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CHARACTERIZATION OF RHIZOME AND PSEUDOSTEM WET ROT OF ORGANIC BANANA (*Musa* sp.) IN PIURA, PERU

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ABSTRACT

Organic banana (*Musa* sp.) is one of the most important export crops in Peru, especially in the Department of Piura. The cultivation of the crop is mainly affected by biotic factors, resulting in important economic losses. The aim of this research was to characterize the causal agent of banana bacteriosis disease in Piura. Bacterial isolates were extracted from rhizome and pseudostem samples of organic bananas with typical symptoms of soft rot from different banana growing areas of Piura. Sixty colonies were obtained, while 10 isolates were selected for presenting cultural and morphological characteristics similar to those of *Pectobacterium* (01MB, 04MB, 05MB, 06MB and 10MB) and *Dickeya* (02MB, 03MB, 08MB, 07MB and 09MB); different biochemical and molecular tests were conducted. The pathogenicity of the isolates was confirmed by *in vitro* and *in vivo* inoculation of rhizome pieces and pseudostems from cultivated plants. The 10 isolates were gram negative and with perimeter flagella. The biochemical tests showed that all the isolates were positive on nutrient agar medium, Mac Conkey, catalase, gelatin hydrolysis and growth at 37°C, whereas they were negative on King's B medium, nitrate reductase and oxidase tests. Isolates 02MB, 03MB, 08MB, 07MB and 09MB also tested positive for erythromycin and indole, while isolates 01MB, 04MB, 05MB, 06MB and 10MB were positive for CVP (Crystal violet pectate). Molecular identification and phylogenetic analysis confirmed that isolates 01MB, 04MB, 05MB and 10MB corresponded to *Pectobacterium carotovorum* subsp. *carotovorum*; isolates 03MB and 08MB corresponded to *Dickeya chrysanthemi*; and 02MB and 07MB corresponded to *Dickeya paradisiaca* and *Klebsiella variicola*, respectively. It was concluded that rhizome and pseudostem wet rot of banana grown organically in Piura is caused by the bacterial complex formed by *P. carotovorum* subsp. *carotovorum*, *D. chrysanthemi*, *D. paradisiaca* and *K. variicola*. This is valuable information for the implementation of appropriate disease management practices in the area.

Keywords: *Dickeya*, *Pectobacterium*, rot, bacteriosis, banana, characterization.

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INTRODUCTION

Banana (*Musa* sp.) is an herbaceous plant belonging to the Musaceae family. It is an excellent source of potassium, fiber, and vitamins (Lal et al., 2017; Voora, 2020), being one of the most important food crops worldwide. It ranks fourth after rice, wheat, and corn (Elbehri et al., 2015), while it occupies the second place worldwide in fresh consumption with 50 million tons. It is produced in all tropical and subtropical regions, and its cultivation spans more than a hundred countries, being of fundamental importance for the economies of many developing countries (Blomme et al., 2017; FAO, 2020; Voora, 2020). In Peru, it is the most important cultivated fruit, with 15,000 hectares dedicated to the cultivation of organic bananas; 12,800 hectares are concentrated in the Department of Piura, mainly in the province of Sullana, followed by Lambayeque, Tumbes and La Libertad (MINAGRI, 2018). The total number of producers dedicated to the production of organic bananas with high quality standards is approximately 9,500 and the export variety is Cavendish Valery (MINAGRI, 2020). In fact, Peru is among the main producers and exporters of organic bananas, with exports of 221,266,136 kg (US$ 152,342,846), being Europe, the United States and Asia the main markets (MINAGRI, 2018; MINAGRI, 2020).

In Piura, organic banana production is increasing, but the crop is affected by abiotic and biotic factors, resulting in important economic losses. Biotic factors include diseases such as rhizome and pseudostem wet rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Gardan et al., 2003) (syn. *Erwinia carotovora* ssp. *carotovora*) or *Dickeya chrisanthemy* (Samson et.al 2005) (syn. *Erwinia chrysanthemi*), and bacterial wilt caused by *Ralstonia solanacearum*, which are the main bacteria that affect the crop.

Rhizome soft rot is a serious disease in different countries of the world (Ma et al., 2007; Snehalatharani and Khan, 2010; Gokul et al., 2019), with an incidence of more than 75% in severely infected fields (Dita et al., 2013; Blomme et al., 2017). The development of the disease is associated with the two causal agents that can act together or separately, depending on the variety of banana and the place of incidence. *P. carotovorum* subsp. *carotovorum* has been reported as a causal agent in cv. Grand Naine (AAA) in India (Rajamanickam et al., 2018), while *Dickeya* sp. (formerly *Pectobacterium chrysanthemi*) has been identified in *Musa sapientum* (cv. ABB) in China (Lin et al., 2010). Symptoms include watery soft rot with an unpleasant foul odor from the rhizome and internal decay of the pseudostem as infection progresses upward, decreased growth, leaf yellowing, and eventually plant drop (Nagaraj et al., 2012; Hugouvieux-Cotte-Pattat et al., 2014; Blomme et al., 2017). *Dickeya* and *Pectobacterium* can seriously affect the crop at any phenological stage of the plant, and thus both pathogens are of great importance given their distribution in all regions where Musaceae are grown (Snehalatharani and Khan, 2010; Nagaraj et al., 2012; Charkowski, 2018). These phytopathogenic bacteria, being natural inhabitants of the soil, are associated with rhizome and pseudostem wet rot, and represent a high-risk potential for banana production (Mansfield, 2012; Blomme et al., 2017). Therefore, an in-depth understanding of the causal agent is required to classify the pathogen(s), and also to develop appropriate disease management practices. The objective of this study was to identify and characterize the causal agent of rhizome and pseudostem wet rot of banana grown organically in Piura, Peru.

