Peruvian Journal of Agronomy 3(3): 144–152 (2019)
ISSN: 2616–4477 (Versión electrónica)
DOI: http://dx.doi.org/10.21704/pja.v3i3.1367
http://revistas.lamolina.edu.pe/index.php/jpagronomy/index

Received for publication: 08 December 2019
Accepted for publication: 31 December 2019

First report of *Enterobacter cloacae* as a causative agent of soft rot disease in dragon fruit (*Hylocereus undatus*) stems in Peru

Primer reporte de *Enterobacter cloacae* como causante de la pudricion blanda en tallos de pitahaya (*Hylocereus undatus*) en Perú

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Abstract

The objective of the present research was to identify the causal agent of soft rot disease in the stems of pitahaya plants (*Hylocereus undatus* (Haw.) Britton & Rose), also known as dragon fruit, in two production areas of Peru. Typical symptoms observed include rotting and soft consistency in stems, as well as yellowish colourations, which usually begin at the tips and outer edges of the stems and extend until they are completely decomposed. Symptomatic samples of pitahaya stalks were collected from two commercial fields, the first from the district of Independencia, Pisco, Ica, and the second from the district of Naranjos, Rioja, San Martin. The collected samples were transferred to the phytopathology laboratory of the National Agrarian University – La Molina where 19 bacterial colonies isolated from symptomatic stem tissue were processed. Nine bacterial colonies were selected from the initial 19 for further analysis. The nine selected colonies were gram negative, positive for the catalase enzyme, negative for the enzyme oxidase, and positive for pectinase production, which causes soft rot in potato tubers. These results indicated that these isolates correspond to the Enterobacteriaceae family. According to molecular tests and analysis of the 16s region of ribosomal DNA, all bacteria corresponded to a single taxonomic genus, Enterobacter. This bacterium presented a 99.85% homology with *Enterobacter cloacae* (accession number MH788982.1). The sequences from the nine selected isolates were entered into GenBank under accession number MN784371. In the pathogenicity test, 100% infection was obtained in the pitahaya stems and plants inoculated with *E. cloacae*, and the symptomatology that developed was the same as that observed in the two fields from where the samples were collected. Given these results, it can be concluded that *E. cloacae* is a causative agent of soft rot disease of the stems of *H. undatus* in the districts of Independencia and Naranjos, and this is also the first report of this bacterium as a pathogen of pitahaya in Peru.

Keywords: *soft rot disease, pitahaya, dragon fruit, Hylocereus, Enterobacter*

Resumen

El objetivo del presente trabajo de investigación fue identificar el agente causal de la pudrición blanda de tallos de plantas de pitahaya (*Hylocereus undatus*) en dos zonas productoras en el Perú. La sintomatología observada son pudriciones de consistencia blanda en tallos y con coloraciones amarillentas, las que por lo general inician en las puntas y en los bordes externos de los cladiolos y se van extendiendo hasta la descomposición total de los mismos. Se recolectaron muestras de tallos de dos campos comerciales del cultivo, la primera muestra fue del distrito de Independencia, en Pisco, Ica y la segunda muestra del distrito de Naranjos, en Rioja, San Martin. Las muestras colectadas se trasladaron al laboratorio de Fitopatología de la Universidad Nacional Agraria La Molina donde se procesaron y aislaron 19 colonias bacterianas de los tallos con los síntomas de pudrición blanda. Se seleccionaron nueve colonias bacterianas de las 19 iniciales. Las nueve seleccionadas fueron las gram negativas, las cuales resultaron positivas a la enzima catalasa, negativas a la enzima oxidasa y positivas a la producción de pectinasas ocasionando también pudriciones blandas en tubérculos de papa, las que demostraron que estos aislamientos corresponden a la familia Enterobacteriaceae. Según las pruebas moleculares con el análisis de la región 16s de ADN ribosomal, todas las bacterias corresponden a un solo género taxonómico, *Enterobacter*. Esta bacteria presentó un 99.85 % de homología con *Enterobacter cloacae* (número de accesión MH788982.1). Las secuencias provenientes de los nueve aislamientos seleccionados fueron introducidas en el GenBank con el número de accesión MN784371. En la prueba de patogenicidad se obtuvo un 100 % de infección en los tallos y plantas inoculadas con *E. cloacae*, y la sintomatología desarrollada fue igual a la observada en los dos campos de donde se procedieron las muestras. Se concluye que *Enterobacter cloacae* como causante de la pudrición blanda de los tallos de *H. undatus* en los distritos de Independencia y Naranjos, siendo este el primer reporte de esta bacteria como patógeno de pitahaya en el Perú.

