

Pectobacterium brasiliense, causal agent of rhizome rot in ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Objective: To isolate and identify the causal agent of ginger rhizome rot in Jalpan, Puebla and to evaluate its pathogenicity and *in vitro* sensitivity to bactericides.

Design/methodology/approach: Bacteria with similar morphology were isolated from ginger rhizomes with rot. Strain CPB04 was biochemically characterized and identified by 16S rRNA gene sequencing. *In vitro* sensitivity was evaluated with nine commercial bactericides and pathogenicity in ginger rhizomes and organs of 11 plant species by injection of a suspension containing 3×10^8 CFU mL⁻¹.

Results: The biochemical characterization of strain CPB04 showed similarity with *Pectobacterium*. Sequencing of the 16S rRNA gene of strain CPB04 identified it with 100% similarity to *Pectobacterium brasiliense* strain CP047495.1. *P. brasiliense* CPB04 was sensitive *in vitro* to copper formulations and resistant to kasugamycin. It caused organ rots in sweet potato (*Ipomoea batatas*), onion (*Allium cepa*), chili (*Capsicum annum*), jicama (*Pachyrhizus erosus*), tomato (*Solanum lycopersicum*), ginger (*Zingiber officinale*), potato (*Solanum tuberosum*) and radish (*Raphanus sativus*).

Implications of the study: *Pectobacterium brasiliense* is a highly virulent pathogen with potential to infect new hosts. Rot diseases are a global problem in agriculture. This is the first report in Mexico of *P. brasiliense* as a causal agent of rot in ginger.

Findings/conclusions: *Pectobacterium brasiliense* is the causal agent of ginger rhizome rot in Jalpan, Puebla. The tuberous root of jicama may be a new host of *Pectobacterium brasiliense*. *Pectobacterium brasiliense* is sensitive *in vitro* to copper oxychloride.

Keywords: *Pectobacterium brasiliense*, ginger, rot, 16S rRNA.

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INTRODUCTION

Ginger (*Zingiber officinale*) is a monocotyledonous plant belonging to the family Zingiberaceae. The underground rhizomes of ginger are consumed as fresh vegetables and spices, and have a long history due to their high economic, nutritional, and medicinal value (Chawla *et al.*, 2021). Globally, India is the leading ginger producer with 1,844,000 tons. Mexico ranks 21st in ginger production with 4,405 tons and 17th as an exporter with 1,673 tons (FAO, 2023).

In Mexico, ginger is mainly cultivated in the states of Oaxaca, Guerrero, and Puebla (SIAP, 2023). However, around 70% of the ginger consumed in Mexico is produced in the



northern Sierra of Puebla state, with an approximate production of 2,870 tons, highlighting the municipalities of Jalpan, Pantepec, and Xicotepéc as the main producers (SIAP, 2023). The most well-known and widely cultivated ginger in Mexico is common ginger (*Zingiber officinale*). Due to its high nutritional and medicinal value, the demand for production and consumption has increased in recent years (García *et al.*, 2023).

Globally, research on diseases affecting ginger cultivation is scarce compared to other agricultural crops. However, ginger is known to be highly susceptible to both bacterial and fungal diseases that affect the plant and its rhizome (Kyaw *et al.*, 2022; Meenu & Kaushal, 2017). Among these, rhizome rot caused by bacteria is a highly destructive disease that significantly impacts rhizome yield and postharvest quality in all countries where it has been reported (Huang *et al.*, 2020). Bacterial pathogens identified worldwide as causal agents of rhizome rot include *Enterobacter cloacae* in China and the United States (Liu *et al.*, 2021; Nishijima *et al.*, 2004; Zhao *et al.*, 2022); *Bacillus pumilus* and *Serratia marcescens* in China (Huang *et al.*, 2020; Peng *et al.*, 2013); *Erwinia chrysanthemi* in the United States (Stirling, 2002); and *Ralstonia solanacearum* in Thailand (Kyaw *et al.*, 2022).