MATERIALS AND METHODS

Isolation and purification of bacteria

Organic banana rhizomes and pseudostems with typical symptoms of rot were collected from the Valleys of Chira (25), Alto Piura (16) and San Lorenzo (2), which are the main banana areas of the Department of Piura, Peru (Table 1). The samples were placed and coded in hermetic bags to be processed and analyzed separately. The rhizomes and pseudostems were washed with plenty of water, disinfected with 1.0% sodium hypochlorite for 2 minutes, rinsed with sterile distilled water, sectioned into small segments to be immersed in a test tube containing 9 mL of sterile distilled water, and then left to rest for 10 minutes to form a bacterial suspension. These were sown in Petri dishes containing nutrient agar (NA) culture medium, using the sterile Kolle loop streak method, and incubated inverted at 28°C for 24 hours until growth of bacterial colonies was achieved. After the last sowing line, colonies showing bacterial growth similar to that of the *Dickeya* and *Pectobacterium* genera were striated in Petri dishes containing NA culture medium and incubated at 28°C for 24 hours. The bacterial colonies obtained were purified in tubes containing NA culture medium and kept under refrigeration (French and Hebert, 1980) until analysis.

Pathogenicity tests

The pathogenicity of the identified isolates was evaluated in potatoes (*Solanum tuberosum*), and banana pseudostem and seedlings. In potatoes, healthy tubers were washed with plenty of water, disinfected with 1.5% sodium hypochlorite for

Table 1. Rhizome and pseudostem samples with symptoms of rot from the banana growing areas of Alto Piura, Peru.

Valley	Province	District	Sector	Georeferential área	N° of samples
Chira	Sullana	Bella Vista	Chalacala	4°47'4.99"S, 80°33'11.88"O	2
		Marcavelica	Marcavelica	4°52'54.00"S, 80°42'12.00"O	2
			La Quinta	4°51'15.01"S, 80°43'30.00"O	5
			Mallares	-4.86389S, -80.7733º	3
		Querecotillo	Montenegro Bajo	-4,84179S, -80,640228º	3
		Salitral	Santa Rosa 3	4°51'26.81"S, 80°40'52.33"O	3
			Cabo Verde Bajo	4°49'34.00"S, 80°40'48.00"O	2
			Tangarará	4°52'60.00"S, 80°50'8.00"O	2
	Paita	La Huaca	Macacará	4°55'5.02"S, 80°51'29.16"O	3
Alto Piura	Morropon	Buenos Aires	El Olguín	5°15'54.00"S, 79°55'18.12"O	7
			Piedra Herrada	5°14'12.01"S, 79°56'9.96"O	2
		La Matanza	Carrasquillo	5°13'59.99"S, 80° 1'59.88"O	3
			Los Rosos	5°11'12.01"S, 79°58'9.84"O	2
			Santa Marcela	5°12'38.00"S, 80° 5'18.00"O	2
San Lorenzo	Piura	Las Lomas	El Partidor	4°43'28.99"S, 80°17'21.84"O	2
Total					43

five minutes, and rinsed with sterile distilled water. Subsequently, they were dried with absorbent paper towels, cut into small pieces of approximately 1 cm and placed in disinfected plastic trays containing wet paper towels to provide humidity for the development of the disease (French and Hebert, 1980). Then, a small cut was made in the central part of the surface and, by using a sterile Pasteur pipette, the bacterial suspension was added at a concentration of $1x10^6$ cells $mL^{-1.}$ The evaluation was carried out on daily basis for three days, observing the progress of the rot.

Banana pseudostems were washed with plenty of water, cut into cylindrical pieces of approximately 10 cm long, disinfected with 1.0% sodium hypochlorite for 2 minutes and rinsed with sterile distilled water. Subsequently, they were placed in disinfected plastic trays containing damp paper towels. By means of the injection method; the bacterial suspension was then inoculated in the center of the pseudostem at a concentration of approximately $1x10^6$ cells $mL^{-1.}$ Three pieces of properly coded pseudostem were used for each isolate. The evaluation was carried out on daily basis for three days, observing the progress of the rot. Likewise, two-month-old banana seedlings of the Williams variety from in vitro multiplication were used. The inoculation was carried out by means of the injection method to the peudostem with 0.5 mL and saturating the soil with 100 mL of bacterial suspension at a concentration of $1x10^6$ cells $mL^{-1.}$ The plants remained without irrigation for 24 hours before inoculation; three plants were inoculated or each bacterial strain. The inoculated plants remained under controlled temperature conditions (between 24°C and 33°C,) with a relative humidity of 60%. The evaluation was carried out on daily basis for 30 days. In all the cases, the causal agent was isolated again to comply with Koch's postulates (Agrios, 2008). The concentration adjustment of the bacterial suspensions used in the pathogenicity tests was made by absorbance readings of 0.1 with a wavelength of 650 nm, measured in a spectrophotometer (Coleman Mod. Junior II 6-20, Illinois, USA).

