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Scientific Article

Pantoea vagans, causal agent of stem rot in pitahaya (*Selenicereus undatus*, syn. *Hylocereus undatus*) in Puebla, Mexico

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ABSTRACT

Background/Objective. The cultivation of pitahaya (*Selenicereus undatus*) has expanded in recent years in Mexico. In 2023, symptoms of stem rot were observed in commercial pitahaya orchards in the state of Puebla. The objective was to characterize and identify the causal agent of stem rot, evaluate the pathogenicity of the causal agent in different pitahaya species as well as other plant species, and assess its *in vitro* sensitivity to commercial bactericide formulations.

Materials and Methods. Ten rotten stems from Huitziltepec, Puebla, were analyzed. Tissue samples from each stem were disinfected and macerated in 500 µL of sterile distilled water; from there, 100 µL were seeded in Wilbrink's and King's B culture media. From the bacterial growth, strain CPHU23 was biochemically characterized and identified by multilocus sequence analysis (MLSA) and phylogenetic analysis of the *rpo*B, *gyr*B, *leu*S, and *fus*A genes. The pathogenicity of CPHU23 was evaluated by infiltration of 3×10^8 CFU mL^{-1} in five species of the genus *Selenicereus*: *S. ocamponis*, *S. purpusi*, *Selenicereus* sp. "Golden," *Selenicereus* sp. "Solferina" and *S. undatus*, as well as in *Agave cupreata*, *A. angustifolia*, *Aloe vera*, *Allium cepa, Solanum lycopersicum,* and *Capsicum annuum*. *In vitro* sensitivity was determined by the disk diffusion method with 10 commercial bactericide formulations.

Results. Three bacterial morphotypes were isolated from the 10 stems. The CPHU23 morphotype was the most common, with yellow, round colonies with smooth edges and a mucoid appearance. Biochemical characterization of the CPHU23 strain showed 93% similarity to *Pantoea vagans*. MLSA analysis phylogenetically grouped the CPHU23 isolate within the *Pantoea vagans* clade, phylogenetically related to the type strains of *P. vagans* LMG 24199, YI-1, and MA I6050 with bootstrap support greater than 85%. Inoculation with *P. vagans* CPHU23 caused stem rot in the five species of the genus *Selenicereus*, in leaves of *A. cupreata* and *A. vera,* but not in bulbs of *A. cepa* and fruits of *S. lycopersicum* and *C. annuum*. *Pantoea vagans* CPHU23 was sensitive *in vitro* to copper oxychloride, gentamicin sulfate, oxytetracycline hydrochloride, and streptomycin, but resistant to kasugamycin.

Conclusion. *Pantoea vagans* is the causal agent of stem rot in pitahaya in Puebla. All five pitahaya varieties are susceptible to stem rot. *Agave cupreata* and *Aloe vera* could be potential hosts for *P. vagans*. The use of copper oxychloride may be an efficient management strategy for *P. vagans* in pitahaya cultivation in Mexico.

Keywords: Enterobacteriaceae, Dragon fruit, Etiology, Multilocus MLSA, Identification

INTRODUCTION

Pitahaya (*Selenicereus undatus*) is a semi-epiphytic cactus with three-angled cladodes, aerial roots, and fragrant nocturnal flowers, belonging to the family Cactaceae. The genus *Selenicereus* is native to Mexico, Central America, and northern South America (Bravo-Hollis, 1978) and includes 18 species that grow in temperate, tropical, subtropical, and semi-arid regions (Britton and Rose, 1963; Anderson, 2001). Species within this genus are characterized by crassulacean acid metabolism (CAM), which confers high photosynthetic efficiency under conditions of limited water availability. Consequently, pitahaya cultivation has expanded in recent decades due to its ability to adapt and grow in degraded soils that are unsuitable for other agricultural crops (Niechayev *et al*., 2019; Montalvo *et al*., 2023).

Globally, pitahaya, or "dragon fruit," production has increased due to growing demand and acceptance within the niche market for exotic fruits in European, Asian, and American import markets, primarily because of its flavor, nutritional content, phytochemicals, and antioxidants associated with a reduced risk of developing diseases such as diabetes, cancer, high blood pressure, and obesity, among others (Lu *et al*., 2025). In addition, pitahaya is the only commercial fruit that accumulates high levels of betalains during fruit ripening, including red betacyanins and yellow betaxanthins. Consequently, fruits with white and red pulp are currently recognized, due to the high concentration of betaxanthins and betacyanins in the fruit (Montalvo *et al*., 2023).

Worldwide, one of the main constraints to optimal pitahaya production is disease. In this context, Balendres and Bengoa (2019) reported 17 genera and 25 species of phytopathogens as causal agents of diseases affecting stems, flowers, and fruits of *Selenicereus* spp. Most of these diseases are caused by fungi (22 species), and only two are of bacterial etiology. In *S. undatus*, the bacteria *Enterobacter cloacae* have been identified in Malaysia and Peru (Masyahit *et al*., 2009; Soto *et al*., 2019), and *Paenibacillus polymyxa* in China (Zhang *et al*., 2017), while in *S. costaricensis*, *Enterobacter hormaechei* was identified in Costa Rica (Retana-Sánchez *et al*., 2019); both bacteria were reported as causal agents of stem and fruit rot. Bacterial stem rot in pitahaya is estimated to severely affect the production of this fruit (Balendres and Bengoa, 2019).