In 2024, in the community of La Garza, municipality of Jalpan, Puebla, ginger producers were affected by rhizome rot both in the field and in storage, with an estimated incidence of 30%. This disease is currently considered the most important one, causing significant economic losses for local producers. Infected rhizomes initially exhibit external water-soaked lesions, followed by internal tissue rot. Based on the characteristics of these symptoms, this study assumes that the rhizome rot is caused by bacteria. To date, no research has been conducted on diseases affecting ginger cultivated in Mexico, particularly on rhizome rot. The objective of this study was to isolate and identify the causal agent of ginger rhizome rot in Jalpan, Puebla, Mexico, and to evaluate its pathogenicity and *in vitro* sensitivity to bactericides.

MATERIALS AND METHODS

Sample Collection

In 2024, four composite samples, each consisting of five ginger rhizomes showing symptoms of rot (Figure 1A), were collected in the community of La Garza, municipality of Jalpan, located in northern Puebla state (20° 23' 24" N and 98° 00' 54" W) (Figure 1B). The samples were analyzed at the Phytopathogenic Bacteria Laboratory of the Colegio de Postgraduados, Montecillo campus, in Texcoco, State of Mexico.

Bacterial Isolation

Ginger rhizomes were surface-disinfested with 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. The rhizomes were then cut longitudinally, and 0.5 cm segments were taken from the internal tissue at the interface between healthy and diseased areas showing rot symptoms. The tissue segments were macerated in a mortar with 500 μ L of sterile distilled water, and 20 μ L of the suspension were plated onto Petri dishes containing Wilbrink's agar medium (Koike, 1965). The plates were incubated at 28 °C for 48 h. Individual bacterial colonies were purified from the resulting growth and

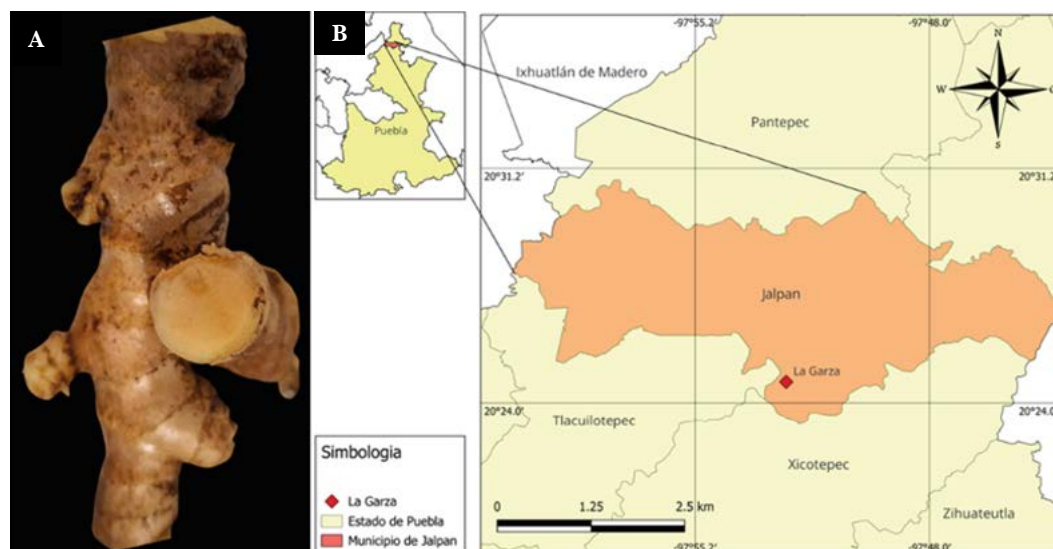


Figure 1. (A) Ginger rhizome with rot symptoms collected from the community of La Garza; (B) Municipality of Jalpan, Puebla.

preserved at $-80\text{ }^{\circ}\text{C}$ in nutrient broth with 20% glycerol. From these bacterial isolates, strain CPB024 was selected for further studies.

