with 250 µL of 1×10^8 CFU mL^{-1} of bacteria. The *P. brasiliense* isolate was used as a positive control strain because it has been reported to affect ornamental plants and other vegetables (van der Wolf et al. 2017; CABI 2020b). Sterile distilled water was injected as a negative control. Each isolate was inoculated in triplicate in a completely randomized design. After inoculation, the leaves were watered with distilled water and the plants were covered with a transparent plastic bag for 48 h to ensure humid conditions. Evaluations were carried out every two days for 15 days, counting from the day the plastic bag was taken off, and symptoms associated with the bacterial soft rot disease in cyclamen plants were recorded. This was carried out in a greenhouse at 18°C and 71% relative humidity conditions.

Physiological and biochemical characterization of the cyclamen bacterial isolates

Isolates that generated rotting symptoms on the petioles were subjected to physiological and biochemical characterization, and the result was compared with the profiles of *Erwinia* spp., *Pectobacterium* spp. and *Pseudomonas* spp. reported in Bergey's Manual of Systematics of Archaea and Bacteria (Garrity et al. 2005; Imhoff 2005), and in the Laboratory Guide for Identification of Plant Pathogenic Bacteria (Schaad et al. 2001). The physiological and biochemical characterization drew on assessments of parameters, such as oxygen requirement, using Hugh and Leifson's medium; sugar use and sulphide release, using the TSI (Triple Sugar Iron) test; tryptophanase activity, using the Indole test; in addition to gelatin hydrolysis, starch hydrolysis, fluorescent pigments production in King-B medium, yellow colonies production in yeast extract dextrose CaCO3 (YDC) medium, and the BBL Crystal enteric/nonfermenter (E/NF) identification (ID) system (Wauters et al. 1995).

Pathogenicity tests on cyclamen plants

The biochemically characterized isolates matching the profile of phytopathogenic bacteria causing soft rot were used to complete Koch's postulates on 10-month-old mini-type cyclamen plants of a red cultivar, in a completely random design, which included seven bacterial isolates inoculated on corm in triplicate (Romero and Rivera 2005). The inoculation procedure was carried out using a 1 mL syringe with a bacterial suspension of 250 µL at 1×10^8 CFU mL^{-1}. As a rot positive control strain, *P. brasiliense* was used; and sterile distilled water was injected as a negative control. After inoculation, the leaves were watered with distilled water and the plants were covered with a transparent plastic bag for 48 h to ensure humid conditions. Evaluations were carried out every two days for 15 days, counting from the day the plastic bag was taken off, and the symptoms associated with bacterial soft rot of cyclamen plants were recorded. This was done in a greenhouse at 18°C and 71% relative humidity. From the symptomatic plants, bacterial agents were re-isolated and biochemically re-identified, as described in the physiological and biochemical characterization procedure, thus fulfiling Koch's postulates.

Statistical analysis

The results obtained from the morphological, physiological, biochemical, and pathogenic profiles of the isolates and the *P. brasiliense* rot positive control strain were subjected to a multiple correspondence analysis (MCA) to evaluate similarity among them. Statistical analysis was performed using R software v. 3.6.1 GUI 1.70.

DNA extraction, polymerase chain reaction, and sequencing

Once the bacterial isolates causing the disease had been identified, they were subjected to DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega Corporation), following the manufacturer's instructions. PCR amplification of the 16S rDNA region was performed using the U1QUGP Fn6 and U2QUGP primers (Barghouthi 2011) (Table 1). The reactions were conducted in a 15 µL volume containing 0.85 U Taq DNA polymerase, 0.1 mM dNTPs, 1.5 mM $MgCl_2$, 1X Buffer, 0.2 mM of each primer and 2 µL of diluted DNA (1:10), and were run in a Biorad C1000 thermocycler. Reaction

Table 1. Oligonucleotide primers used for PCR amplification and sequencing of bacterial strains from cyclamen plants.

Gene	Primer	Primer sequence 5´- 3´	Sequence length (pb)[a]	Reference
16S rADN	U1QUGP-Fn6	CCAGCAGCCGCGGTAATAC	995	Barghouthi (2011)
	U2QUGP-Rn1	GGCTACCTTGTTACGACTTC		Barghouthi (2011)
gapA	gapA326F	ATC TTC CTG ACC GAC GAA ACT GC	450	Ma et al. (2007)
	gapA845R	ACG TCA TCT TCG GTG TAA CCC AG		Ma et al. (2007)
icdA	icdA400F	GGT GGT ATC CGT TCT CTG AAC G	520	Ma et al. (2007)
	icdA977R	TAG TCG CCG TTC AGG TTC ATA CA		Ma et al. (2007)
mdh	mdh86F	CCC AGC TTC CTT CAG GTT CAG A	460	Ma et al. (2007)
	mdh628R	CTG CAT TCT GAA TAC GTT TGG TCA		Ma et al. (2007)
pgi	pgi815F	TGG GTC GGC GGC CGT TAC TC	520	Ma et al. (2007)
	pgi1396R	TGC CTT CGA ATA CTT TGA ACG GC		Ma et al. (2007)
rpoB	Vic3	GGC GAA ATG GCW GAG AAC CA	501	Delétoile et al. (2009)
	Vic2	GAG TCT TCG AAG TTG TAA CC		Delétoile et al. (2009)
rpoD	*70 F*	ACG ACT GAC CCG GTA CGC ATG TAY ATG MGN GAR ATG GGN ACN GT	889	Mulet et al. (2009)
	70 R	ATA GAA ATA ACC AGA CGT AAG TTN GCY TCN ACC ATY TCY TTY TT		Mulet et al. (2009)
	PsEG30F	ATY GAA ATC GCC AAR CG	736	Mulet et al. (2009)
	PsEG790R	CGG TTG ATK TCC TTG A		Mulet et al. (2009)
	70 Fs	ACG ACT GAC CCG GTA CGC ATG TA		Mulet et al. (2009)
	70 Rs	ATA GAA ATA ACC AGA CGT AAG TT		Mulet et al. (2009)

[a]Base pair: bp

conditions included: (i) denaturation at 94°C for 2 min; (ii) 32 cycles at 94°C for 1.5 min, 50°C for 35 sec, and 72°C for 1.45 min; and (iii) a final extension phase at 72°C for 3 min (Barghouthi 2011). For the identification of the enterobacteria at the species level, the following housekeeping genes were amplified: glyceraldehyde-3-phosphate dehydrogenase A (*gapA*); isocitrate dehydrogenase, specific for NADP+ (*icdA*); malate dehydrogenase (*mdh*); and glucose-6-phosphate isomerase (*pgi*) (Ma et al. 2007). For the *Pseudomonas* genus, β subunit RNA polymerase (*rpoB*) and 70 factor subunit of the DNA polymerase (*rpoD*) genes were amplified (Gomila et al. 2015) using, in each case, the respective primers (Table 1). PCR analysis for the genes *gapA, icdA, mdh* and *pgi* was conducted in a 15 µL volume containing 0.5 U of Taq DNA polymerase, 0.4 mM dNTPs, 3 mM $MgCl_2$, 1X Buffer, 0.8 mM of each primer, and 2 µL of diluted DNA (1:10). Reactions were run under the following amplification conditions: (i) initial denaturation at 95°C for 3 min; (ii) 30 cycles at 94°C for 5 min, 52°C for 0.5 min and 72°C for 1 min; and (iii) one final extension phase at 72°C for 6 min (Ma et al. 2007). The amplification of the *rpoB* gene of *Pseudomonas* isolates was performed in a 15 µL volume containing 0.85 U Taq DNA polymerase, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1X Buffer, 0.32 mM of each primer and 2 µL of diluted DNA (1:10). The PCRs were run under the following amplification conditions: (i) initial denaturation at 94°C for 4 min; (ii) 31 cycles of 94°C for 0.5 min, 50°C for 30 sec and 72°C for 30 sec and (iii) a final extension phase of 72°C for 5 min (Delétoile et al. 2009). The amplification of the *rpoD* gene of *Pseudomonas* was carried out through a nested PCR where the first round was done using the 70F/70R primers, followed by the PsEG30F/PsEG790R primers in the second round, and both PCRs were performed under the same conditions. The reactions were conducted in a 15 µL volume containing 1.5 U Taq DNA polymerase, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1X Buffer, 0.5 mM of each primer, and 2 µL of diluted DNA (1:10). PCR was run in a thermocycler according to the following setting: (i) starting at 94°C for 5 min; (ii) 30 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min; and (iii) one extension phase at 72°C for 10 min (Mulet et al. 2009).