Pitahaya cultivation represents an alternative for regions with water scarcity and poor soils. In Mexico, pitahaya production is concentrated in Quintana Roo, Yucatan, and Puebla. In Puebla, the planted area reached 152 ha distributed across 14 municipalities in the regions of La Cañada, the Tehuacán Valley, and the Mixteca, with an annual production of 546 t; the municipality of Huitziltepec contributes 53% of the state's total production. Puebla was the first state to export pitahaya fruit to the U.S. and Canadian markets and has consistently ranked among the top three producing states nationwide (SIAP, 2024).

In 2023, in commercial orchards of white-fleshed pitahaya intended for fruit export in the community of Santa Ana, municipality of Huitziltepec, Puebla, symptoms of stem rot were observed with an estimated incidence of 40%, affecting fruit yield and quality. Based on the characteristics of the observed symptoms—initial dry rot that progresses to soft rot with water-soaked tissue—this study assumes that pitahaya stem rot is of bacterial etiology. In Mexico, research on diseases affecting pitahaya cultivation is scarce; therefore, the objective of this study was to characterize and identify the causal agent of pitahaya stem rot in Huitziltepec, Puebla, evaluate the pathogenicity of the causal agent in different pitahaya species, and assess its *in vitro* sensitivity to commercial bactericide formulations.

MATERIALS AND METHODS

Collection of plant material. During the 2023 production cycle, 10 white-fleshed *S. undatus* stems showing rot symptoms were collected through directed sampling from a 2-ha commercial orchard intended for fruit export in the community of Santa Ana, municipality of Huitziltepec, Puebla (18°44'59.6"N 97°53'27.9"W) (Figure 1). Symptomatic stem samples were kept refrigerated in a cooler and analyzed at the Phytopathogenic Bacteria Laboratory of Colegio de Postgraduados, Montecillo campus, Texcoco, State of Mexico.

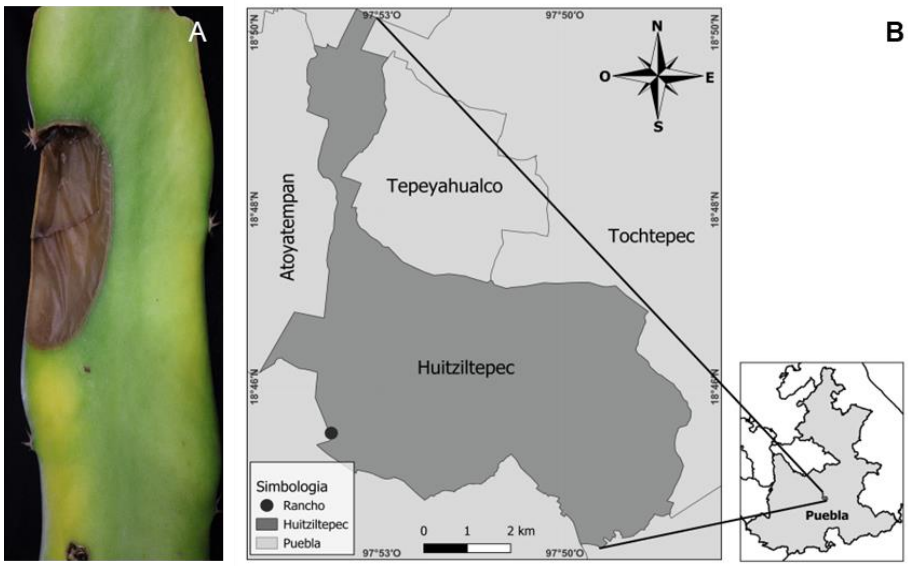

Figure 1. A) Symptom of rot on the stem of pitahaya (*Selenicereus undatus*) in a commercial orchard in the community of Santa Ana; **B)** Location of the orchard in the municipality of Huitziltepec, Puebla.

Bacterial isolation. Stems showing rot symptoms were surface-disinfested with ethanol (70%) for 1 min, followed by three rinses with sterile distilled water. The stem epidermis was then removed, and approximately 3 mm sections of tissue were excised from the interface between healthy and diseased tissue showing rot symptoms. Tissue sections were macerated in 500 µL of sterile distilled water; from this suspension, 100 µL were spread with a Digralsky spatula onto Petri dishes containing Wilbrink's medium ($MgSO_4$ 0.25 g, K_2HPO_4 0.5 g, $NaSO_3$ 0.05 g, sucrose 10 g, meat peptone 5 g, agar 18 g, and 1000 mL distilled water) (Koike, 1965) and King's B medium (K_2HPO_4 1.5 g, $MgSO_4 \cdot 7H_2O$ 1.5 g, proteose peptone 20 g, glycerol 15 mL, agar 18 g, and 1,000 mL distilled water) (Schaad *et al*., 2001). Plates were incubated at 28 °C for 5 days. The bacterial colonies obtained were purified on Wilbrink's medium and preserved in cryogenic storage (−80 °C) in nutrient broth with glycerol (20% v/v).