Physiological and Biochemical Characterization

The physiological and biochemical characterization of strain CPB024 was carried out following the protocols described by Borkar (2017) and Schaad *et al.* (2001). *In vitro* sensitivity to bactericides was evaluated using a modified agar diffusion method (Liu and Filiatrault, 2020): $100\text{ }\mu\text{L}$ of a bacterial suspension containing 10 CFU mL^{-1} of strain CPB024 were inoculated onto square Petri dishes ($120\times 120\text{ mm}$) (Thermo Fisher Scientific, USA) containing nutrient agar medium (BD Bioxon, Mexico). The inoculum was evenly distributed on the surface of the culture medium using a Drigalski loop. Sterile filter paper discs (0.5 cm in diameter) previously soaked in bactericide solutions were then placed on the agar surface, with three replicates each. The plates were incubated for 72 h at $28\text{ }^{\circ}\text{C}$. The sensitivity of strain CPB024 was determined by the formation of an inhibition zone around the filter paper disc containing the bactericide. Seven bactericide formulations were evaluated at the label-recommended dosage: Cobrezate[®] (50% copper oxychloride + 36% mancozeb); Intermicin 500[®] (0.235% oxytetracycline + 71.8% tribasic copper sulfate + 2.2% streptomycin); Kasumin[®] (2.3% kasugamycin); Oxicob[®] (85% copper oxychloride); Quatz[®] (quaternary ammonium compound); Sagol[®] (22% copper oleate); and Copper Sulfate.

Molecular and Phylogenetic Identification

Genomic DNA was extracted using the 2% CTAB method (Doyle and Doyle, 1990) from pure cultures grown on nutrient agar for 48 h at $28\text{ }^{\circ}\text{C}$. Molecular identification was performed by amplification and partial sequencing of the 16S rRNA gene using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R

(5'-GGTTACCTTGTTACGACTT-3') (Heuer *et al.*, 1997). Polymerase chain reactions (PCR) were carried out under the conditions described by Mejía-Sánchez *et al.* (2019) using a Touch C1000 thermal cycler (Bio-Rad). PCR products were sequenced by Macrogen Inc. (South Korea). Consensus sequences were compared with those in the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) database using the BLASTn tool. A phylogenetic tree was constructed based on aligned sequences using the maximum likelihood method, applying the Tamura-Nei evolutionary model and 1,000 bootstrap replicates to assess branch robustness. Phylogenetic analysis was performed using MEGA X software.

Pathogenicity Tests

Pathogenicity tests were conducted using strain CPB024 isolated from ginger rhizomes exhibiting rot symptoms. Ginger rhizomes were surface-disinfested sequentially with soapy water, 70% ethanol, and three rinses with sterile distilled water. Subsequently, rhizomes were inoculated by injecting 50 μL of a bacterial suspension adjusted to 3×10^8 CFU mL^{-1} using the McFarland standard scale. In addition to ginger, the pathogenicity of strain CPB024 was evaluated following the same disinfection and inoculation protocol described above on organs of plant species belonging to 10 botanical families: Amaryllidaceae [onion bulb (*Allium cepa*)], Apiaceae [carrot tuberous root (*Daucus carota*)], Brassicaceae [radish tuberous root (*Raphanus sativus*)], Cactaceae [pitahaya stem (*Hylocereus undatus*)], Convolvulaceae [sweet potato tuber (*Ipomoea batatas*)], Fabaceae [jicama tuberous root (*Pachyrhizus erosus*)], Marantaceae [pencil plant rhizome (*Goepfertia ornata*)], Rosaceae [apple fruit (*Malus domestica*)], Solanaceae [chili pepper fruit (*Capsicum annuum*) and tomato fruit (*Solanum lycopersicum*)], potato tuber (*Solanum tuberosum*), and Zingiberaceae [turmeric rhizome (*Curcuma longa*) and shell ginger (*Alpinia zerumbet*)].

The treatments were maintained in a humid chamber at room temperature (~ 24 °C). Each treatment was performed with three replicates. In the control, the same volume of sterile distilled water was injected instead of the bacterial inoculum.

RESULTS AND DISCUSSION

Bacterial Isolation

From the four ginger samples with rot symptoms collected from the study region, colonies with similar morphological characteristics (circular, convex, mucoid, and moist-appearing colonies) were most frequently isolated on Wilbrinks agar medium. Among these, strain CPB024 was selected for further studies.