Palabras clave*: pudrición blanda, pitahaya, fruta de dragon, Hylocereus, Enterobacter*

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Introduction

Pitahaya or Pitaya is the common name given to several species of plants in the genera *Hylocereus, Selenicereus, Cereus, Leptocereus, Escontria, Myrtilloactos, Stenocereus*, and *Opuntia* within the Cactaceae family. They are native to Central and South America, and there are about 35 species. Several of the species can be consumed as fruits or vegetables or used as forage (Esquivel, 2004; Mizrahi, Mouyal, Nerd, & Sitrit., 2004; Legaria, Alvarado, & Hernández, 2005; Tel, Abbo, Bar, & Mizrahi, 2004). The genus *Hylocereus*, which has 16 recognised species, is the most widely distributed worldwide. The genome is highly polymorphic, which implies a great deal of variation (Legaria et al., 2005). The species *Hylocereus undatus*, *H. polyrhizus, H. costaricensis, H. triangularis*, *and H. purpusii,* traditionally known as red pitahayas and referred to as dragon fruit in English, are grown mainly in Central America and Israel (Esquivel, 2004). The yellow pitahayas, which correspond to 20 species of the genus *Selenicereus*, are distributed across Bolivia, Peru, Ecuador, Colombia, and Venezuela (Tel et al., 2004). Pitahaya is mainly consumed as fresh fruit, but it also contains water-soluble nitrogen pigments called betalains. Furthermore, its broad range of colours, red-violet to yellow, and ability for the natural pigment to remain stable in a wider pH range compared with other natural dyes, such as anthocyanins, make them great potential natural dyes (Stintzing, Schieber, & Carle, 2001; Stintzing, Schieber, & Reinhold, 2002; Strack, Vogt, & Schliemann, 2003; Wybraniec et al., 2001). The consumption of flowers and young shoots as fresh vegetables has also been reported (Cálix de Dios, 2004). The seeds are used as probiotics owing to their oligosaccharide content and are an important ingredient in health foods and nutraceutical products (Wichienchot, Jatupornpipat, & Rastall, 2010).

The growing demand for pitahaya in Peruvian national markets and its potential as fresh fruit to the international market is spurring NA increase in planted production acreage, especially along the coast and in the highlands and jungle of Peru. However, as production has increased, several phytosanitary issues have become evident, including those caused by pathogens, and there are no official reports of the causative agents of the diseases that are being observed in the cultivation of pitahaya in Peru.

Worldwide, there have been reports that the stems and fruits of pitahaya are affected by bacteria, such as *Pectobacterium carotovorum, Xanthomonas campestris,* and *Enterobacter* spp.; by fungi, such as *Colletotrichum gloeosporioides, Botryosphaeria dothidea, Fusarium oxysporum, Neoscytalidium dimidiatum, Curvularia lunata, Fusicoccum* sp.; and other pathogens, such as Cactus virus X (Valencia, Sandoval, & Cardenas, 2004; Wright, Rivera, Ghirlanda, & Lori, 2007; Palmateer, Ploetz Van Santen, & Correll, 2007; Masyahit, Sijam, Awan & Ghazali, 2009; Mohd, Salleh, & Zakaria, 2013; Retana, Castro, Blanco, & Quesada, 2019).