Physiological and biochemical characterization. Physiological and biochemical characterization was performed according to the protocols described by Schaad *et al.* (2001), including Gram staining; oxidase and catalase tests; glucose oxidation and fermentation; nitrate reduction; starch and gelatin hydrolysis; arginine dihydrolase activity; levan and indole production; gas production from glucose; carbon source assimilation; pectinolytic activity in potato (*Solanum tuberosum*); and hypersensitivity reaction in tobacco (*Nicotiana tabacum* cv. xanthi) (Borkar, 2017). Additionally, the bacterial isolates

were characterized using API 20E (bioMérieux catalog, Durham, NC, U.S.A.) following the manufacturer's instructions.

Multilocus molecular identification and phylogenetic analysis. Genomic DNA was extracted from pure cultures incubated for 48 h at 28 ºC using the 2% CTAB method (Doyle and Doyle, 1990). DNA concentration and purity were assessed with a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific). The isolates were subjected to multilocus sequence analysis (MLSA) using the housekeeping genes 16S rRNA, *rpo*B, *gyr*B, *leu*S, and *fus*A (De Armas *et al*., 2022). Polymerase chain reaction (PCR) was performed with 100 ng μL^{-1} of DNA using the primers and PCR conditions described in Table 1.

Table 1. Primers and PCR conditions used for multilocus identification of bacteria isolated from pitahaya stem rot symptom**.**

Gen	Primer	Sequence (5´-3´)	Amplicon (bp)	PCR conditions	Reference
16S rRNA (*16S ribosomal RNA*)	8F	AGAGTTTGATCCTGGCTCAG	1 500	95 °C-30 s; (30 cycles) 95 °C-2 min, 60 °C-1 min, 72 °C-2 min; 72 °C-5 min	Galkiewicz y Kellogg, 2008
	1492R	GGTTACCTTGTTACGACTT			
***rpo*B** (*RNA polymerase subunit β*)	vic2	GAG TCT TCG AAG TTG TAA CC	498	95 °C-30 s; (30 cycles) 95 °C-30 s, 50 °C- 1 min , 68 °C-1 min; 68 °C- 5 min	Delétoile *et al.,* 2009
	vic3	GGC GAA ATG GCW GAG AAC CA			
***gyr*B** (*DNA gyrase subunit* β)	gyrB3	GCG TAA GCG CCC GGG TAT GTA	405	95 °C-30 s; (30 cycles) 95 °C-30 s, 58 °C-1min, 68 °C-1 min; 68 °C- 5 min	
	gyrB4	CCG TCG ACG TCC GCA TCG GTC AT			
***leu*S** (*Leucil-tRNA synthetase)*	leuS3	CAG ACC GTG CTG GCC AAC GAR CAR GT	586	95 °C-30 s; (30 cycles) 95 °C-30 s, 58 °C- 1 min, 68 °C-1 min; 68 °C- 5 min	Salerno *et al.,* 2007
	leuS4	CGG CGC GCC CCA RTA RCG CT			
***fus*A** (*Elongation factor G (EF-G*)	fusA3	CAT CGG TAT CAG TGC KCA CAT CGA	516	95 °C-30 s; (30 cycles) 95 °C-30 s, 58 °C- 1 min, 68 °C-1 min; 68 °C- 5 min	
	fusA4	CAG CAT CGC CTG AAC RCC TTT GTT			

PCR was performed using GoTaq Flexi DNA Polymerase (Cat. M8295, PROMEGA) in 25 µL reaction mixtures containing 5 µL of 5X buffer; 1.5 mM $MgCl_2$; 0.2 mM dNTPs; 10 µmol of each primer; 1 U of Taq DNA polymerase; and 100 ng of genomic DNA. Amplification was carried out in a Mastercycler™ Nexus gradient thermocycler (Eppendorf™). PCR products were visualized on a 2% agarose gel, purified, and bidirectionally sequenced with the five primer pairs by Macrogen Inc. (Seoul, South Korea). The resulting sequences were analyzed and edited using BioEdit (v7.7.1), and the consensus sequence was generated in MEGA12. Consensus sequences were subjected to BLASTn analysis and deposited in the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) database. For phylogenetic analysis, GenBank sequences of the genus *Pantoea* were included along with those generated in this study for the four genes (*rpo*B, *gyr*B, *leu*S, and *fus*A). Alignment and trimming were performed for each gene. The four genes were concatenated using Mesquite 3.6.1. Phylogenetic analysis was conducted using the maximum-likelihood method in IQ-TREE 3 (Wong *et al*., 2025).

ModelFinder was used for evolutionary model selection (Kalyaanamoorthy *et al*., 2017). *Tatumella punctata* was used as the outgroup. Nodal support was estimated using ultrafast bootstrap (UFBoot) (1,000 replicates) (Hoang *et al*., 2018), and the tree was visualized and edited in iTOL (Letunic and Bork, 2024).

Pathogenicity tests. Strain CPHU23, identified as *P. vagans* in this study, was used. Pathogenicity tests were conducted on healthy stems of five pitahaya species: *Selenicereus ocamponis*, *S. purpusi*, *Selenicereus* sp. "Golden," *Selenicereus* sp. "Solferina," and *S. undatus*, which are extensively cultivated in the municipality of Huitziltepec. Stems were surface-disinfested with 70% alcohol, followed by three rinses with sterile distilled water; they were then inoculated by injection with a hypodermic syringe containing 500 µL of a suspension at 3×10^8 CFU mL^{-1}, according to the McFarland turbidity standard (Isenberg, 2004).