Physiological and Biochemical Characterization

Strain CPB024 was Gram-negative, catalase positive, oxidase negative, exhibited oxidative and fermentative metabolism of glucose, reduced nitrates, produced H_2S ; it utilized arabinose, mannose, and sucrose as carbon sources and showed pectinolytic activity on potato slices (Table 1). This metabolic profile is characteristic of bacteria in the Enterobacteriaceae family, which includes the genus *Pectobacterium* (Borkar *et al.*, 2017; Czajkowski *et al.*, 2015; Schaad *et al.*, 2001), a pathogen with a broad host range

Table 1. Physiological and biochemical characterization, pathogenicity, and *in vitro* sensitivity to bactericides of *Pectobacterium brasiliense* CPB024 isolated from ginger rhizomes with rot symptoms.

Test	Strain		Pathogenicity CPB024			In vitro sensitivity CPB024
	CPB024 Ginger ¹	PbTet5 Cactacea ²	Family	Specie	Organ	
Gram stain	-	-	Amaryllidaceae	Onion (<i>Allium cepa</i>)	Bulb	+ Cobrezate (Copper oxychloride + Mancozeb)
Levana	-	ND	Apiaceae	Carrot (<i>Daucus carota</i>)	Tuberous root	+ Intermicin 500 (Oxytetracycline + Tribasic copper sulfate + Streptomycine)
Oxidase	-	-	Brassicaceae	Rábano (<i>Raphanus sativus</i>)	Tuberous root	+ Oxicob (Copper oxychloride)
Arginine	-	-	Cactaceae	Pitahaya (<i>Hylocereus undatus</i>)	Stem	- Kasumin (Kasugamycin)
Catalase	+	+	Convolvulaceae	Sweet potato (<i>Ipomoea batatas</i>)	Tuberous root	+ Quatz (Quaternary ammonium)
Nitrate reduction	+	ND	Fabaceae	Jicama (<i>Pachyrhizus erosus</i>)	Tuberous root	+ Sagol (Cupric oleate)
Starch hydrolysis	-	ND	Marantaceae	Calathia (<i>Goepertia ornata</i>)	Rhizome	- Cupric sulfate
Oxidative/fermentative metabolism	+/+	+/+	Rosaceae	Apple (<i>Malus domestica</i>)	Fruit	-
Phosphatase	-	-		Chili (<i>Capsicum annuum</i>)	Fruit	+
Gelatin hydrolysis	+	ND	Solanaceae	Tomato (<i>Solanum lycopersicum</i>)	Fruit	+
Gas production	-	ND		Potato (<i>Solanum tuberosum</i>)	Tuber	+
Indole	-	-		Turmeric (<i>Curcuma longa</i>)	Rhizome	-
Growth at 7% NaCl	+	+	Zingiberaceae	Ginger (<i>Zingiber officinale</i>)	Rhizome	+
Growth at 8% NaCl	+	ND		Shell ginger (<i>Alpinia zerumbet</i>)	Rhizome	-
Growth at 36 °C	+	+				
H ₂ S production	+	ND				
Esculin	+	ND				
-Methyl glucoside	-	+				
Arabinose	+	ND				
Cellobiose	+	ND				
D-glucose	+	ND				
Dulcitol	-	ND				
Inositol	+	ND				
Lactose	+	+				
Maltose	-	+				
Mannitol	+	ND				
Mannose	+	ND				
Melezitose	-	ND				
Melibiosa	+	ND				
Sorbitol	-	-				
Sucrose	+	ND				
Trehalose	+	+				
Xylose	+	ND				

¹ *Pectobacterium brasiliense* CPB024 isolated from ginger.

² *Pectobacterium brasiliense* Tet5 isolated from *Neobuxbaumia tetetzo*. Source: Mejía-Sánchez et al. (2019).
ND=Not determined.

affecting at least 35% of angiosperms (Toth *et al.*, 2021), and is distributed worldwide across diverse ecological niches (van der Wolf *et al.*, 2021). Additionally, strain CPB024 showed 87% similarity with the metabolic profile of strain Tet5 (accession No. MF403054) of *Pectobacterium carotovorum* subsp. *brasiliense* isolated from *Neobuxbaumia tetetzo* in Puebla, Mexico (Mejía-Sánchez *et al.*, 2019); which was reclassified as *Pectobacterium brasiliense* (Portier *et al.*, 2019) (Table 1).