In Mexico, the United States of America, and Costa Rica, there have been reports of the identification of new species of enterobacteria that cause soft rot on the stems of pitahaya in commercial fields (Valencia, Cruz, & Rodríguez, 2003; Retana et al., 2019). The same rotting symptom has been observed in several production areas of pitahaya in Peru, and the disease is negatively impacting crop production during some seasons of the year. For these reasons, the present research was conducted with the objective of identifying the causative agent of the soft rot of pitahaya stems in two production areas of Peru.

Materials and methods

Sample selection and collection

Pitayaha samples (*H. undatus*) were collected from two production areas of Peru, one sample in the district of Independencia, Pisco, Ica, and another in the district of Los Naranjos, Rioja, San Martin. At the commercial plantations, pitahaya plants whose stems exhibited symptoms of soft rot at different states were located, and a description of the symptoms was taken. Fifteen symptomatic stem samples were collected from each plantation, and the material was transferred to the phytopathology laboratory of the Universidad Nacional Agraria La Molina.

Isolation of the causative agent

To isolate the causative agent, the methodology described by French and Hebert (1980) was used to isolate both fungi and bacteria. The pitahaya stems were washed with clean, running water to remove any impurities from the field and then cut into small pieces, which were placed in a biosecurity booth II Biobase. Inside the biosecurity cabin, the stem pieces were immersed in a solution of 1% sodium hypochlorite for 3 min, then rinsed with sterile distilled water and allowed to air-dry for 15 min on sterile paper towel in order to remove excess water. Segments of approximately 0.5 cm were removed from the internal part of the stem pieces that covered both diseased and healthy tissues. Five tissue segments were placed in various Petri dishes containing the NA, potato dextrose agar (PDA), and corn meal agar with antibiotics and benomyl (CMAPARB) media.

Another isolation methodology used was to macerate small portions of symptomatic tissue with 10 mL of sterile, distilled water in ceramic mortars using a pestle. Aliquots of the macerated juice were extracted and distributed into Petri dishes containing either NA, PDA, or CMAPARB. All Petri dishes were placed in a Thermo Scientific incubator for 48 h at a temperature of 30 °C. Once the bacterial isolates were obtained, visually distinct colonies were transferred to individual Petri dishes with NA media using a sewing needle. A drop of sterile, distilled water was added to the sample to facilitate streaking of the plate. Plates of the selected and isolated colonies were incubated

for 24 h at 30 °C. These purified colonies were used to perform the corresponding identification tests.

Phenotypic Identification Tests

Gram staining

The Gram staining test was conducted in order to discriminate gram-positive from gram-negative bacteria. It was conducted using the methodology described by Schaad (2001). After staining on slides, the preparations were observed on a Leica DM500 compound microscope at a magnification of 1000× per immersion to determine, by colouring, whether the isolates were gram positive or gram negative. Additionally, the shapes of the bacterial cells were observed (Garcia de la Guarda & Alvarado, 2012)

Oxidase enzyme test

To determine the presence of the cytochrome C oxidase enzyme, each isolated bacterial colony was sub-sampled and placed on filter paper with tetramethyl-p-phenylenediamine. Whether or not the colour of the filter paper changed from white to purplish blue was observed, which is indicative of the presence of enzyme (Schaad, 2001).

Catalase enzyme tests

To determine the presence of the catalase enzyme, the methodology described by Rodríguez, Gamboa, Hernández, and García (2005) was used. Each isolated bacterial colony was sub-sampled and placed on a slide with a drop of 3% hydrogen peroxide (H_2O_2). Oxygen release was evaluated by the production of effervescence (Schaad, 2001).

Pectinase test

To determine the presence of pectinase enzymes, isolated and selected colonies were carried onto crystal violet pectate (CVP) media, which was used to inoculate slices of potato (*Solanum tuberosum*) tubers (Schaad, 2001).

Planting in half CVP

To determine the activity of the pectinmethylgalacturonase and/or pectinliase enzymes, the selected bacterial isolates were placed at five points equidistant from each other on a Petri plate containing CVP media. The inoculated plates were incubated for 48 h at 30 °C, after which a hole formation in the middle of the points was observed (Schaad, 2001).