In addition to pitahaya, the pathogenicity of strain CPHU23 was evaluated on agave leaves (*Agave cupreata* and *A. angustifolia*), aloe (*Aloe vera*), onion bulb (*Allium cepa*), tomato fruits (*Solanum lycopersicum*), and chili pepper fruits (*Capsicum annuum* 'Serrano'), following the same inoculation protocol described above. For each plant species, inoculation was performed with three replicates, and samples were maintained in a humid chamber (relative humidity >90%) at a constant temperature of 28 ºC for 20 days. In the control treatment, the same volume of sterile distilled water was injected instead of the bacterial inoculum. The pathogenicity of strain CPHU23 was determined by maceration or necrosis in the inoculated tissue area.

***In vitro* sensitivity to bactericides.** *In vitro* sensitivity to bactericides was qualitatively evaluated for strain CPHU23 using the disk diffusion method (Sosa-Pérez *et al*., 2025). Ten commercial bactericide formulations were tested at the label-recommended dose: Kasumin® (kasugamycin 2.3%); Sagol® (copper oleate 22%); Cobre Control® (copper sulfate 30%); Quatz® (quaternary ammonium); Manto® 80 PH (mancozeb [ethylenebisdithiocarbamate] 80%); Oxicob® (copper oxychloride 85%); Bordocop® (cupricalcium sulfate 42.4%: mixture of copper sulfate + calcium hydroxide); Cobrezate® M (copper oxychloride 50% + mancozeb 30%); Final Bacter (gentamicin sulfate 2% + oxytetracycline hydrochloride 6%); Intermicin 500® (oxytetracycline 0.235% + streptomycin 2.2% + tribasic copper sulfate 71.8%). *In vitro* sensitivity to the bactericides was determined by the presence of a bacterial growth inhibition halo around the filter paper disc impregnated with 10 µL of the bactericide suspension. The assay was repeated three times.

RESULTS AND DISCUSSION

Bacterial isolation. From the 10 *S. undatus* stems showing rot symptoms, three bacterial morphotypes were isolated. Morphotype CPHU23 was the most frequent, producing yellow, round colonies with smooth margins and a mucoid appearance on Wilbrink's medium incubated at 28 °C for 72 h (Figure 2A). On King's B medium, the yellow pigmentation of the colonies was more intense compared with Wilbrink's medium at the same temperature and incubation period (Figures 2B and 2C). These phenotypic characteristics are similar to those described for bacterial colonies of the genus *Pantoea* (Brady *et al*., 2009; Stice *et al*., 2021).

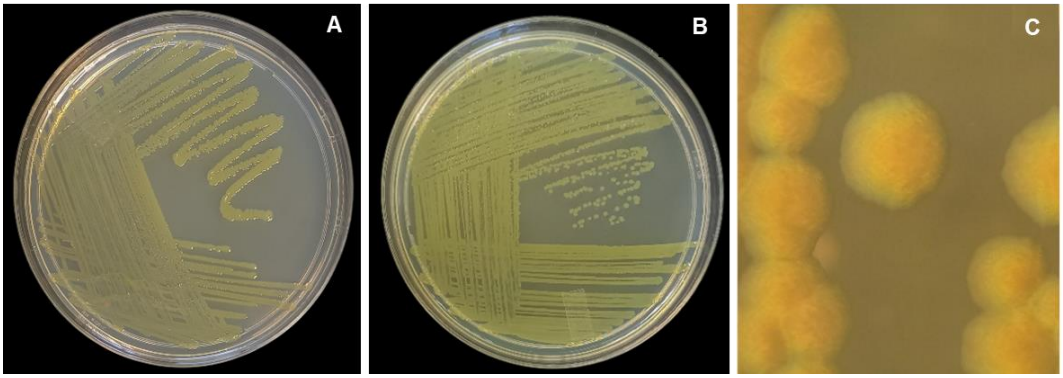

Figure 2. Morphology of bacterial colonies isolated from the stem of *Selenicereus undatus* with symptoms of rot. Strain CPHU23 on Wilbrink's medium (**A**) and King's medium (**B, C**) incubated at 28 °C for 72 h.

Physiological and biochemical characterization. Strain CPHU23 was Gram-negative, oxidase-negative, catalase-positive, and exhibited a fermentative metabolism; these characteristics are typical of the family Enterobacteriaceae, which includes the genus *Pantoea* (Walterson and Stavrinides, 2015). Additionally, strain CPHU23 caused rot in potato slices and induced a hypersensitive reaction in tobacco leaves 72 h after infiltration.

Pantoea is widely distributed in nature. Various species of this genus have been isolated and identified from plants, soil, water, animals, and humans; in plants, *Pantoea* species can establish either epiphytic or pathogenic relationships (Adeolu *et al*., 2016; Brady *et al*., 2008). In recent years, *Pantoea* species have gained attention for causing emerging diseases worldwide in important crops such as rice (*Oryza sativa*) (Duan *et al*., 2025), strawberry (*Fragaria* sp.) (Wang *et al*., 2025) in China, agave (*Agave angustifolia*) in Mexico (Palemón-Alberto *et al*., 2021), and onion (*Allium cepa*) in Taiwan (Chang *et al*., 2018).