Molecular and Phylogenetic Identification

The partial amplification of the 16S rRNA gene of strain CPB024, isolated from ginger rhizome, was deposited in GenBank under accession number PV017467. BLASTn analysis of this sequence showed 100% identity with various sequences belonging to *Pectobacterium brasiliense*, among which is sequence CP020350 (strain 1692), identified as the causal agent of cucumber fruit rot and considered highly virulent in a wide range of plant species in Shanxi Province, China (Meng *et al.*, 2017), and phylogenetically related to the strain isolated from ginger in this study (Figure 2).

Pathogenicity Tests

Inoculation of strain CPB024, isolated from ginger with rot symptoms (identified as *Pectobacterium brasiliense* in this study), caused rot in ginger rhizomes seven days after inoculation (dai) (Figure 3). The symptoms were similar to those observed in rhizomes from Jalpan, Puebla. No symptoms were observed in the control rhizomes injected with sterile distilled water. The bacteria re-isolated from the rotted rhizomes exhibited the same morphological characteristics as the inoculated strain, fulfilling Koch's postulates.

In ginger, rhizome rot has been associated with infections by the fungi *Fusarium* spp. and *Pythium* spp. (Meenu and Kaushal, 2017); however, *Pectobacterium brasiliense* is considered the most destructive and important pathogen causing ginger rhizome rot, leading to

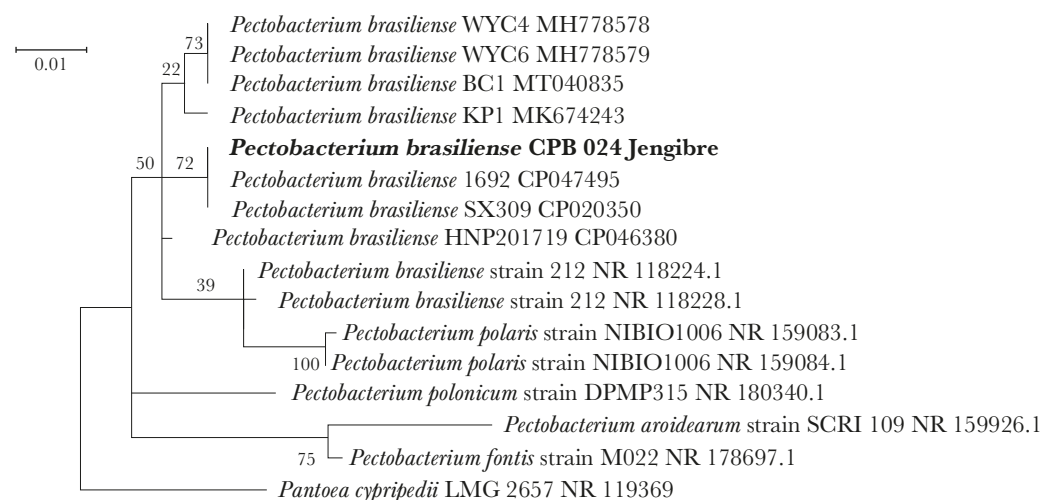


Figure 2. Phylogenetic tree based on the partial sequencing of the 16S rRNA gene of the genus *Pectobacterium* using the Maximum Likelihood method, constructed in MEGA X, with 1000 bootstrap replicates. The ginger sequence from this study is highlighted in bold (GenBank accession number PV017467). *Pantoea cyripedii* was used as the outgroup.

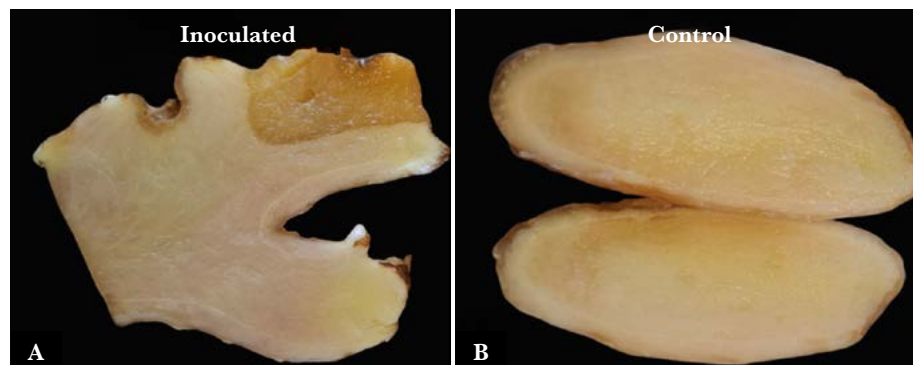


Figure 3. Rot symptoms on ginger rhizome at 7 days post-inoculation (dpi) with strain CPB024 of *Pectobacterium brasiliense*.