Cultural and morphological characterization of bacterial isolates

Bacterial isolates were characterized by their response to morphological, biochemical, and physiological characteristics. The characteristics of shape, size, edge shape, elevation, color, transparency, and production of fluorescent pigments of each of the isolated bacterial colonies were observed and studied in Petri dishes containing nutrient agar, MacConkey agar and King's B agar culture media. (MacConkey, 1905; King et al., 1954; Schaad et al., 2001).

Biochemical and physiological characterization of bacterial isolates

The cellular morphology of the bacterial isolations was determined using the Gram staining and flagella techniques following the methodology of Schaad, et al. (2001). Tests for

catalase, oxidase, sensitivity to erythromycin, reduction of nitrate, production of indole acid, hydrolysis of gelatin, degradation of the medium in crystal violet pectate (CVP) and growth at temperatures of 37 and 45°C were performed using the methodology proposed by Schaad et al. (2001) and Hélias et al. (2012). All the tests were performed in replicates.

Molecular analysis

DNA extraction and polymerase chain reaction of bacterial isolates

For the molecular analysis of the pathogens determined by biochemical tests, the pure bacterial isolates were cultured in 1mL of nutrient broth at 30ºC for 24 hours. The tubes were centrifuged at 13,000 RPM (DLAB Scientific Co., China) for 2 min and then used for genomic DNA extraction, which was determined using the CTAB protocol, described by Worden (2009). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using primers 27F(5'AGAGTTTGATCMTGGCTC3') and 1492R(5'TACGGYTACCTTGTTACGACTT3'). The PCR reactions were performed using the DreamTaq DNA polymerase kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. The amplification cycles were performed in a thermocycler (MiniAmp™, Applied Biosystems, USA) under the following conditions: an initial pre-denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 58°C for 45 s, 72°C for 90 s and a final elongation step of 72°C for 6 min. The PCR products were verified by 1.0% agarose gel electrophoresis.

Sequencing and bioinformatic analysis of bacterial isolates

For molecular identification, 30 µl of each amplified product were double-sequenced at MACROGEN laboratories (Korea), using the Sanger sequencing method. For the identification of bacterial isolates, the consensus sequences of the 16S rRNA regions were aligned with the MUSCLE tool included in the MEGA X bioinformatic software (hhttps://www.megasoftware.net/) (Kumar et al., 2018), and then uploaded to the BLAST (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/). Finally, the sequence alignment was subjected to a phylogenetic analysis for the construction of a tree using the Neighbor Joining Tree method.

Host range assessment

The evaluation was conducted using carrot (*Daucus carota*), onion (*Allium cepa*), gherkin (*Cucumis sativus*), eggplant (*Solanum melongena*), sweet potato (*Ipomoea batata*) and banana (*Musa paradisiaca*). The structures were washed with plenty of water, disinfected with 1.5% sodium hypochlorite for 2 minutes and rinsed with sterile distilled water. Then, they were cut into slices of approximately 1cm and placed in plastic trays disinfected containing wet paper towels to promote the development of the disease. Subsequently, a small cut was made in the central part of the surface using a sterile scalpel and, by using a sterile Pasteur pipette, the bacterial suspension was inoculated at a concentration of $1x10^6$ cells mL^{-1}, adjusted to 0.1 absorbance and 650 nm in wavelength in a spectrophotometer (Coleman Mod. Junior II 6-20, Illinois, USA). In the banana fruits, the bacterial suspension was inoculated in the middle with a hypodermic needle. The evaluation was carried out on daily basis for three days.

RESULTS AND DISCUSSION

Bacterial isolation

From the samples collected in the field, 60 colonies were isolated in NA culture medium, 34 isolates were from the Chira Valley (Bellavista, Marcavelica, Querecotillo, Salitral and La Huaca districts); 24 isolates were from the Alto Piura Valley (Buenos Aires and La Matanza); and two isolates from the San Lorenzo Valley (Las Lomas district) (Table 2). All the colonies of the morphologically identical isolates were cream-colored or whitish in the NA culture medium. Of these, 10 isolates were selected because they turned out to be gram negative, positive for catalase and for presenting typical colonies similar to those described for bacteria of the genera *Dickeya* and *Pectobacterium*. The selected isolates were subjected to different biochemical tests and molecular analyses.

Isolate pathogenicity test

The results showed that the 10 isolates produced variable symptoms of aqueous soft rot when inoculated on the surfaces of the plant tissues evaluated after 72 hours of inoculation. In potato tubers, the results obtained were positive for all the bacterial isolates, while it was also observed that the isolates presented enzymatic activity against pectin. The symptoms were creamy exudates in the area of the inoculum, maceration of tissues accompanied by a dark halo and a foul odor (Fig. 1A). This confirmed that the symptoms are typical of *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum*, which coincides with Aremu and Babalola (2015), who mentioned that these bacterial pathogens have pectinolytic

Table 2. Bacterial isolates from the different organic banana zones of Piura, Peru.