Sliced potato tuber inoculation

Selected bacterial isolates were inoculated into slices of disinfected potato tubers and placed into humidity chambers. For inoculation, an aliquot of bacterial suspension from each of them was placed onto the slices. The inoculated slices were incubated for 3 days at 30 °C and then observed for soft rot symptom development (Schaad, 2001).

Molecular identification of bacterial isolates

DNA extraction

The method used was adapted from Ramos and Barboza (2016). For DNA extraction from each of the selected bacterial isolates, tubes containing 2 mL of the nutrient broth medium were prepared, and the isolates were added individually using a small pestle. The tubes were then incubated for 48 h at 30 °C. After the incubation time, a 1 mL aliquot was extracted from each tube and transferred to 1.5 mL Eppendorf vial tubes. The following reagents were then added to each sample: 558 µL of TE buffer, 30 µL of 10% SDS, 6 µL of proteinase K, 6 µL of RNase, 100 µL of 5 M sodium chloride, 80 µL of 2% CTAB buffer (10 mM Tris HCl, 20 mM EDTA, 0.7 M NaCl), and 6 µL of beta mercaptoethanol. The samples were incubated for 15 min at 65 °C in a thermo-agitator, and then 800 µL of isoamyl alcohol chloroform was added (24:1 v/v) and vortexed for 10 s. The samples were allowed to stand for 5 min in the biofreezer at −20 °C, after which 500 µL of isopropanol was added and centrifuged for 7 min at 12000 rpm at 4 °C. After centrifugation, the supernatant was removed from each tube, and 700 µL of 80% ethanol was added. The samples were centrifuged again for 5 min at 12,000 rpm at 4 °C. The supernatant was removed, and the tubes with DNA pellets were allowed to dry inclined overnight. The next day, the DNA pellets were resuspended in TE buffer. To quantitatively determine the concentrations of DNA extracted from each selected isolation, a Nanodrop 2000 spectrophotometer was used, and gel electrophoresis on a 1% agarose gel stained with HydraGreen run at 80 volts for 30 min was performed (Ramos & Barboza, 2016).

Amplification of DNA by polymerase chain reaction (PCR)

The PCR was performed with primers 27F (5´-AGAGTTTGATCMTGGCTCAG-3´) and 1492R (5´ TACGGYTACCTTGTTACGACTT-3´) described by Lane et al. (1985), which amplify the total RNA sequence of the 16S ribosomal gene. The PCRs were performed according to the Aime protocol (2006) with some modifications. The stock solution or master mix was prepared in 25 µL reaction volumes with 5 µL of 5X Green GoTaq® Flexi Buffer (Promega), 2 µL of 25 mM magnesium chloride solution (Promega), 0.5 µL of 2.5 mM triphosphate dinucleotides, 0.5 µL of each of the primers at 20 µM (forward and reverse), 0.125 µL of Mix GoTaq® Flexi DNA Polymerase (Promega), and 1 µL of DNA at a concentration of 30 to 90 ng/µL. A PCR without DNA was used as a negative control. The amplification reaction was performed starting with an initial pre-denaturation of the DNA at 96 °C for 2 min, followed by 35 cycles of denaturation at 96 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 10 min.

The PCR products were separated by electrophoresis with 0.5X TAE buffer in 2% agarose gels for 30 min at 90 V and stained with HydraGreen ™ Safe DNA Dye using a 100 bp DNA size reference ladder (Promega). Fragments were observed using an ultraviolet light transilluminator. The PCR amplified fragments were sequenced in both directions at the Macrogen company in Korea and compared with the National Biotechnology Information Center (NCBI) gene bank database (GenBank) (Ramos & Barboza, 2016).

Phylogenetic analysis

First, nucleotide sequences were edited to eliminate unclear sequences in the electropherogram, and the 5 'and 3' ends of the DNA strands obtained were cut. The edited DNA strand sequences were then aligned to determine the quality of the nucleotide sequences of selected bacterial isolate DNA samples. Bidirectional alignment and comparison with chromatograms were performed using the BioEdit programme (Hall, 1999).