In this study, biochemical characterization of strain CPHU23 isolated from pitahaya showed high metabolic similarity (93%) using both characterization approaches. In the API 20E characterization, the results were compared with the metabolic profile described for the type strain *P. vagans* LMG24199, isolated in Uganda from eucalyptus (*Eucalyptus globulus*) leaves showing blight and dieback symptoms (Brady *et al*., 2009). Using the protocols described by Schaad *et al.* (2001), the results were compared with *P. vagans* MalMa-22, isolated in Mexico from agave with leaf rot symptoms (Table 2) (Rodríguez-Velázquez *et al*., 2024).

Table 2. Physiological and biochemical characterization, pathogenicity, and *in vitro* sensitivity to bactericides of *P. vagans* CPHU23 isolated from stem rot of *S. undatus*.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION					PATHOGENICITY pitahaya CPHU23 strain			*In vitro* SENSITIVITY pitahaya CPHU23 strain		
Test	**CPHU23[a] Pitahaya strain**		***P. vagans* R21566[b] Eucalyptus**	***P. vagans* MalMa-22[c] Agave**	**Plant specie**	**Inoculated plant organ**	**Rot**	**Bactericidal formulation**	**Active ingredient**	**Sensitivity**
	Test[1]	**API 20E**	**Test[2] API**	**Test [1]**						
Gram	-	ND	-	-	Pitahaya *S. undatus*	Stem	+	Intermicin 500 ®	Oxytetracycline 0.235% + streptomycin 2.2% + tribasic copper sulfate 71.8%	+
Catalase	+	ND	ND	ND	Pitahaya *S. purpusi*		+			
Oxidase	-	ND	ND	-	Pitahaya *S. ocamponis*		+			
Levana	-	ND	ND	ND	Pitahaya *Selenicereus* "golden"		+	Kasumin®	Kasugamycin 2.3%	-
Phosphatase	+	ND	ND	ND	Pitahaya *Selenicereus* "solferina"		+	Finalbacter®	Gentamicin sulfate 2% + oxytetracycline hydrochloride 6%	+
β-Galactosidase	ND	-	+	ND	Agave (*Agave angustifolia*)		+			
Arginine dihidrolase	+	+	ND	ND	Agave (*A. cupreata*)	Leaf	+	Bordocop®	Cupricalcium sulfate 42.4%: mixture of copper sulfate + calcium hydroxide	-
Lysine decarboxylase	ND	-	ND	-	Aloe (*Aloe vera*)		+			
Ornithine descarboxylase	ND	-	ND	-	Chili (*Capsicum annuum* 'Serrano')	Fruit	-	Cobre control®	Copper sulfate 30%	-
Citrate utilization	ND	-	ND	ND	Tomato (*Solanum lycopersicum*)		-	Cobrezate® M	Copper oxychloride 50% + mancozeb 30%	+
H_2S production	+	-	-	-	Onion (*Allium cepa*)	Bulb	-			
Urea hydrolysis	-	-	-	-				Manto® 80 ph	Mancozeb 80 %	-

Continuation of the Table 2.

Indole production	-	-	-	-	Oxicob®	Copper oxychloride 85%	-
Gelatin hydrolysis	+	+	+	ND	Sagol®	copper oleate 22%	-
Glucose fermentation (O/F)	+	+	+	-	Quatz®	Quaternary ammonium	+
Gas production	-	ND	ND	ND			
Nitrate reduction	+	ND	ND	ND			
Starch hydrolysis	-	ND	ND	ND			
Esculin hydrolysis	-	ND	-	ND			
Mannitol	+	+	+	ND			
Inositol	+	+	+	ND			
Sorbitol	-	-	-	ND			
Rhamnose	ND	+	+	+			
Sucrose	+	+	+	+			
Maltose	+	+	ND	ND			
Amygdalin	ND	-	-	ND			
Arabinose	+	+	+	+			
Dextrose	+	ND	ND	+			
D-ribose	ND	ND	+	+			
L-tartrate	+	ND	-	ND			
Adonitol	-	ND	-	ND			
Maltose	ND	ND	-	+			
D-xylose	+	ND	+	+			
D-fructose	+	ND	ND	+			
D-mannose	+	ND	ND	+			
D-galactose	+	ND	ND	+			
Trehalose	ND	ND	ND	+			
D-mannitol	+	ND	+	+			

[1] Physiological and biochemical characterization (Schaad *et al.,* 2001).

[2] Physiological and biochemical characterization with API 20E, API 50CHB/E, Biotype-100 (bioMérieux), and Biolog GN plates (Brady *et al.,* 2009).

[a] CPHU23 strain isolated from stem rot in pitahaya (*S. undatus*) in Puebla, Mexico.

[b] *P. vagans* R-21566 T type strain isolated from leaf necrosis in eucalyptus (*Eucalyptus globulus*) in Uganda (Brady *et al.,* 2009).

[c] *P. vagans* MalMa-22 strain isolated from leaf rot in agave (*A. angustifolia*) in Mexico (Rodríguez-Velázquez *et al.,* 2024).

ND= Not determined.