PCR products were purified and sequenced through the Sanger method, and the resulting sequences were edited with MEGAX v. 10.1.7. Sequence analysis was carried out by comparing these with the National Center for Biotechnology Information (NCBI) databases of complete genomes using the Basic Local Alignment Search Tool (BLAST) tool (Altschul et al. 1990). In addition, the sequences of the 16S rDNA region were compared with the EzBioCloud databases using the USEARCH tool (Ultras-Fast sequence analysis) (Yoon et al. 2017).

Multi-locus sequence analysis (MLSA)

MLSA was based on dendrograms built from edited and concatenated sequences of 16S rDNA and housekeeping genes of the seven pathogenic isolates, along with partial and complete sequences of these genes present in reference strains of the species *Dickeya solani, Dickeya dianthicola, Dickeya dadantii, Dickeya chrysanthemi, Pectobacterium brasiliense, Pectobacterium carotovorum, Pectobacterium atrosepticum, Pectobacterium parmentieri*, and *Pectobacterium wasabiae* (Table 2). For the *Pseudomonas* MLSA, the reference sequences used were *Pseudomonas asplenii, Pseudomonas agarici, Pseudomonas marginalis, Pseudomonas viridiflava, Pseudomonas syringae* and *Pseudomonas cichorii* (Table 2). For enterobacteria, the General Time Reversible plus Gamma (GTR) model was used, and for *Pseudomonas*, the Tamura-Nei plus Gamma (TrN) model was used (Tamura et al. 2004) with MODELTEST software (Posada and Crandall 1998). Phylogenetic analysis was performed through the Maximum likelihood method with a bootstrap analysis of 5000 replicates (Ma et al. 2007; Gomila et al. 2015). Sequence editing and alignment were carried out using Clustal W and MEGA X v. 10.1.7 (Tamura et al. 2007).

Results

Description of symptoms and disease progression

The plants collected from nurseries in San Antonio del Tequendama showed: i) wilt symptoms; ii) soft corms; iii) bacterial exudates on the corms; iv) soft rot on the petioles and peduncles; v) necrotic soft rot on the petioles and peduncles; and vi) root rot (Fig. 1). One plant might show one or more symptoms at the same time (Fig. 1*i*). The main symptom observed was wilting, which could affect the entire plant (Fig. 1*b*) or only portions of the plant (Fig. 1*c*). Wilting was sometimes accompanied by chlorotic leaves (Fig. 1*c*) and a soft corm (Fig. 1*d*), which could have bacterial exudates (Fig. 1*e*).

Table 2. Reference enterobacterial soft rot and *Pseudomonas* strains included in the multilocus sequence analysis (MLSA).

Species	16S rADN	*gapA*	*mdh*	*IcdA*	*Pgi*
Dickeya					
D. solani	A37G	IPO 2222*	IPO 2222*	*Ds748-2-2-12*	IPO 2222*
D. dianthicola	CFBP 1200*	M23	M23	Dd44	ME23
D. dadantii	CFBP 1269	Ech600	106 634	Ech600	Ech600
D. chrysanthemi	DSM 4610*	NCPPB 402*	GSPB4610	GSPB4610	GSPB4610
Pectobacterium					
P. parmentieri	RNS 08–42-1A*	RNS 08–42-1A*	RNS 08–42-1A*	RNS 08–42-1A*	RNS 08–42-1A*
P. atrosepticum	LMG 2386*	SCRI107	SCRI107	SCRI107	SCRI107
P. carotovorum	CFBP2046*	M30	M30	M30	M30
P. brasiliense	NZEC1	NZEC1	NZEC1	NZEC1	NZEC1
P. wasabiae	SR91*	SCRI207	SCRI207	SCRI207	SCRI207
Species	**16S rADN**	***rpoB***	***rpoD***		
Pseudomonas					
P. asplenii	LMG 5147	ICMP 11824	LMG 2158 T		
P. agarici	71A	LMG 2112*	NCPPB 1999		
P. marginalis	LMG 2210*	ICMP 3553*	ICMP 3553*		
P. viridiflava	ATCC 13223*	ATCC 13223	PDDCC 2848		
P. syringae	ATCC 19310*	ATCC 19310*	LMG 1247*		
P. cichorii	ATCC 10857*	LMG 2163	NCPPB 943*		
P. tolaasii	NCPPB 2192*	NCPPB 2192*	NCPPB 2192*		

* Strain Type

Apparently healthy plants presented corms with internal necrotic soft rot (Fig. 2*b* and 2*c*). These plants showed two types of soft rot on the petioles and peduncles: one was associated with bleaching of the tissue (Fig. 2*f*) and the other caused necrosis, preceded by an olive colouring (Fig. 2*e*). Plants in advanced stages of the disease, when all aerial organs were affected, developed root rot symptoms, causing the plant to die (Fig. 2*i*).

Isolation and morphological characterization of the bacterial agents associated with the soft rot symptoms in cyclamen plants

Eighty bacterial isolates were recovered from symptomatic plants, soft corms, petioles and peduncles with soft rot, petioles and peduncles with necrotic soft rot, and root rot. No isolates were recovered from asymptomatic plants. The 80 isolates were clustered in 47 different groups according to colony morphology, of which 15 were isolated from soft corms, eight from petioles and peduncles with soft rot, 14 from petioles and peduncles with necrotic soft rot, and 10 from root rot. The bacterial colonies obtained after 48 h incubation at 25°C on NA medium were mainly small, rounded (< 5 mm in diam.), shiny, convex with even edges and a smooth surface; microscopically they were Gram-negative rods (Table 3).

Soft rotting activity test on potato and cyclamen petioles

In the soft rotting activity test, 15 of 47 bacterial isolates from cyclamen caused rot in potato, of which seven were pathogenic on petioles (isolates 2, 3, 7, 9, 12, 13 and 14), showing differences in symptoms and virulence (Fig. 3). In the evaluation of petioles four days post-inoculation (dpi), isolate 2 from soft corms, isolate 12 from petioles with soft rot, and isolate 14 from root rot caused symptoms of soft rot. Isolates 3 and 13 from petioles with necrotic soft rot and isolate 9 from root rot showed the symptoms of necrotic soft rot. Isolate 7 from petioles with soft rot caused localized rot at the inoculation sites (Fig. 3).

Physiological and biochemical characterization of bacterial isolates from cyclamen plants

The criteria for isolate identification included the percentages of physiological and biochemical parameters in common with the bacterial genera *Erwinia, Pectobacterium* and *Pseudomonas*. Isolates 2, 3, 12, 13 and 14 were associated with the *Dickeya* genus, isolate 9 and the rot positive control strain with the *Pectobacterium* genus, and isolate 7 with the *Pseudomonas* genus (Table 3). The isolates that were associated with the *Dickeya* genus showed over 81% similarity with the profile of *Erwinia* spp.; isolate 9