The phylogenetic analysis was conducted using the MEGA 7.0 software in which the "closest neighbour" and "maximum likelihood" tests were performed with 1000 bootstraps. Consensus sequences of the aligned isolates were also determined using MUSCLE and used to query the NCBI GenBank using the Basic Local Alignment Search Tool (BLAST).

Pathogenicity test

The stems of pitahaya plants (*H. undatus*) were planted in propagation bags with sterile substrate and allowed to root, after which they were grown for 60 days. Pathogenicity tests were conducted on healthy pitahaya plants under two different conditions: 1) in excised stems in humidity chambers or 2) in plants in bags in the greenhouse.

For the first set of conditions, healthy stems of pitahaya plants were cut and transferred to the phytopathology laboratory, washed with clean water, disinfected in 1% sodium hypochlorite, and then placed in the humidity chambers. Stems were inoculated in a biosafety cabin II with sterile toothpicks. Bacteria were collected from each of the isolated colony plates with individual toothpicks, and several parts of the pitahaya stems were perforated to inoculate the stems. The control stems were punctured with the tip of toothpicks only containing half NA. The humidity chambers were sealed and set at 30 °C.

For the pathogenicity test under greenhouse conditions, pure bacterial isolates grown in Petri dishes with NA medium were taken to the phytopathology greenhouse. Healthy pitahaya plant stems were inoculated using the same methodology as in the laboratory using sterile toothpicks. The inoculated plants were covered with plastic sheets to maintain a warm, humid environment for 20 days.

Results

Symptomology observed in the field

Symptomatic stems at different stages of development were observed at the commercial pitahaya plantations starting with small, wet, yellow spots that later developed into soft, watery rot with a yellowish, pre-necrotic margin.

The rot typically starts at the tips or on the protruding edges of the stems and extends towards the stem center (Fig. 1A, B, and C).

Figure 1. Symptoms at different stages of soft rot caused by *Enterobacter cloacae* in pitahaya Isolations obtained and selected

In some stems, the rot appears restricted or limited in sectors, whereas complete rots occur in others. When the rot is restricted, the decomposed tissue detaches from the healthy tissue by its own weight, leaving its respective epidermis and vascular ducts attached to the rest of the plant's healthy stem. The remaining epidermis, when dried, has a soft, sometimes crusty and whitish, texture and can be easily removed when manipulated (Fig. 1D).

Nineteen isolated bacterial colonies obtained appeared to differ morphologically from each other. Following gram staining tests, nine gram-negative bacterial colonies were selected. The nine gram-negative colonies selected also tested positive for 3% KOH, and all had coccobacillus forms and were catalase positive and oxidase negative (Fig. 2A, B, and C). The nine selected colonies also tested positive for pectinase enzymatic activity, which was confirmed by observing the formation of shallow holes on inoculated tissue. The nine bacterial colonies also caused an aqueous or soft rot in the potato tubers where they were inoculated (Fig. 2D, E and Table 1).

Of the nine bacterial isolates selected, five (Sot1, Sot2, Sot3, Sot 4, and Sot5) were from Independencia in the district of Pisco, Ica, and four (Sot6, Sot7, Sot8, and Sot9) were from Naranjos in the district of Rioja, San Martin.