Bacterial Isolates	Código	Organ affected	Banana Zones
Bp2	01MB	Pseudotallo	Buenos Aires
Bc2	02MB	Pseudotallo	Bella Vista
Mm1	03MB	Pseudotallo	Marcavelica
Lr1	04MB	Pseudotallo	La Matanza
Ls1	05MB	Rizoma	La Matanza
Lh1	06MB	Pseudotallo	La Huaca
Sc4	07MB	Pseudotallo	Salitral
Qp1	08MB	Pseudotallo	Querecotillo
Br1	09MB	Rizoma	Buenos Aires
Lp1	10MB	Rizoma	Las Lomas

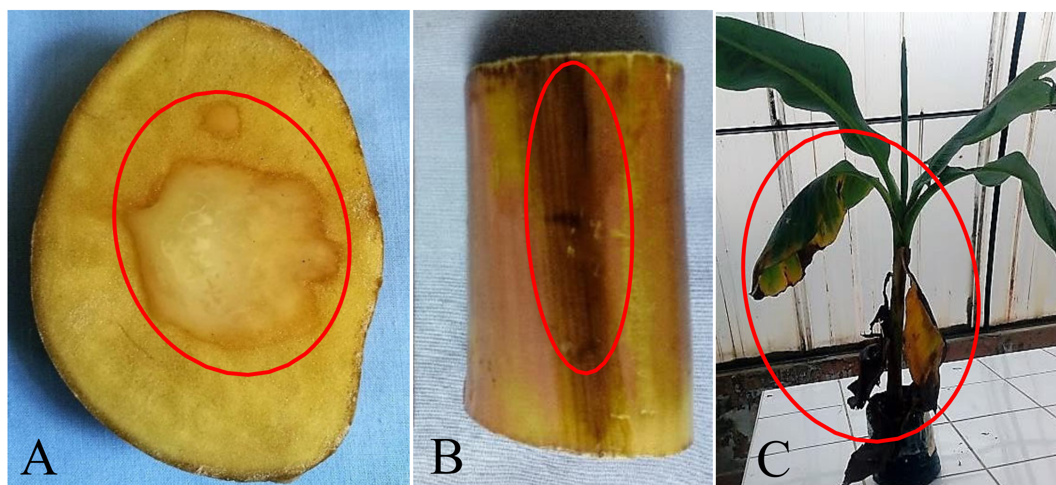

Fig. 1. Pathogenicity test in potato tubers (A), banana pseudostem (B), and banana plants (C).

enzymes and that, when isolated from rotting organisms, the pathogenicity can be assessed with this test.

The pathogenicity test of the 10 isolates from banana pseudostem produced typical symptoms of soft and wet rot similar to that caused by *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* (Fig. 1B). Symptoms appeared two and three days after inoculation. Likewise, the pathogenicity results in banana seedlings were also positive for the 10 bacterial isolates evaluated. The isolates produced typical symptoms of rot. Leaf flaccidity symptoms developed ten days after inoculation. Small translucent watery spots, yellowing of the pseudostem and chlorosis were observed in two or more leaves between 15 and 20 days after inoculation, while more severe symptoms, such as soft and wet rot of the pseudostem, wilting, death of old leaves and cracking of the surrounding tissues, were observed after 27 days (Fig. 1C). Re-isolation of affected potato tissue, rhizomes, and pseudostem from banana seedlings produced bacterial colonies that were identical to the original colonies of the isolates used for inoculation. These pathogenicity tests allowed determining that the isolates obtained correspond to the bacteria *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum*. Similar results were found by Vasundhara and Thammaiah (2017), who reported that symptoms of the disease by *D. chrysanthemi* appeared 25 to 30 after inoculation in banana seedlings. Furthermore, Gokul et al. (2019) and Ragavi et al. (2019) reported that symptoms of complete rot and plant drop caused by *P. carotovorum* subsp. *carotovorum* occurred 16 days after inoculation.

Cultural, morphological, biochemical, and physiological characterization of the isolates

The results of the cultural, morphological,

biochemical, and physiological tests for the characterization of the obtained isolates are shown in Table 3. The 10 isolates sown in NA culture medium presented colonies with typical characteristics of *Dickeya* spp. and *P. carotovorum* subsp. *carotovorum*. All the bacterial colonies were cream-colored, circular in shape, convex, with a smooth surface and translucent after 24 hours after seeding (Fig. 2A and 2B). Colonies of *D. chrysanthemi*, *D. paradisiaca* and *P. carotovorum* subsp. *carotovorum* were isolated from different crops, including bananas using NA culture medium, with similar results to those obtained in the present work (Nagrale et al., 2013; Akbar et al., 2015; Czajkowski et al., 2015; Vasundhara and Thammaiah, 2017).

Likewise, all the isolates were gram negative (Fig. 3A), mobile, and presented flagella of the perimeter type (Table 3), which was determined through flagellar staining and observed by light microscope (Carl Zeiss, Mod. Primo Star, Germany). These bacterial pathogens are characterized by presenting perimeter flagella (Agrios, 2008; Aremu and Babalola, 2015; Czajkowski et al., 2015; Blomme et al., 2017; Vasundhara and Thammaiah, 2017), which was observed in all the isolates in the present work.