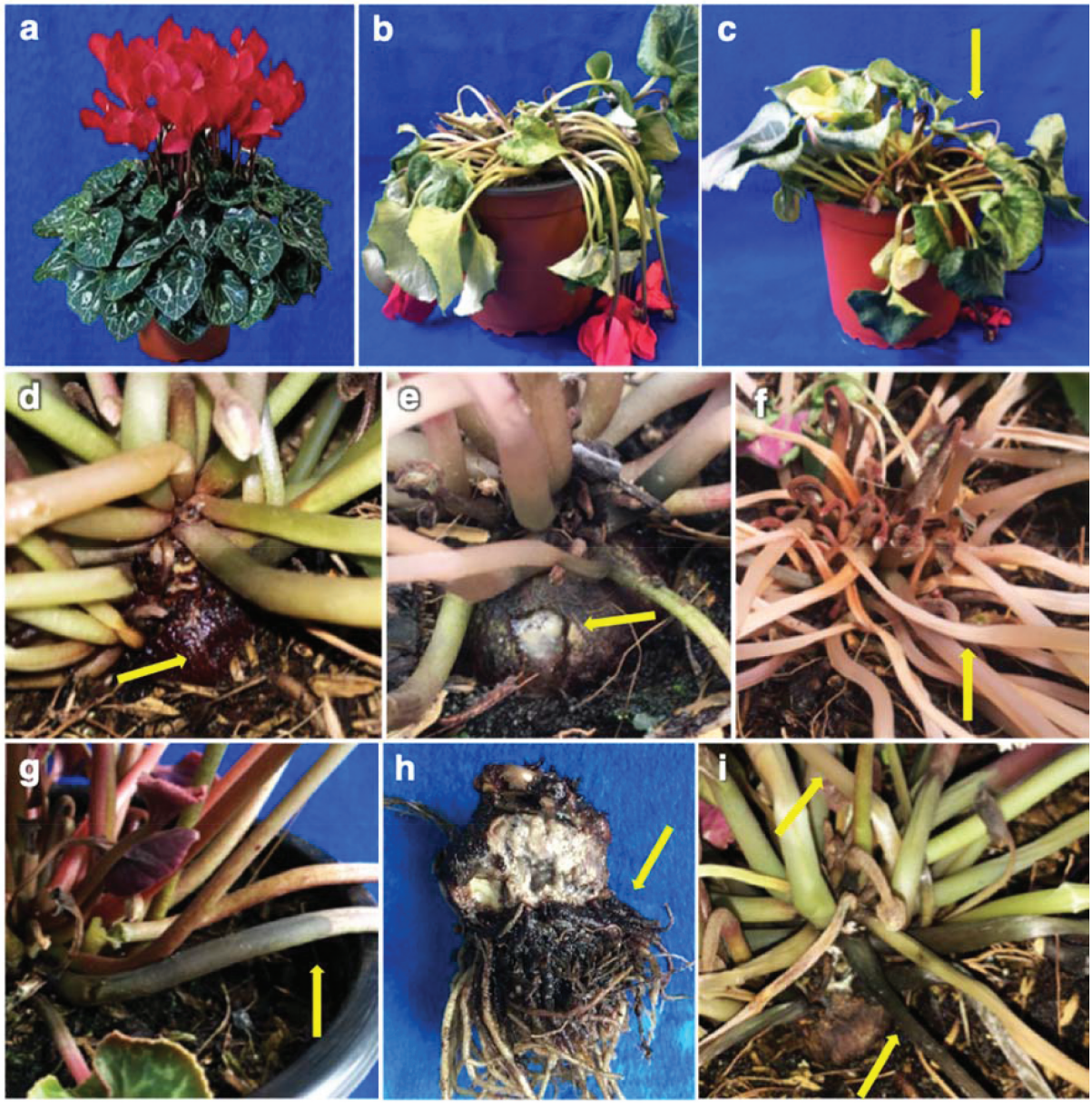

Fig. 1 Bacterial soft rot symptoms on cyclamen plants: **(a)** Healthy plant. **(b)** Wilt **(c)**. Partial plant wilt. **(d)** Soft corm. **(e)** Bacterial exudates on corms **(f)**. Soft rot on petioles and peduncles. **(g)** Necrotic soft rot on petioles and peduncles. **(h)** Soft rot on corm and root. **(i)** Soft rot and necrotic soft rot on petioles and peduncles on the same plant.

showed 81.4% similarity with *Pectobacterium* spp., and 84.5% with *P. brasiliense* (rot positive control strain), while isolate 7 showed 87.5% similarity with *Pseudomonas* spp. The latter was the only one that showed a fluorescent pigment in King-B medium, hydrolysed urea and proline nitroanilide, but not aesculin, p-n-p α-β-glucoside, p-n-p-β-galactoside or p-n-p bis-phosphate; in addition, it did not metabolize arabinose, sucrose, or mannitol. Isolates 2, 3, 12, 13 and 14 belonging to the *Dickeya* genus and isolate 9 to the *Pectobacterium* genus were the only ones that metabolized arabinose, mannose, sucrose and mannitol, and hydrolysed p-n-p α-β-glucoside, p-n-p-β-galactoside, p-n-p-bis-phosphate and aesculin. In contrast, isolates from the *Dickeya* genus degraded malonic acid and reduced Triphenyl Tetrazolium chloride, and those of

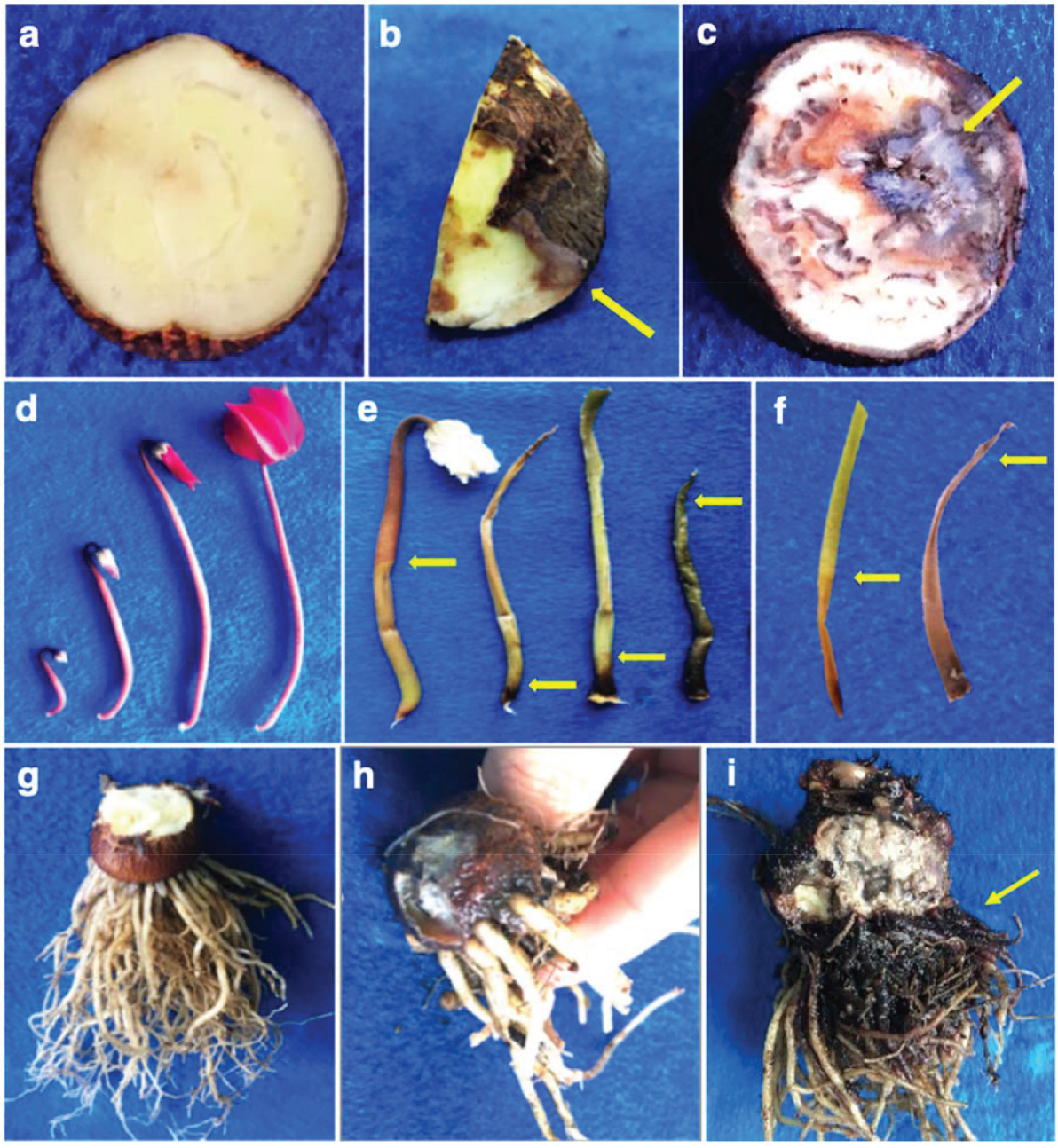

Fig. 2 Disease progression of cyclamen bacterial soft rot. **(a)** Cross-section of healthy corm. **(b)** Longitudinal section of corm from apparently healthy plant. **(c)** Cross-section of affected corm. **(d)** Petioles of healthy cyclamen plant. **(e)** Progression of necrotic soft rot on peduncles. **(f)** Progression of soft rot on petioles. **(g)** Cross-section of healthy corm and root. **(h)** Soft corm with bacterial exudates and apparently healthy root. **(i)**. Soft corm with soft root. Yellow arrows indicate the symptom location.

the *Pectobacterium* genus hydrolysed γ-L-glutamyl p-nitroanilide (Table 3).