Multilocus molecular identification and phylogenetic analysis. Analysis of the 16S rRNA sequence (Accession number: PX596407) identified strain CPHU23 isolated from *S. undatus* as *Pantoea* sp., without species-level resolution. However, it was identified as *Pantoea vagans* based on the concatenated phylogenetic tree of the four analyzed genes: *rpo*B (PX641593), *gyr*B (PX641591), *leu*S (PX641592), and *fus*A (PX641590), together with sequences derived from different reference strains of the genus *Pantoea*, inferred by maximum likelihood. Multilocus analysis grouped isolate CPHU23 within the *Pantoea vagans* clade along with the type reference strains LMG 24199, YI-1, and MA I6050, with bootstrap support values greater than 85%, indicating high robustness of the phylogenetic inference (Figure 3) (Hoang *et al*., 2018).

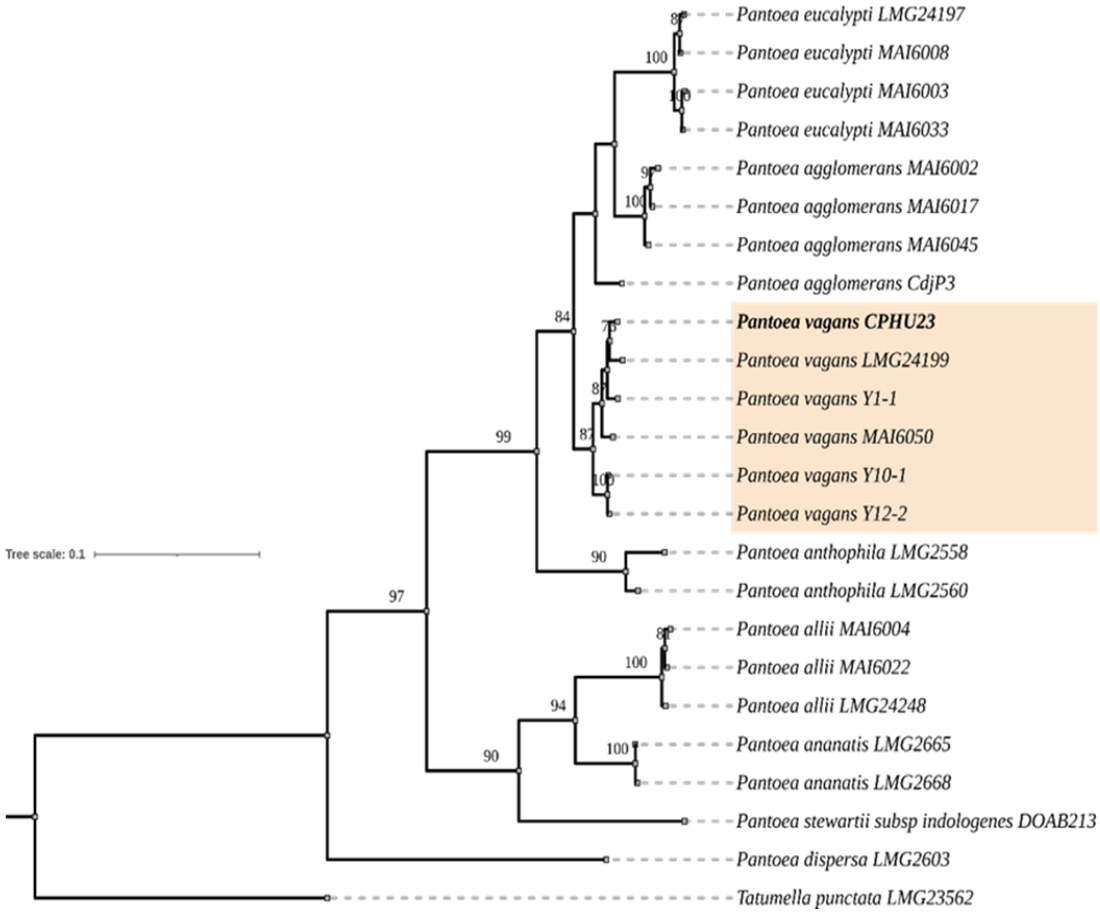

Figure 3. Maximum-likelihood phylogenetic tree based on the TIM2+I+G4 model, derived from the concatenation of four housekeeping genes (*fus*A, *gyr*B, *rpo*B, and *leu*S) from strains of the genus *Pantoea*. Ultrafast bootstrap values >70% (generated from 1000 replicates) are shown at branch nodes. *Tatumella punctata* LMG23562 was used as an outgroup. The strain CPHU23 isolated from *S. undatus* in this study is shown in bold.

In this study, strain *P. vagans* CPHU23 isolated from *S. undatus* was phylogenetically related to *P. vagans* LMG 24199, isolated in Uganda from eucalyptus leaves with blight and dieback symptoms (Brady *et al*., 2009); *P. vagans* YI-1, isolated in China from mango leaves with necrosis symptoms (Chen *et al*., 2023); and *P. vagans* MA I6050, isolated in Uruguay from leaves with blight symptoms and onion bulb rot (De Armas

et al., 2022). This suggests that they share an evolutionary origin and possible virulence factors as phytopathogens. Accurate differentiation among *Pantoea* species is considered difficult based solely on physiological and biochemical characterization and partial sequencing of the 16S rRNA gene; it is estimated that in the past, *Pantoea* species were frequently misidentified using this approach. Therefore, multilocus analysis has been shown to be an efficient method for accurately defining both pathogenic and non-pathogenic *Pantoea* species (Chang *et al*., 2018; Yao *et al*., 2023) and for inferring their phylogenetic and evolutionary relationships (Anwer *et al*., 2025).