In MacConkey agar medium, all the isolates were positive and presented growth and staining characteristics that were similar to those described for *Dickeya* spp. and *P. carotovorum* subsp. *carotovorum* (Table 3). The results showed that all the isolates had the capacity to ferment lactose and presented pink colonies (Fig. 2C). This typical coloration of *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* was previously reported by Corzo-López and Quiñones-Pantoja (2017), who indicated that the isolated colonies on MacConkey agar reacted positively and were pink, being characteristic of lactose fermenting bacteria. This gives the MacConkey medium a selective and differentiating property (MacConkey, 1905).

The results of the growth test in CVP medium showed a positive response only for the isolates 01MB, 04MB, 05MB, 06MB and 10MB, which formed holes or deep cavities 48 hours after planting (Fig. 2D) because of the degradation of pectate (Ma et al., 2007; Snehalatharani and Khan, 2010). This confirms that these isolates belonged to *P. carotovorum* subsp. *carotovorum*. In fact, the pathogen was isolated, differentiated and identified through this test. This agrees with previous studies that have described that isolates of *P. carotovorum* subsp. *carotovorum* can degrade pectate (Hélias et al., 2012; Gokul et al., 2019; Ragavi et al., 2019).

The present study showed that all the selected isolates were positive for the catalase test (Table 3 and Fig. 3B). However, none of them presented fluorescent pigments, which clearly indicates that they do not belong to *Pseudomonas* because members of this genus can generate fluorescence when exposed to a UV length of 360 nm, while *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* do not fluoresce (King et al., 1954; Lamichhane and Varvaro, 2013). In this sense, the results obtained in the present study are in agreement with the findings of Ashmawy et al. (2015), who observed that the isolates

Table 3. Morphological, biochemical, and physiological characterization of the bacterial isolates.

Tests	Bacterial isolates									
	01MB	02MB	03MB	04MB	05MB	06MB	07MB	08MB	09MB	10MB
Growth on agar MacConkey	+	+	+	+	+	+	+	+	+	+
Growth on NA	+	+	+	+	+	+	+	+	+	+
Fluorescence on agar King B	-	-	-	-	-	-	-	-	-	-
Gram stain	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
Erythromycin	-	+	+	-	-	-	+	+	+	-
Nitrate reductase	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Indole	-	+	+	-	-	-	+	+	+	-
CVP	+	-	-	+	+	+	-	-	-	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Growth at 45°	-	+	+	-	-	-	+	+	+	-
Staining flagella	Perimeter type									

+: positive test reaction. -: negative test reaction.

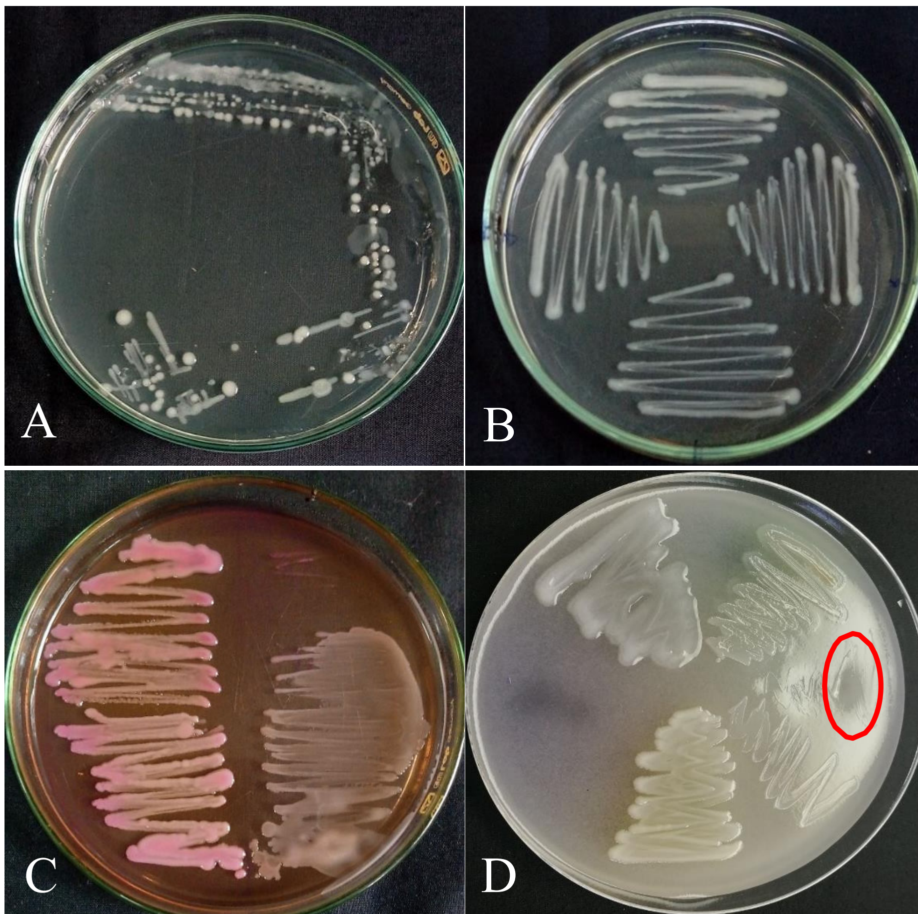

Fig. 2. Growth and development of bacterial isolates of organic banana on nutrient agar (AB), Mac Conkey medium (C), and CVP medium (D).