Pathogenicity tests on cyclamen plants

Isolates of the genera *Dickeya* (2, 3, 12, 13 and 14) and *Pectobacterium* (9 and the *P. brasiliense* rot positive control strain) developed symptoms 7 dpi, in contrast to the *Pseudomonas* isolate (isolate 7) that developed symptoms 15 dpi (Fig. 4). All isolates generated soft rot symptoms on corms, but they developed different types of soft rot on the petioles and peduncles. Isolates 2, 12 and 14 (*Dickeya*) collected from soft corms, petioles with soft rot, and

Table 3. Identification of bacterial isolates collected from cyclamen according to diagnostic characteristics of the bacterial genera *Pseudomonas, Dickeya* and *Pectobacterium.*

Genera	*Dickeya*					*Pectobacterium*		*Pseudomonas*
ID[a]	**2**	**3**	**13**	**12**	**14**	**9**	***PB***[b]	**7**
Origen								
Affected organ	Corm	Petiole	Petiole	Petiole	Root	Root	Potato	Petiole
Symptom	Soft	Necrotic soft	Necrotic soft	Soft	Soft	Soft	Soft	Soft
Pathogenicity test								
Pathogenicity on petiole	+	+	+	+	+	+	+	+
Symptom of rot on petiole	Soft	Necrotic soft	Necrotic soft	Soft	Soft	Necrotic soft	Necrotic soft	located soft
Pathogenicity in planta	+	+	+	+	+	+	+	+
Wilt plant	+	+	+	+	+	+	+	+
Soft corm	+	+	+	+	+	+	+	Located
Soft petiole	Soft	Necrotic soft	Necrotic soft	Soft	Soft	Necrotic soft	Necrotic soft	-
Bacterial exudate on the corm	+	+	+	+	+	-	+	+
Morphological and biochemical features								
Motility	+	+	+	+	+	+	+	+
Rod morphology	+	+	+	+	+	+	+	+
Convex colonies	+	+	+	+	+	+	+	+
Smooth colonies	+	+	+	+	+	+	+	+
Shiny colonies	+	+	+	+	+	+	+	+
Potato soft rot test	+	+	+	+	+	+	+	+
Anaerobic grow	+	+	+	+	+	+	+	-
White colonies	translucent	translucent	translucent	translucent	Creamy-white	translucent	translucent	Creamy-white
Colonies with entire margin	+	+	+	-	+	+	+	+
Colonies with circular form	+	+	Punctiform	+	+	+	+	+
p-n-p-phosphate	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	-
Mannose	+	+	+	+	+	+	+	-
Sucrose	+	+	+	+	+	+	+	-
Mannitol	+	+	+	+	+	+	+	-
p-n-p α-β-glucoside	+	+	+	+	+	+	+	-
p-n-p-β-galactoside	+	+	+	+	+	+	+	-
p-n-p bis-phosphate	+	+	+	+	+	+	+	-
Aesculin	+	+	+	+	+	+	+	-
Malonic acid	+	+	+	+	+	-	-	+
Triphenyl Tetrazolium chloride	+	+	+	+	+	-	-	+
Melibiose	-	+	+	+	+	-	+	-
γ-L-glutamyl p-nitroanilide	-	-	-	-	-	+	+	-
Glycine	-	-	-	-	-	-	+	+
Proline nitroanilide	-	-	-	-	-	-	-	+
Urea	-	-	-	-	-	-	-	+
Fluorescent pigments (King B medium)	-	-	-	-	-	-	-	+
Arginine	-	-	-	-	-	-	-	-
Gram test	-	-	-	-	-	-	-	-
Hydrogen Sulphide (TSI medium)	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-
Production of H_2S (Indole test)	-	-	-	-	-	-	-	-
Yellow Colonies (YDC medium)	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-
p-n-p-β-glucuronide	-	-	-	-	-	-	-	-
p-n-p-N-acetyl glucosaminide	-	-	-	-	-	-	-	-
p-nitro-DL-phenylalanine	-	-	-	-	-	-	-	-

(*Continued*)

Table 3. (Continued.)

Genera	*Dickeya*					*Pectobacterium*		*Pseudomonas*
Lysine	-	-	-	-	-	-	-	-
Colonies Size (mm)	1–4	1–4	1–4	1–4	1–4	1–4	< 1	< 5
Starch	-	-	-	-	-	ND[c]	ND	-
Oxidative/fermentation (Hugh Leifson medium)	Fermentative	Glucose not metabolized	Glucose not metabolized	Fermentative	Fermentative	Fermentative	Fermentative	Oxidative
Gelatin hydrolysis	+	+	+	-	-	ND	ND	ND
p-n-p-phosphorylcholine	+	+	+	-	-	-	-	-
p-n-p-xyloside	+	-	-	+	+	-	-	-
Rhamnose	+	-	-	+	+	+	+	-
p-n-p-α-arabinoside	-	+	+	+	+	+	+	-
Galactose	-	+	+	+	+	-	-	-
Glucose (TSI medium)	+	-	-	+	+	+	+	-
Sucrose (TSI medium)	-	+	-	+	+	+	-	-
Lactose (TSI medium)	-	+	-	+	+	+	-	-
CO2 production (TSI medium)	-	-	-	+	+	-	-	-

[a]Identification code: ID
[b]Rot positive control strain of *P. brasiliense*: (PB)
[c]Not determined: ND

root rot, respectively, caused soft rot on petioles and peduncles, while isolates 13 and 3 (*Dickeya*) collected from petioles and peduncles with necrotic soft rot, isolate 9 (*Pectobacterium* genus) from tissue with root rot, and the *P. brasiliense* rot positive control strain all caused necrotic soft rot on the petioles and peduncles (Fig. 4).

Statistical analysis

Multiple correspondence analysis (MCA) of the morphological, physiological, and pathogenic characteristics of the isolates showed that 63.7% of the total variance observed in the biochemical tests was explained in two dimensions, where 21 of the evaluated variables contributed to dimension 1 (Fig. S1) and 33 to dimension 2 (Fig. S2). The MCA clustered the seven isolates and the rot positive control strain of *P. brasiliense* in four clusters, A, B, C and D, with isolates of *Dickeya* being located in clusters A (2, 12 and 14) and C (3 and 13); isolates of *Pectobacterium* (9 and the *P. brasiliense* rot positive control strain) in cluster B; and isolate of *Pseudomonas* (7) in cluster D (Fig. 5). However, the MCA dendrogram showed a subgroup inside cluster A, differentiating isolate 2 from *Dickeya* isolates 12 and 14 (Fig. 6)

Molecular identification from the 16S rDNA region

Analysis of the 16S rDNA region from biochemically identified isolates associated with bacterial soft rot in cyclamen plants confirmed their identity at the genus level. Within the *Dickeya* genus, two species were identified, namely *D. solani* (isolates 2 and 3) and *D. chrysanthemi* (isolates 12, 13 and 14). The *Pectobacterium* isolate (9) had the same identity percentage as *P. atrosepticum* and *P. parmentieri;* the *Pseudomonas* isolate (7) corresponded to *P. asplenii* (*P. fuscovaginae*) (Tohya et al. 2020), and the rot positive control strain was confirmed as *P. brasiliense* (Table 4).