The results of this study identified *P. vagans* CPHU23 isolated from *S. undatus* through concatenation of four housekeeping genes (*fus*A, *gyr*B, *rpo*B, and *leu*S) and confirmed the high percentage of metabolic profile similarity (93%) of *P. vagans* CPHU23 with the reference strains LMG 24199 and MalMa-22 of *P. vagans*, isolated from eucalyptus and agave, respectively (Table 1). The nucleotide sequences from the multilocus phylogenetic analysis of strain *P. vagans* CPHU23 were deposited in the NCBI GenBank under the accession numbers: *rpo*B (PX641593), *gyr*B (PX641591), *leu*S (PX641592), and *fus*A (PX641590).

Pathogenicity tests. Inoculation by infiltration with strain *P. vagans* CPHU23 caused rot in the five pitahaya species (Figure 4), as well as in agave and aloe (Figure 5) 14 days after inoculation. From these symptoms, bacterial colonies with the same morphology as the inoculated strain *P. vagans* CPHU23 were re-isolated. No symptoms were observed in the non-inoculated controls.

Figure 4. Rot in pitahaya stems inoculated by infiltration with a suspension containing 3×10^8 CFU mL^{-1} of *Pantoea vagans* CPHU23. **A)** *Selenicereus undatus*, **B)** *Selenicereus* sp. "Golden," **C)** *Selenicereus purpusi*, **D)** *Selenicereus ocamponis*, and **E)** *Selenicereus* "Solferina". T= control, I= inoculated.

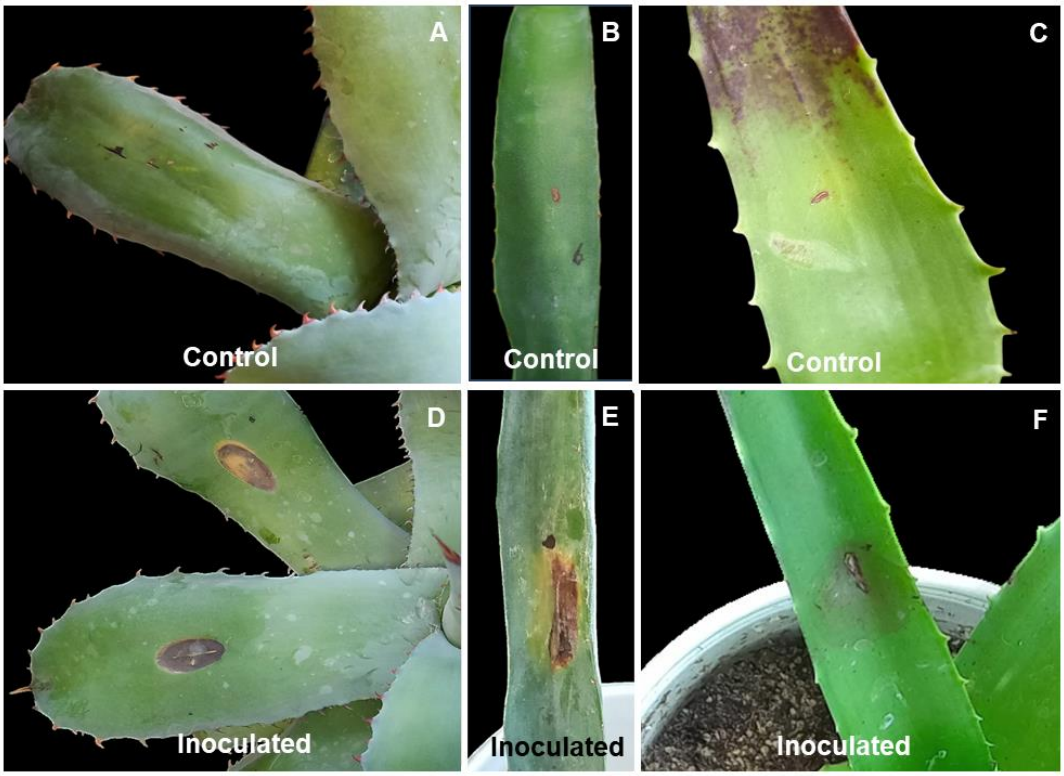

Figure 5. Leaf rot in **D)** *Aloe vera*, **E)** *Agave angustifolia*, and **F)** *Agave cupreata* inoculated by infiltration with a suspension containing 3×10^8 CFU mL^{-1} of *Pantoea vagans* CPHU23. **A**, **B**, and **C** = controls.

In pitahaya, the symptoms were identical to those observed in *S. undatus* stems in Huitziltepec, Puebla. Among the five inoculated species, greater severity (greater extent of damaged tissue) was observed in *Selenicereus undatus* and *Selenicereus* "Golden" (Figures 4A and 4B), both of which produce white-fleshed fruit; this is consistent with the original isolation of *P. vagans* CPHU23 from *S. undatus*.