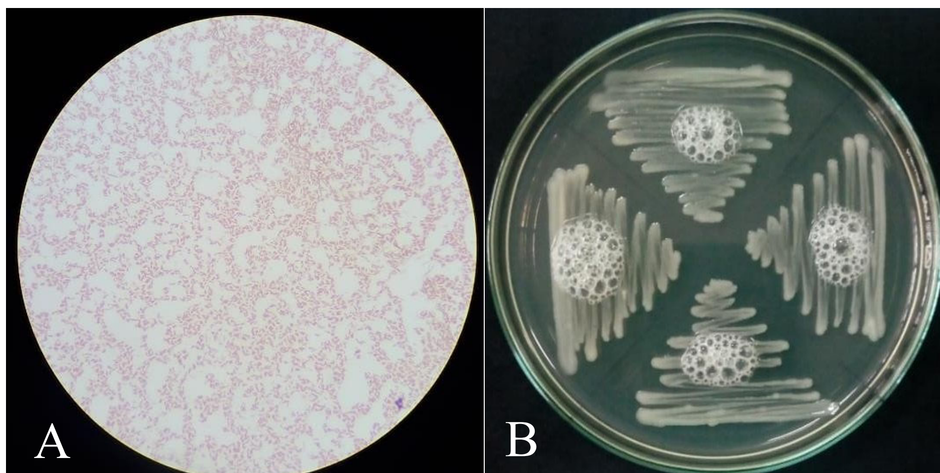

Fig. 3. Gram stain (A) and positive reaction to the catalase test (B) of the bacterial isolates.

of *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* gave positive and negative reactions to catalase when they evaluated the production of fluorescents in KB.

With respect to the gelatin hydrolysis test, the results show that the 10 isolates selected were positive when liquefying the gelatin medium (Table 3). Similar results were observed by Ashmawy et al. (2015), who found that *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* reacted positively to gelatinase. Hydrolysis occurs because such microorganisms have the capacity to produce proteolytic enzymes like gelatinases, which liquefy and hydrolyze gelatin causing characteristic changes (Schaad et al., 2001).

In the case of the indole acid production test, isolates 02MB, 03MB, 07MB, 08MB were positive (Table 3), observing a blue-green coloration in the medium after 10 seconds. The coloration occurs as a result of reductive deamination of tryptophan by the action of tryptophanases (Schaad et al., 2001), which confirms that these isolates belong to the species *D. chrysanthemi*. These results agree with those of Nagrale et al. (2013) for *E. chrysanthemi* pv. *paradisiaca*, Ashmawy et al. (2015), Bhupendra and Yogendra (2015), and Vasundhara and Thammaiah (2017), who observed that *D. chrysanthemi* caused production of indole acid when performing this biochemical test to characterize bacterial isolates.

In the erythromycin sensitivity test, the results show that this antibiotic showed high sensitivity against isolates 02MB, 03MB, 07MB, 08MB and 09MB (Table 3), observing a zone of inhibition (Fig. 4A) around the disc of filter paper containing erythromycin (15 μg). However, isolates 01MB, 04MB, 05MB, 06MB and 10MB were resistant to this antibiotic. These results agree with previous studies. For instance, Nagrale et al. (2013) reported sensitivity to erythromycin in *E. chrysanthemi* pv. *paradisiaca*., while Ali et al. (2014) and Ashmawy et al. (2015) observed that strains of *P. carotovorum* subsp. *carotovorum* were not affected by erythromycin, but reported sensitivity to this antibiotic in *D. chrysanthemi* strains. Furthermore, *P. carotovorum* subsp. *carotovorum* has been described as resistant to erythromycin, while *D. chrysanthemi* has been regarded as susceptible (Snehalatharani and Khan, 2010; Dipak et al., 2013; Akbar et al., 2015; Dana et al., 2015; Gokul et al., 2019).

In the sensitivity test at temperatures of 37 and 45°C, the 10 selected isolates had normal growth at 37°C (Fig. 4B). However, only isolates 02MB, 03MB, 07MB, 08MB and 09MB grew at a temperature of 45°C after 24 hours after being sown in NA medium (Table 3). These growth characteristics at different temperatures are similar to those for *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum*. These results agree with Dana et al. (2015) and Ragavi et al. (2019), who evaluated growth of isolates at different temperatures, reporting growth of *P. carotovorum* subsp. *carotovorum* at 36 and 37°C. Conversely, Gokul et al. (2019) reported that *P. carotovorum* subsp. *carotovorum* reached maximum growth at 27°C. In addition, Nagrale et al. (2013) and Vasundhara and Thammaiah (2017) reported that *E. chrysanthemi* pv. *paradisiaca* and *D. chrysanthemi* developed at 36 and 37°C, respectively.