Multilocus sequence analysis (MLSA)

Of the seven isolates identified at the morphological, physiological, biochemical, and molecular (16rDNA) levels, five profiles were obtained and one isolate of each profile was subjected to MLSA analyses. Isolate 7 (*P. asplenii*), isolate 9 (*P. atrosepticum*), isolate 2 (*D. solani*), isolate 13 (*D. chrysanthemi*) and isolate 12 (*D. chrysanthemi*) were selected. The sequence analysis of the *rpoB* and *rpoD* genes confirmed isolate 7 as *P. asplenii*, while analysis of the *gapA, icdA, mdh and pgi* genes identified isolates 2, 12 and 13 as *D. solani*; however, isolates 12 and 13 had been previously identified as *D. chrysanthemi* by 16S rDNA analysis. Isolate 9, identified by 16S rDNA analysis as *P. atrosepticum/ P. parmentieri*, showed identity with different species according to the analyzed gene; the *gapA* and *icdA* genes identified it as *P. parmentieri*, while *mdh and pgi* suggested it was *P. atrosepticum*. The rot positive control strain of *P. brasiliense* was confirmed as such.

The MLSA carried out from sequences obtained from the cyclamen bacterial soft rot isolates and reference

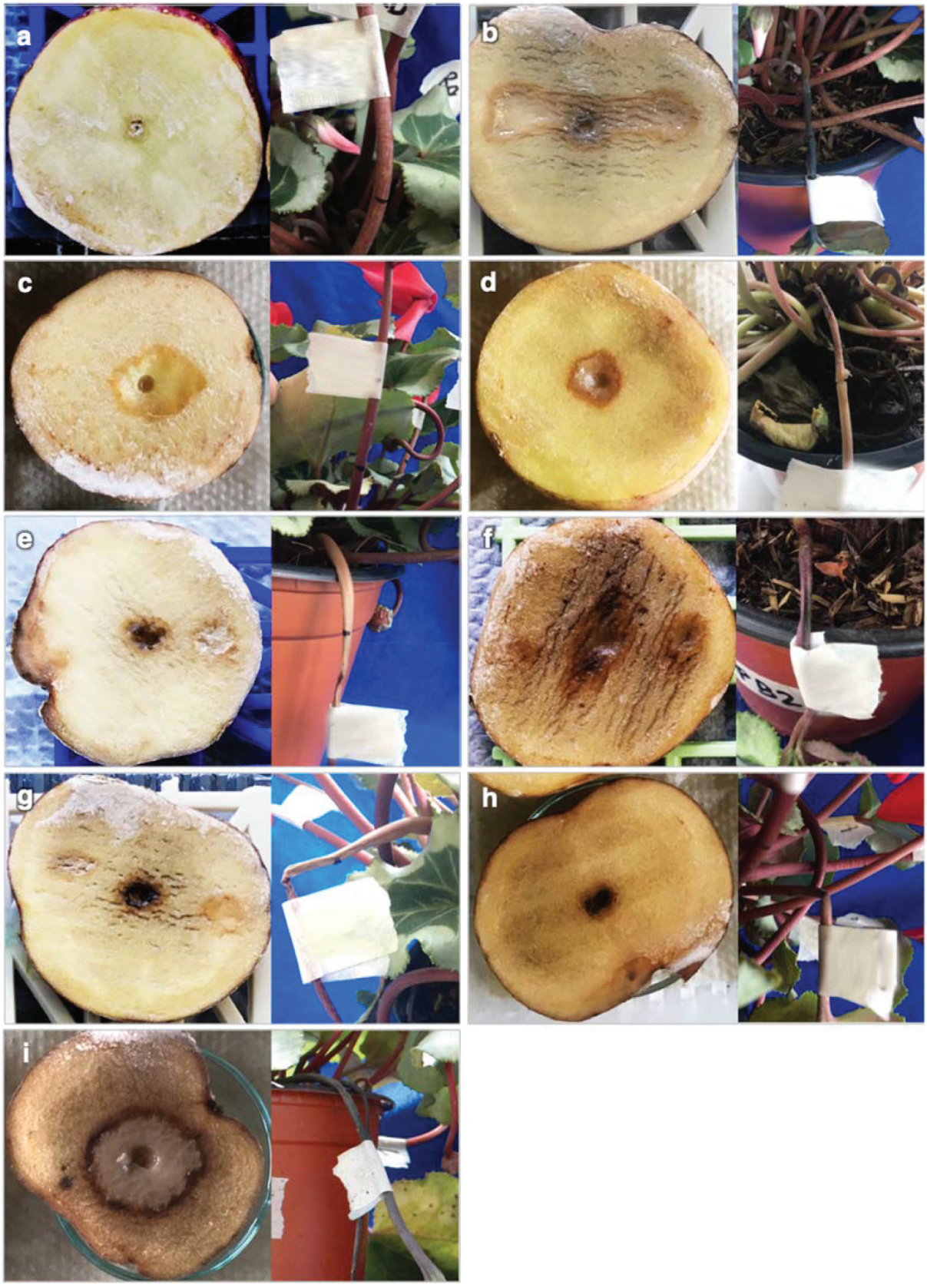

Fig. 3 Features of bacterial soft rotting activity test on potato sections (2 days post-inoculation (dpi)) and cyclamen petioles (4 dpi). **(a)** Negative control. **(b)** Rot positive control strain of *Pectobacterium brasiliense*. **(c)** Cyclamen-collected Isolate 7. **(d)** Isolate 2. **(e)** Isolate 12. **(f)** Isolate 13. **(g)** Isolate 14. **(h)** Isolate 3. **(i)** Isolate 9.

Fig. 4 Symptoms on cyclamen plants caused by bacterial isolates (7 days post-inoculation). **(a)** Negative control. **(b)** *P. brasiliense* (rot positive control strain). **(c)** *Pseudomonas* sp. (isolate 7). **(d)** *Dickeya* sp. (isolate 2). **(e)** *Dickeya* sp. (isolate 12). **(f)** *Dickeya* sp. (isolate 13). **(g)** *Dickeya* sp. (isolate 14). **(h)** *Dickeya* sp. (isolate 3). **(i)** *Pectobacterium* sp. (isolate 9).

strains of phytopathogenic bacteria (four *Dickeya*, five *Pectobacterium* and six *Pseudomonas*) resulted in a phylogenetic tree in which the cyclamen isolates were clustered along with a reference species (Figs 7, 8). The *Pseudomonas* MLSA dendrogram showed two clades: the first one corresponding to the group *P. fluorescens* and the second one to the group *P. syringae*. In the first group, three sub-groups were found: subgroup *P. asplenii* with the isolate (7) identified as *P. asplenii*; sub-group *P. fluorescens* with strains of *P. tolaasii* and *P. marginalis*; and the subgroup formed by the *P. agarici* strain (Mulet et al. 2010) (Fig. 7). The second group of the dendrogram included the reference strains of *P. syringae* and *P. cichorii*, belonging to the group *P. syringae* (Mulet et al. 2010) (Fig. 7).

The dendrogram of the enterobacteria differentiated two large groups associated with the *Dickeya* and *Pectobacterium* genera. Inside the *Dickeya* group, the reference strains of *D. dianthicola* and *D. dadanthi* formed one group, opposite *D. chrysanthemi* and *D. solani*, which formed different groups. The cyclamen isolates identified as *D. solani* (2, 12 and 13) were in the *D. solani* cluster, but not all in the same group; isolate 2 was closer to the reference strain of *D. solani*, but isolates 12 and 13 formed a different sub-group (Fig. 8). The

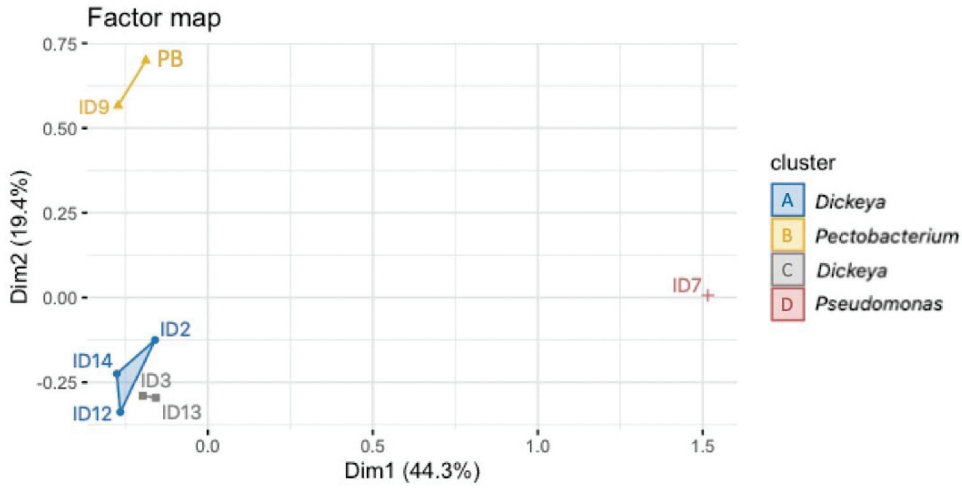

Fig. 5 Multiple correspondence analysis according to morphological, physiological, biochemical, and pathogenic features of bacterial isolates collected from cyclamen plants. Cluster A – *Dickeya* sp. (ID2, 12, and 14). Cluster B – *Pectobacterium* sp. (ID9 and rot positive control strain of *P. brasiliense* PB). Cluster C – *Dickeya* sp. (ID 3 and 13). Cluster D – *Pseudomonas* sp. (ID 7).