production losses ranging from 50 to 90% (Wang *et al.*, 2022, 2024). The identification of *P. brasiliense* as the causal agent of ginger rhizome rot in Jalpan, Puebla, is consistent with the first report of *P. brasiliense* causing ginger rhizome rot in China (Wang *et al.*, 2022). Until now, *P. brasiliense* had been reported in Mexico causing rot in the cactus *Neobuxbaumia tetetzo* in Zapotitlán de las Salinas, Puebla (Mejía-Sánchez *et al.*, 2019; Vargas-Peralta *et al.*, 2021).

Inoculation of *Pectobacterium brasiliense* CPB024 on plant organs from 10 different families caused rot symptoms at 2 days post-inoculation (dpi) in tomato, chili pepper, onion, sweet potato, jicama, radish, and potato; however, no symptoms were observed in apple, pitahaya, carrot, calathea, shell ginger, and turmeric (Table 1) (Figure 4).

Until 2021, *P. brasiliense* had been reported as a pathogen on 19 plant species across 10 families; since then, its host range has expanded to include additional species within the families Aizoaceae, Cucurbitaceae, and Zingiberaceae (Park *et al.*, 2023a; Song *et al.*, 2023; Wang *et al.*, 2022), making it an emerging global problem (Oulghazi *et al.*, 2021).

In this study, the rot caused by the inoculation of *P. brasiliense* CPB024 is consistent with results reported in other investigations involving *P. brasiliense* strains inoculated in tomato, chili pepper, and potato (Duarte *et al.*, 2004; Mejía-Sánchez *et al.*, 2019), as well as

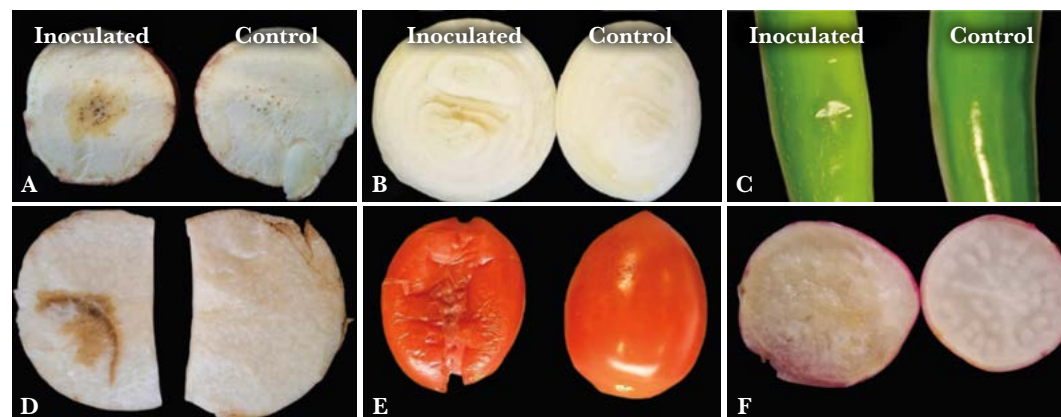


Figure 4. Rot symptoms in: A) Sweet potato, B) Onion, C) Serrano chili, D) Jicama, E) Tomato, and F) Radish at 48 days post-inoculation (dpi) with *P. brasiliense* CPB024.

in radish, onion, and sweet potato (Li-ping *et al.*, 2020; Mejía-Sánchez *et al.*, 2019; Park *et al.*, 2023b). However, to our knowledge, there are no reports of rot caused by *P. brasiliense* inoculation in the tuberous root of jicama.

The worldwide distribution of *Pectobacterium brasiliense* suggests that it adapts to both tropical and temperate climates as well as high humidity conditions (Wang *et al.*, 2022). Therefore, the prevailing environmental conditions in Puebla and other states where ginger is cultivated could favor the establishment and spread of this pathogen in Mexico.