Ali et al. (2014) indicated that morphological

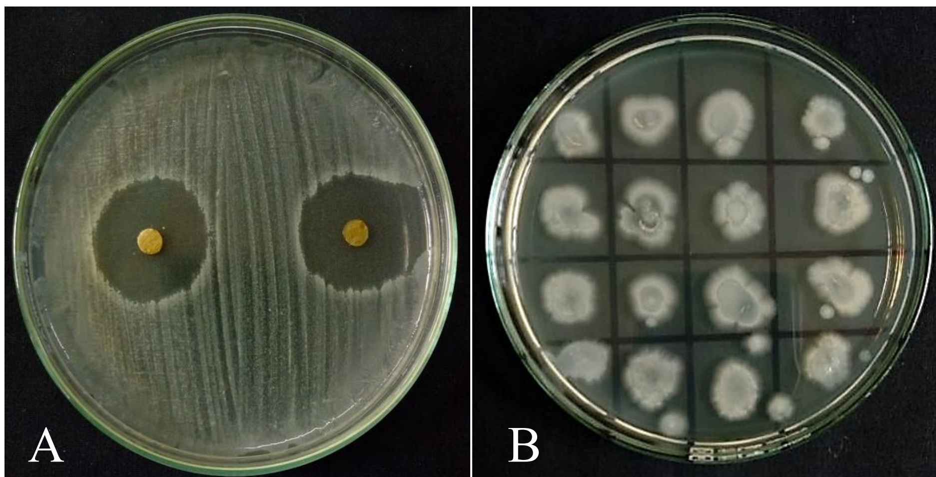

Fig. 4. Sensitivity to erythromycin (A) and growth of bacterial isolates at 37°C after 24 hours of incubation (B).

and physio-biochemical tests can be used for the identification of *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* as it occurred in the present study. The pathological, cultural, and biochemical characteristics are fundamental because they play an important role in the identification of the pathogenic agents of soft and wet rot disease. Recently, *P. carotovorum* subsp. *carotovorum* was reported as a causal agent of wet rot in banana plants (Basim et al., 2019). Likewise, a study conducted in banana plants under greenhouse conditions in Turkey indicated that severe wet rot disease was caused by *P. carotovorum* subsp. *carotovorum* (Basim et al., 2019). Previous studies on the bacterial pathogens *D. chrysanthemi* and *P. carotovorum* subsp. *carotovorum* have been isolated and identified based on their morphological, cultural, physiological, and biochemical characteristics, and pathogenicity tests (Snehalatharani and Khan, 2010; Himel et al., 2016; Vasundhara and Thammaiah, 2017; Gokul et al., 2019).

Molecular and phylogenetic identification of isolates

The results of the molecular and phylogenetic identification of the bacterial isolates are shown in Table 4 and Fig. 5. Ten bacterial isolates were identified based on the partial sequencing of the 16S rRNA. The regions revealed identities with the NCBI GenBank sequences between 90.55% and 99.57%. In relation to the phylogenetic tree of the isolates, only the species associated with the symptoms of soft rot of banana pseudostem were related (Fig. 5).

The partial sequencing of the 16S rRNA gene allowed the identification of 10 bacterial isolates associated with symptoms of pseudostem rot of banana. All the isolates were gram-negative bacteria, and seven of them belonged to the Pectobacteriaceae family. *P. carotovorum* subsp. *carotovorum* (01MB, 04MB, 05MB and 10MB), which has been related to the causal agent of soft rot in several crops, including Musaceas (Premabati and De Mandal, 2020), was also identified. Banana plants are susceptible to *P. carotovorum* infection (Maisuria and Nerurkar, 2013). Likewise, two species of the Dickeya genus were identified, *D. chrysanthemi* (03MB and 08MB) and *D. paradisiaca* (02MB). Previous reports have identified the species *D. chrysanthemi*, which is directly related to pseudostem wet rot of banana (Blomme et al., 2017), and *D. paradisiaca*, which is associated with pseudostem and rhizome wet rot with a fetid odor, causing vascular internal decomposition accompanied by discoloration in banana (Premabati and De Mandal, 2020). Similar results were obtained in previous works carried out by Ramirez et al. (2014), Basim et al. (2019), and Kabir et al. (2020), and a recent study conducted by and Suarez et al. (2022), who diagnosed and identified isolates corresponding to the genera *Pectobacterium* and *Dickeya* using molecular analysis.

In the present study, two members of the Enterobacteriaceae family, *Citrobacter* sp. (09MB) and *Klebsiella variicola* (07MB) were also identified. This agrees with the findings of Fan et al. (2016) and Loganathan et al. (2021), who reported the presence of *K. variicola* as a pathogen associated with banana soft rot disease complex in China and India, respectively. *Pseudomonas vangonensis* (06MB) was also identified, but there is no evidence in the literature indicating *Pseud. vangonensis* is part of the bacterial complex that causes rot of banana. However, there are reports

Table 4. Identification of bacterial isolates according to partial sequencing of the 16S rRNA gene.

Bacterial isolates	Scientific name	Per. Ident.	Accession*
01MB	*Pectobacterium carotovorum*	97.24%	MN393954.1
02MB	*Dickeya paradisiaca*	99.36%	NR_173610.1
03MB	*Dickeya chrysanthemi*	98.09%	DQ418491.1
04MB	*Pectobacterium carotovorum*	96.78%	Z96091.1
05MB	*Pectobacterium carotovorum*	96.27%	MK058440.1
06MB	*Pseudomonas yangonensis*	90.55%	KX364008.1
07MB	*Klebsiella variicola*	99.47%	MZ575142.1
08MB	*Dickeya chrysanthemi*	98.09%	AF373203.1
09MB	*Citrobacter* sp.	99.57%	KU597520.1
10MB	*Pectobacterium carotovorum*	98.30%	MF787368.1

* The accession number corresponds to the species in the NCBI database that matched sequences.