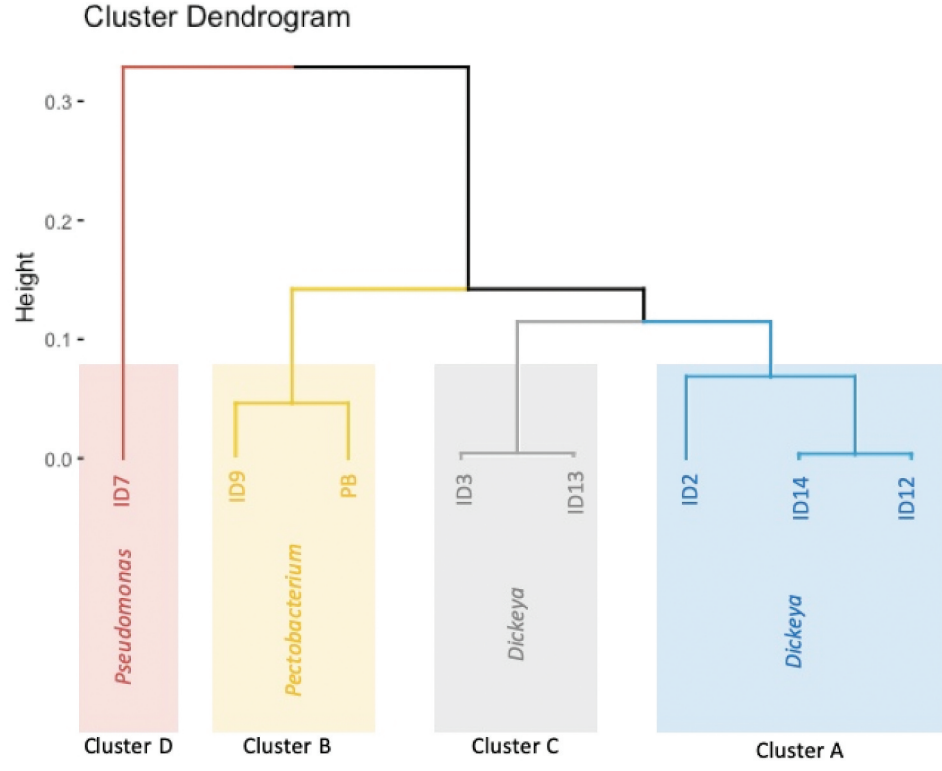

Fig. 6 Dendrogram generated from the multiple correspondence analysis based on the morphological, physiological, biochemical, and pathogenic features of bacterial isolates collected from cyclamen plants. Cluster A – *Dickeya* sp. (ID2, 12, and 14). Cluster B – *Pectobacterium* sp. (ID9 and rot positive control strain *P. brasiliense* PB). Cluster C – *Dickeya* sp. (ID 3 and 13). Cluster D – *Pseudomonas* sp. (ID 7).

Table 4. Molecular identification of bacterial isolates collected from cyclamen based on 16S rDNA sequence analysis.

ID[a]	Identification	Similarity	Similarity
Isolate		BLAST	USEARCH
2	*Dickeya solani*	100%	100%
3	*Dickeya solani*	99,80%	100%
12	*Dickeya chrysanthemi*	99,40%	99,40%
13	*Dickeya chrysanthemi*	97,30%	99.1%
14	*Dickeya chrysanthemi*	99,30%	99,40%
9	*Pectobacterium parmentieri*	98,90%	99,20%
	Pectobacterium atrosepticum	98,70%	99%
7	*Pseudomonas asplenii*	99,10%	99,40%
PB	*Pectobacterium brasiliense*	99,90%	99,30%

[a]Identification code: ID

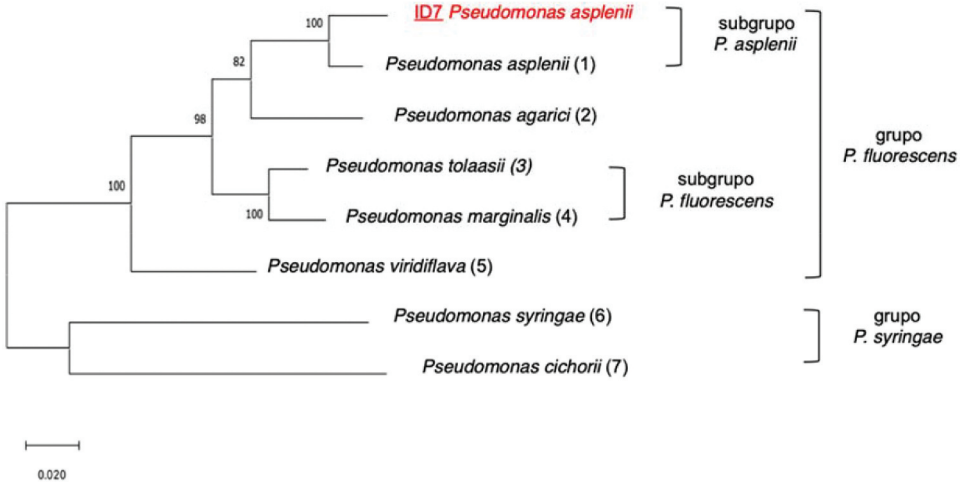

Fig. 7 Maximum-likelihood dendrogram generated using concatenated 16SrADN, *rpoB*, and *rpoD* gene sequences of plant pathogenic *Pseudomonas* and bacterial isolate 7 collected from cyclamen. The percentages of the replicate trees in which the associated taxa clustered together in the bootstrap test (5000 replicates) are shown above the branches. Numbers in parentheses indicate the reference strain and the partial sequences genes concatenated. (1) *Pseudomonas asplenii* LMG 5147 (16S rADN), ICMP 11824 (*rpoB*) and LMG 2158 T (*rpoD*). (2) *Pseudomonas agarici* 71A (16S rADN), LMG 2112 (*rpoB*) and NCPPB 1999 (*rpoD*). (3) *Pseudomonas tolaasii* NCPPB 2192 (16S rADN, *rpoB* and *rpoD*). (4) *Pseudomonas marginalis* LMG 2210 (16S rADN), ICMP 3553 (*rpoB* and *rpoD*). (5) *Pseudomonas viridiflava* ATCC 13223 (16S rADN), ATCC 13223 (*rpoB*) and PDDCC 2848 (*rpoD*). (6) *Pseudomonas syringae* ATCC 19310 (16S rADN and *rpoB*) and LMG 1247 (*rpoD*). (7) *Pseudomonas cichorii* ATCC 10857 (16S rADN), LMG 2163 (*rpoB*) and NCPPB 943 (*rpoD*).

Pectobacterium genus formed two groups: the first with strains of *P. carotovorum* and *P. brasiliense* (the reference and the rot positive control strains), and the second with strains of *P. atrosepticum, P. parmentieri, P. wasabiae*, and the cyclamen isolate (9) identified as *Pectobacterium* sp. (Fig. 8).