In Jalpan, Puebla, the origin of the planted rhizomes as well as the source of *P. brasiliense* inoculum are unknown. Previous studies have documented that *P. brasiliense* has been isolated from water and the rhizosphere of weeds, and can be easily transmitted to other cultivation areas through irrigation systems, soil, and during the storage of planting material (Oulghazi *et al.*, 2021). In Mexico, Mejía-Sánchez *et al.* (2021) detected *P. brasiliense* in the intestines of larvae and adults of the insect *Lagocheirus lugubris* Dillon (Coleoptera: Cerambycidae) and in adults of *Glyphidops flavifrons* (Bigot) (Diptera: Neriidae), both closely associated with the rot disease of the columnar cactus Tetecho in Zapotitlán, Puebla; highlighting the possible role of insects as potential vectors of *P. brasiliense*. Other factors identified that favor infection in other *Pectobacterium* species include saturated or flooded soils, wounds caused to planting material during harvest, and infection by other soil pathogens such as fungi and nematodes (Stirling *et al.*, 2002; Charkowski, 2018).

Interestingly, in this study, the control ginger rhizomes injected with sterile distilled water showed rot symptoms similar to those inoculated (7 days after the start of the assay); from the tissue of the rotten rhizomes, colonies with the same morphological and metabolic characteristics of *P. brasiliense* were isolated. Studies on the endophytic microbiota in ginger rhizomes with rot symptoms and asymptomatic rhizomes revealed the presence of bacteria from the Enterobacteriaceae family, including *P. brasiliense*, in both types of rhizomes; however, a higher relative abundance of these populations was found in the rotten rhizomes compared to the asymptomatic ones (Huang *et al.*, 2022; Wang *et al.*, 2024). This suggests that *P. brasiliense* could be an endophyte in ginger rhizomes, which would be relevant in the *P. brasiliense*-ginger pathosystem due to the asexual propagation of this crop and the use of rhizomes for new plantations in other cultivation areas.

Currently, no effective control method against *P. brasiliense* has been documented, making it necessary to develop management strategies against this pathogen. Control recommendations based on copper sprays have been frequently suggested for managing diseases caused by *Pectobacterium* spp., along with other strategies such as the use of healthy planting material, sanitary practices, and biological control (Azaiez *et al.*, 2018; Charkowski, 2018). In this study, *P. brasiliense* CPB024 was sensitive *in vitro* to the bactericidal formulations Intermicin 500 (Oxytetracycline+tribasic copper sulfate+streptomycin), Cobrezate (Copper oxychloride+Mancozeb), Oxicob (Copper oxychloride), and Quatz (Quaternary ammonium) (Table 1). Quaternary ammonium salts are an emerging group of antibacterial agents used as disinfectants that inhibit bacterial biofilm formation on surfaces (Nadagouda *et al.*, 2022); however, they have been little studied in agriculture except as disinfectants in greenhouse structures. In contrast, the bactericidal activity of copper is widely documented globally in agriculture (Charkowski, 2018; Czajkowski *et al.*,

2015). Therefore, it is important to evaluate the biological effectiveness in the field of the copper-based formulations identified in this study for the control of *P. brasiliense* in ginger cultivation in Jalpan, Puebla. Likewise, it is necessary to deepen the understanding of the *P. brasiliense*-ginger pathosystem, particularly regarding the epidemiology, distribution, and genetic variability of this pathogen, as well as the susceptibility of the cultivated ginger varieties. This is crucial for the development of efficient control strategies against *P. brasiliense* in Puebla and other states in Mexico where ginger is grown.

CONCLUSIONS

Pectobacterium brasiliense is the causal agent of rhizome rot in ginger plants from Jalpan, Puebla. Plant organs of species belonging to the Solanaceae family are more susceptible to rot caused by *P. brasiliense*. Jicama, which belongs to the Fabaceae family, may be a new potential host of *P. brasiliense*. *P. brasiliense* is resistant to antibiotics such as kasugamycin and sensitive to copper-based compounds; among them, formulations containing copper oxochloride may be an effective tool for managing *P. brasiliense* in ginger crops in Mexico. This is the first report of *P. brasiliense* causing rhizome rot in ginger in Mexico.

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