In addition, inoculation with *P. vagans* CPHU23 caused rot in leaves of *Agave angustifolia*, *Agave cupreata*, and *Aloe vera* (Figure 5), but not in onion bulbs or tomato and chili fruits (Table 2). Therefore, *P. vagans* could represent a potential phytosanitary problem in agave and aloe, as these species are cultivated in Huitziltepec, Puebla. In Mexico, *P. vagans* has been reported as the causal agent of rot in *Agave angustifolia* (Rodríguez-Velázquez *et al*., 2024); however, to our knowledge, there are no reports of *P. vagans* causing rot in *A. cupreata* and *A. vera*.

Populations of *P. vagans* are considered to exhibit high ecological plasticity, as they can establish interactions with plants as endophytes, epiphytes, and even, in some strains, as biocontrol agents (Walterson and Stavrinides, 2015). *P. vagans* was first identified as a phytopathogen in Uganda, causing blight and dieback of eucalyptus (Brady *et al*., 2008). However, it has recently been identified more frequently as a phytopathogen, and its incidence is increasing globally. In the past four years, *P. vagans* has been reported as the causal agent of rot in cabbage (*Brassica* sp.) in Korea (Lee *et al*., 2022), foliar necrosis and onion bulb rot in Uruguay (De Armas *et al*., 2022), foliar necrosis in mango (*Mangifera indica*) in China (Chen *et al*., 2023), and foliar and stem necrosis in cotton (*Gossypium* sp.) in Pakistan (Anwer *et al*., 2025). In Mexico, *P. vagans* has been identified as the causal

agent of rot in *Agave angustifolia* (Rodríguez-Velázquez *et al*., 2024), which is consistent with the results of this study.

Knowledge of the virulence factors used by *P. vagans* during infection of host plants is limited. In a comparative genomic study, a family of LPP-1 (Large *Pantoea* Plasmid) plasmids distributed among seven *Pantoea* species, including *P. vagans*, was identified and associated with metabolism, colonization, and pathogenesis (Walterson and Stavrinides, 2015). Additionally, in *P. vagans* populations, genes encoding the type III secretion system (T3SS) and type VI secretion system (T6SS) have been highlighted for their important role in infection and pathogenicity (Lv *et al*., 2022; Yao *et al*., 2023). In this study, *P. vagans* CPHU23 isolated from pitahaya (*S. undatus*) induced a hypersensitive reaction in tobacco, which is closely associated with the hypersensitivity and pathogenicity gene cluster and the T3SS in Gram-negative phytopathogenic bacteria (Chang *et al*., 2014).

In this study, the *in vitro* sensitivity of *P. vagans* CPHU23 isolated from pitahaya to bactericides notably showed *in vitro* resistance to most of the evaluated bactericides; however, it was sensitive to Intermicin® 500 (oxytetracycline + streptomycin + tribasic copper sulfate), Finalbacter® (gentamicin sulfate + oxytetracycline hydrochloride), Cobrezate® M (copper oxychloride + mancozeb), and Quatz® (quaternary ammonium), and resistant to kasugamycin (Table 2). The effectiveness of tetracycline analogs for the control of *Pantoea agglomerans* has been demonstrated in fruit crops (Zhou *et al*., 2025); however, to our knowledge, no bactericidal chemical strategies have been reported globally for the control of *P. vagans*. Therefore, this study demonstrates the *in vitro* sensitivity of *P. vagans* CPHU23 to copper-based formulations and antibiotics for the development of future management strategies in pitahaya cultivation. The use of copper has been widely documented as an effective management strategy for the control of enterobacteria; it has been reported that mixing copper with mancozeb improves copper solubility and bacterial control (Charkowski, 2018). *P. vagans* CPHU23 was sensitive to Quatz® (Table 2); quaternary ammonium salts are potent biocides that have been little studied in agriculture as pesticides; however, they have been recommended as disinfectants for greenhouse surfaces (Peyneau *et al*., 2022), suggesting their evaluation for the disinfection of pruning and harvesting tools in pitahaya cultivation.

The number of reports of *P. vagans* as a causal agent of diseases in crops has increased in recent years; therefore, the development of future research on the epidemiology and infection mechanisms of *P. vagans* in pitahaya cultivation in Mexico and other potential hosts is warranted, as well as the evaluation of effective management strategies to reduce its impact on fruit production. This study represents the first report of *P. vagans* as the causal agent of stem rot in pitahaya.

CONCLUSIONS

Physiological and biochemical characterization, multilocus sequence analysis, and pathogenicity tests confirmed that *Pantoea vagans* is the causal agent of stem rot in pitahaya in Puebla. Pitahaya species cultivated in the municipality of Huitziltepec are susceptible; among them, red-fleshed pitahaya species are less susceptible to stem rot caused by *P. vagans*. In addition, *Agave cupreata* and *Aloe vera* are susceptible to rot caused by *P. vagans*, and therefore could serve as potential hosts of this pathogen. *P. vagans* is resistant to kasugamycin but sensitive to copper-based products and other antibiotics. The biological effectiveness of copper oxychloride + mancozeb should be

evaluated under field conditions, as the use of this combined formulation may represent an efficient management strategy for *P. vagans* in pitahaya cultivation in Mexico.

Conflict of interest. The authors declare that there is no conflict of interest related to this research.

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