Discussion

Bacterial soft rot is a disease that affects cyclamen plants worldwide, including in the main cyclamen production zone in Colombia. Nonetheless, Colombian growers associate the symptoms with other causes, leading to inadequate disease management. This study characterized bacterial soft rot in cyclamen plants with respect to various symptoms reported for this disease in other countries, including wilt, chlorotic leaves, soft rot on the petioles, peduncles, and roots and corms with a foul smell (Butcher 1934; Nicolas and Aggery 1937; Amani 1967; Panagopoulos and Psallidas 1970; Lemattre 1973; Chandrashekar and Diriwaechter 1983; Carta 1993; Romero and Rivera 2005; Elmer and Daughtrey 2018). This is the first report that details the progression of

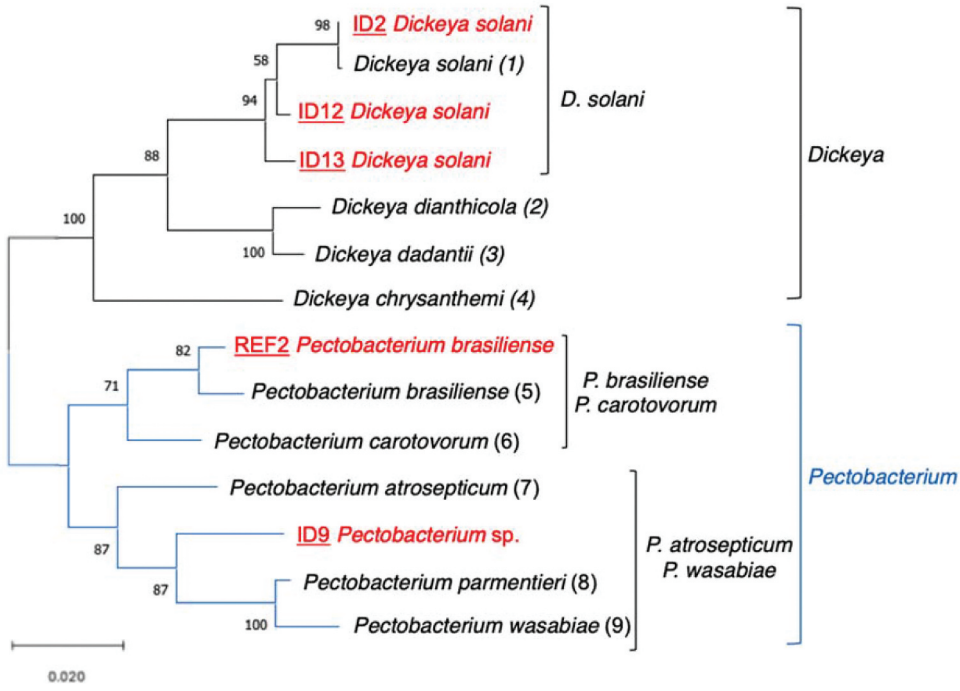

Fig. 8 Maximum-likelihood dendrogram generated using concatenated 16S rADN, *gapA, icdA, mdh*, and *pgi* gene sequences of *Dickeya* and *Pectobacterium* species, and bacterial isolates collected from cyclamen plants. The percentages of the replicate trees in which the associated taxa clustered together in the bootstrap test (5000 replicates) are shown above the branches. Numbers in parentheses indicate the reference strain and the partial sequences genes concatenated. (1) *Dickeya solani*. A37G (16S rADN), IPO2222 (*gapA, mdh* and *pgi*) and Ds748-2-2-12 (*icdA*); *Dickeya dianthicola* (2) CFBP 1200 (16S rADN), M23 (*gapA, mdh* and *pgi*) and Dd44 (*icdA*); *Dickeya dadantii* (3) CFBP 1269 (16S rADN), Ech600 (*gapA, icdA* and *pgi*) and 106 634 (*mdh*); *Dickeya chrysanthemi* (4) DSM 4610 (16S rADN), NCPPB 402 (*gapA*) and GSPB4610 (*icdA, mdh and pgi*); *Pectobacterium brasiliense* (5) NZEC1 (16S rADN, *gapA, icdA, mdh* and *pgi*); *Pectobacterium carotovorum* (6) CFBP2046 (16S rADN) and M30 (*gapA, icdA, mdh* and pgi); *Pectobacterium atrosepticum* (7) LMG 2386 (16S rADN) and SCRI1071 (*gapA, icdA, mdh* and *pgi); Pectobacterium parmentieri* (8) RNS 08–42-1A (16S rADN, *gapA, icdA, mdh* and *pgi); Pectobacterium wasabiae* (9) SR91 (16S rADN) and SCRI207 (*gapA, icdA, mdh and pgi*).

symptoms in the different plant organs and describes two types of soft rot on petioles and peduncles, one of them accompanied by tissue bleaching and the other one by necrosis. Cross-sections of corms from asymptomatic plants revealed internal wet necrotic rot, suggesting that the onset of the disease occurs in the corm. In addition, this study found that soft rot on petioles and peduncles progresses from the corm to the leaves and flowers; root rot is present only in plants where corms, petioles and peduncles have advanced rot, and none of the plants presented only root rot. The bacterial isolates collected from symptomatic tissues were initially characterized at the morphological and biochemical levels using various tests that allowed us to differentiate between the *Pectobacterium* and *Dickeya* genera. However, for the *Pseudomonas* genus, its biochemical profile made identification easier, as it is not an enterobacterium (Schaad et al. 2001; Garrity et al. 2005; Imhoff 2005). *Erwinia, Dickeya, Pectobacterium* and *Pseudomonas* species have been associated with this disease (Van Assche ; Elmer and Daughtrey 2018). Cyclamen isolates identified as *Pectobacterium* sp. and *Dickeya* sp. were more virulent and caused symptoms in plants at 7 dpi, in contrast with the *Pseudomonas* isolate which caused symptoms at 15 dpi. Similar results in cyclamen were previously reported for *E. carotovora* (*P. carotovorum*) (Chandrashekar and Diriwaechter 1984), but not for *E. chrysanthemi* (*Dickeya* spp.), which caused visible symptoms at 21 dpi (Romero and Rivera 2005).

The MCA clustered the bacterial isolates based on morphological, biochemical, and pathogenic characteristics, and formed four clusters for the three bacterial genera identified: one for *Pectobacterium* (isolate 9 and the *P. brasiliense* rot positive control strain), another for *Pseudomonas* (isolate 7) and two others for *Dickeya*. The gelatin hydrolysis p-n-p-phosphorylcholine, p-n-p-xiloside, p-n-p-α-arabinoside and the ability to metabolize rhamnose, galactose, glucose, sucrose, and

lactose were variable among the isolates identified as *Dickeya*. However, to this point, the biochemical characterization procedure did not identify isolates at the species level, this being a limiting factor for enterobacteria identification (Brenner and Farmer 2005; Octavia and Lan 2014). To confirm the identity of the causal agent of cyclamen bacterial soft rot, molecular tools were used, including 16S rDNA sequencing (Adeolu et al. 2016) and MLSA markers based on housekeeping genes of phytopathogenic enterobacteria (Ma et al. 2007; Nabhan et al. 2012) and species of the *Pseudomonas* genus (Mulet 2009; Gomila et al. 2015). Sequencing of the analyzed genes confirmed the bacterial genus of the cyclamen isolates which had already been identified biochemically; however, differences at the species level were detected depending on the analyzed genomic region. In the case of isolate 7, biochemically identified as *Pseudomonas* sp., both the 16S rDNA region and the housekeeping genes identified it as *P. asplenii*, confirming the presence of this genus, as previously reported (Elmer and Daughtrey 2018). Isolate 2 (*Dickeya* sp.), was identified as *D. solani* using all the molecular markers, in contrast with isolates 12 and 13 (*Dickeya* sp.) which were identified as *D. chrysanthemi* based on the 16S rDNA region and as *D. solani* by housekeeping genes. The classification of enterobacteria changed when multiple housekeeping genes were analyzed. This indicated that the use of housekeeping genes for species identification is preferred relative to the low discriminatory power of the 16S rDNA region at the species level (Nabhan et al. 2012; Adeolu et al. 2016). However, isolate 9, biochemically identified as *Pectobacterium* sp., showed similarity with the species *P. atrosepticum* and *P. parmentieri* based on the housekeeping genes analyzed, confirming they are close phylogenetic species (Khayi et al. 2016).