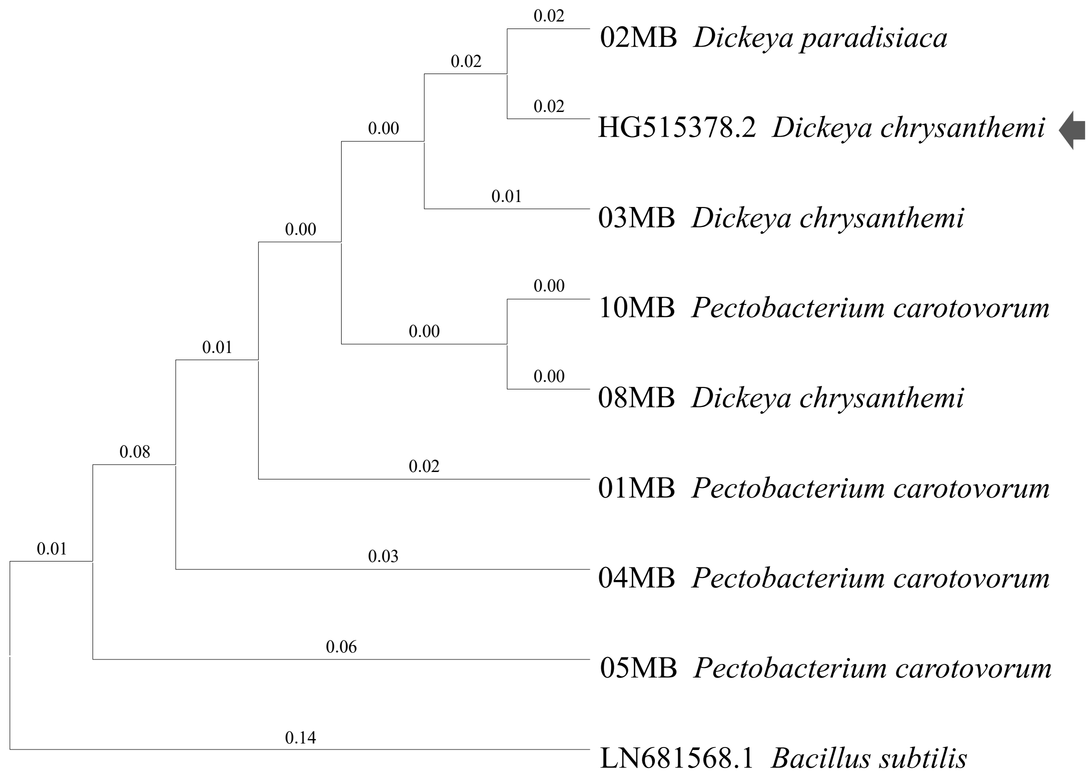

Fig. 5. Phylogenetic reconstruction of the sequences (16S rRNA) of seven bacterial isolates from samples with symptoms of pseudostem rot. The Neighbor-Joining method allowed relating the isolates in 5 clades. The arrow indicates the sequence of the type of strain for *Dickeya chrysanthemi* (syn. *Erwinia chrysanthemi*) (Accession Number: HG515378.2) and underlined *Bacillus subtilis* (Accession Number: LN681568.1) as outgroup.

of *Pseud. cichorii* and *Pseud. solanacearum* acting as phytopathogens in banana plants (Stover, 2020; Choi et al., 1988).

Host range

The results of the host range of the isolates show that the bacterial isolates obtained in the present work infected the evaluated hosts (onion, carrot, cucumber, and banana), except for eggplant and sweet potato, indicating that the isolates differ in their pathogenicity (Table 5). Symptoms appeared 72 hours after inoculation and corresponded to constant and typical soft rot in the different plant organs evaluated. These results coincide with Zhang et al. (2014), Aremu and Babalola (2015) and Liu et al. (2016), who reported that the *D. chrysanthemi*, *D. paradisiaca* y *P. carotovorum* subsp. *carotovorum* have a wide host range, being considered as the main tissue macerating pathogens because they cause cell degradation and induce soft rot in different plant species such as carrot, onion, cabbage, tomato and pickle.

CONCLUSIONS

The characterization of the isolates, which was conducted by using pathogenicity tests followed by cultural, morphological, biochemical, physiological, and molecular methods, indicated that the bacterial isolates correspond to *Pectobacterium carotovorum* subsp. *carotovorum*, *Dickeya chrysanthemi*, *Dickeya paradisiaca* and *Klebsiella variicola*. All the identified pathogens cause rhizome and pseudostem wet rot of banana. This represents valuable information for the implementation of appropriate disease management practices for organic banana in the area under study.

Table 5. Determination of the host range of the bacterial isolates.

Bacterial isolates	Host					
	Allium cepa	*Daucus carota*	*Cucumis sativus*	*Solanum melongena*	*Musa paradisiaca*	*Ipomoea batata*
01MB	+	+	+	-	+	-
02MB	+	+	+	-	+	-
03MB	+	+	+	-	+	-
04MB	+	+	+	-	+	-
05MB	+	+	+	-	+	-
06MB	+	+	+	-	+	-
07MB	+	+	+	-	+	-
08MB	+	+	+	-	+	-
09MB	+	+	+	-	+	-
10MB	+	+	+	-	+	-

+: positive test reaction. -: negative test reaction.

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