To confirm the species identification of the cyclamen isolates, a nMLSA was generated, concatenating the sequences obtained from the 16S rDNA and housekeeping genes. To complete the analysis, partial and complete sequences of the same genes from different species of *Dickeya, Pectobacterium* and *Pseudomonas* were used. The *Pseudomonas* dendrogram formed two clusters with the main taxonomic groups of this genus (Gomila et al. 2015). Isolate 7 from cyclamen plants was clustered with the reference strain *P. asplenii*, confirming its identification. It is worth noting that this pathogen is known as *P. fuscovaginae*, which is associated with the brown sheath rot disease in rice (CABI 2019c); nevertheless, *P. asplenii* has been reported to cause bacterial leaf blight in bird's-nest fern (*Asplenium nidus*) (Chase et al. 1984), but is not reported from other ornamental plants. In the cyclamen plants studied, the symptom caused by this bacterium was a localized rot that did not spread through the petiole, probably due to the low aggressiveness of the pathogen, but together with *Pectobacterium* and *Dickeya*, it favoured the development of the disease. The dendrogram built for enterobacteria showed two groups: one corresponding to the *Dickeya* genus and another to the *Pectobacterium* genus, respecting the topology of the phylogenetic analysis for these bacteria (Ma et al. 2007; Nabhan et al. 2012). In the *D. solani* group, two groups were formed: the first with isolates 12 and 13, and the second with isolate 2 and the reference strain *D. solani*, suggesting that there are differences in the nucleotide sequence between isolates of the same species. Similar results have been reported with pectinolytic bacteria isolated from potato, which were reclassified from *D. chrysanthemi* to *D. solani* through a multilocus analysis using different genes like *dnaN, fusA, gapA, purA* and *rplB*, also generating sub-groups inside the same species (van der Wolf et al. 2014). The species *D. solani* has been associated with symptoms of blackleg, general wilt, and tuber rot in potato plants (CABI 2019a). However, it has also been reported from a wide range of ornamental hosts like *Kalanchoe blossfeldiana, Dianthus caryophyllus, Chrysanthemum morifolium, Dahlia* sp. *Begonia bertinii, Sedum* sp. and *Hyacinthus orientalis* (Parkinson et al. 2015). The molecular analysis, based on the variable number of tandem repeat (VNTR) showed that some isolates of *D. solani* from ornamental and potato plants shared the same profile at the nucleotide level, suggesting that these can infect both hosts and cause cross-infections (Parkinson et al. 2015).

These variations at the nucleotide level among isolates of the same species could explain the two groups obtained with the *D. solani* isolates from cyclamen and confirm the species diversity. This bacterial species was detected in different countries from Asia and Europe, while in the Americas, it has only been reported from Brazil (Degefu et al. 2013; Potrykus et al. 2016; Cardoza 2017). Nonetheless, there are reports of other *Dickeya* species like *D. chrysanthemi* affecting ornamental plants in South America, including Colombia (Bradbury 1986). Therefore, this is the first report of the presence of *D. solani* in Colombia and as a causal agent of cyclamen bacterial soft rot.

The enterobacteria dendrogram showed, in the *Pectobacterium* group, two clusters: the first including *P. carotovorum* and *P. brasiliense* (*P. carotovorum* subsp. *brasiliense*) (Portier et al. 2019), and the second

including isolate 9 from cyclamen and the strains *P. atrosepticum, P. wasabiae* and *P. parmentieri*. The species *P. brasiliense* is widespread in Europe, Asia, Africa, Australia, North and South America, and has been reported to affect ornamental plants, potato tubers and other vegetables due to its adaptability to a wide range of temperatures and hosts (van der Wolf et al. 2017; CABI 2020b). In Colombia, there is one report of *P. brasiliense* affecting tomato (Jaramillo et al. 2017), but it had not been identified from ornamental plants. However, the symptoms caused by this bacterium are indistinguishable from those generated by other *Pectobacterium* and *Dickeya* species (CABI 2020b). While *P. brasiliense* was not isolated from diseased cyclamen plants, a strain previously isolated from potato plants was used as a rot positive control. It caused bacterial soft rot symptoms in cyclamen, thus confirming that this species is able to infect this ornamental plant. The species diversity of *Pectobacterium* have been widely studied with different molecular tools, such as amplification of Repe-titive Element Palindromic PCR (REP-PCR), Restriction Fragment Length Polymorphism (RFLP), Amplified Frag-ment Length Polymorphism (AFLP), Multilocus Sequence Typing (MLST), Random Amplification of Polymorphic DNA (RAPD), Fatty Acid Methyl Ester (FAME), CRISPR-Cas, average nucleotide identity values (ANI), and entire genome sequencing (Waleron et al. 2002; Czajkowski et al. 2015; Khayi et al. 2016; Dees et al. 2017; Li et al. 2018). These strategies have allowed an updating of the genus taxonomy, leading some *P. carotovorum* subspecies to be regarded as new species, for instance *P. atrosepticum, P. betavasculorum, P. wasabiae* (Gardan et al. 2003) and *P. odoriferum* (Portier et al. 2019). Moreover, other *P. carotovorum* strains have been reclassified as *P. polaris* and *P. peruviense*, and *P. wasabiae* and *P. parmentieri* (Khayi et al. 2016; Waleron et al. 2018; Portier et al. 2019), showing the great diversity of the genus.

At the molecular level, isolate 9 showed similarity with *P. parmentieri* and *P. atrosepticum*, related species, but the high similarity depended on the gene sequence analyzed; nonetheless, this isolate was clearly differentiated from the *P. carotovorum* and *P. brasilense* cluster. Therefore, to perform species-level identification of isolates of the *Pectobacterium* genus, a combined analysis of biochemical and molecular characteristics is required, as the assimilation of certain sugars like sucrose, rhamnose and galactose, and α-glucoside hydrolysis, has been demonstrated to differ among the species *P. atrosepticum, P. wasabiae* and *P. parmentieri* (Khayi et al. 2016; Waleron et al. 2018). In this study, isolate 9 metabolized sucrose and rhamnose and hydrolysed α-glucoside, identifying it as *P. atrosepticum*, as it is the only species from these three that shows this activity. *Pectobacterium wasabiae* was isolated only from symptomatic horseradish in Japan (Goto and Matsumoto 1987; Zoledowska et al. 2018). However, strains formerly classified as *P. wasabiae* and isolated only from potato were later reclassified as *P. parmentieri* (Khayi et al. 2016; Zoledowska et al. 2018).

Pectobacterium parmentieri has been detected in Canada, New Zealand, Iran, South Africa, Zimbabwe, Finland, France, and United States, affecting potato plants, and producing blackleg symptoms and wet rot tubers (Khayi et al. 2016; CABI 2019b). On the other hand, both species have not been reported as phytopathogens on ornamentals. *Pectobacterium atrosepticum* is a pathogen of potato, tomato and other vegetables, and has been detected in ornamentals like lupin, poinsettia, sunflower and African violet (CABI 2020). In Colombia, according to CABI (CABI 2020), this species was reported in the 'Guide to Plant Pathogenic Bacteria' as the causal agent of blackleg of potato (Bradbury 1986), but to date there is no additional or updated information regarding this pathogen. This likely is reflective of the scarcity of studies at the molecular level confirming the presence of bacterial species causing rot in hosts other than potato.

Based on the results of this study, the bacterial species *Dickeya solani, Pectobacterium atrosepticum* and *Pseudomonas asplenii* are causal agents of bacterial soft rot disease in cyclamen plants in Colombia. However, in other countries, *E. chrysanthemi* (*Dickeya* spp.) (Romero and Rivera 2005), *E. rhapontici* (Elmer and Daughtrey 2018), *P. carotovorum* (*E. carotovora* subsp. *carotovora*) (Beaumont 1953; Amani 1967; Panagopoulos and Psallidas 1970; Lemattre 1973) and *Pseudomonas* sp. (Elmer and Daughtrey 2018), have been identified as causal agents of this disease.

Acknowledgements

We are grateful to the Plant Health Laboratory and the "Antonio Angarita Zerda" Plant Biotechnology Laboratory of Universidad Nacional de Colombia, Bogotá, for allowing the use of its facilities and equipment.

Funding

This work was part of the project 'Identification of the etiological agent of the bacterial rot disease in cyclamen

plants (*Cyclamen persicum* Mill.)' under code Research Projects between Universidad de Cundinamarca and Universidad Nacional de Colombia [39398], financed by the Call for research projects between Universidad de Cundinamarca and Universidad Nacional de Colombia, Bogotá, 2017.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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