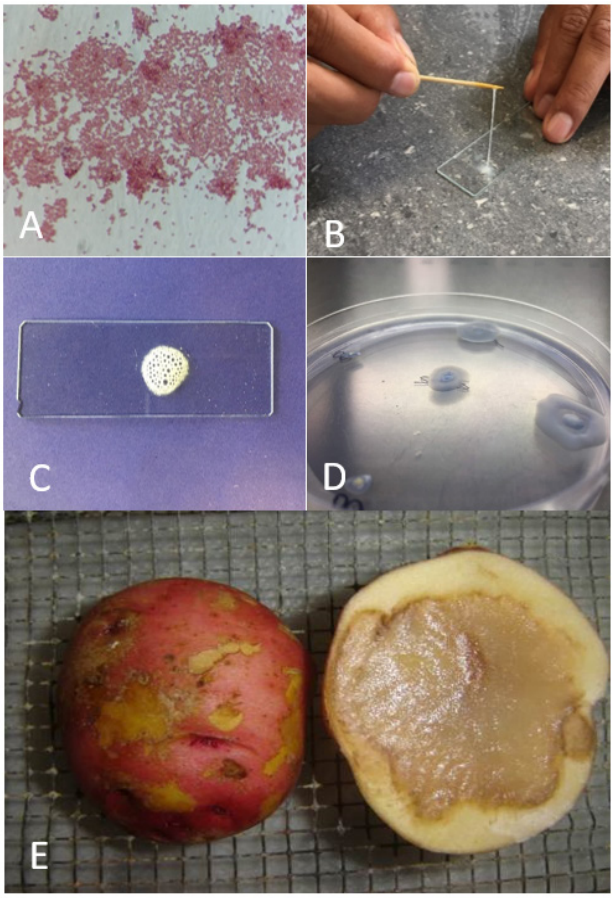

Figure 2. A. Results of phenotypic tests on bacteria with reddish colour. gram negative. B. positive to 3% potassium hydroxide. C. positive catalase. D. degradation of pectin in CPV medium. E. Sliced Potato Rot

Table 1. Phenotypic test results

Prueba	Resultado
Colour and shape of the colony	Light beige, small round colonies
Gram staining	Reddish
3% KOH	Positive
Catalase	Positive
Oxidase	Negative
Violet pectate crystal (CPV)	Smooth hole formation in the middle
Potato tuber rot	Positive

Molecular identification of the nine selected bacterial isolates

The results of the amplifications of the ribosomal DNAs of the 16s region using primers 27F and 1492R generated an amplicon of 1500 bp and are presented in Figure 3. After performing a BLAST search with the 16s region to GenBank, all nine bacterial isolates selected corresponded all to a single taxonomic genus, *Enterobacter*. These isolates shared a 99.85% homology with *Enterobacter cloacae* (accession number MH788982.1). The sequences from our selected isolates were introduced into GenBank under accession number MN784371 (Fig. 4). The results of the phylogenetic analysis using the sequences of the selected isolates show clusters corresponding to *E. cloacae* (red and purple squares) and distant relationship with *E. hormaechei* (green triangles). *Agrobacterium tumefaciens* is included as an outgroup species.

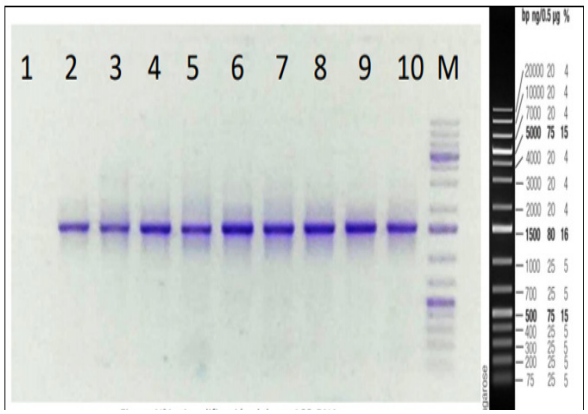

Figure 3 Amplifications of ribosomal DNA regions with primers 27F and 1492R. (1) White sequence (2. Sot 1, 3. Sot 2, 4. Sot3, 5. Sot4, 6. Sot5, 7. Sot6, 8. Sot7, 9. Sot8, 10. Sot9) Sequences (M) Ladder 1 Kb

Pathogenicity test

In the pathogenicity testing conducted inside a humidity chamber, *E. cloacae* developed symptoms of soft rot in all inoculated stems. After 24 h, small, yellow, wet spots of approximately 5 mm in diameter were observed from the point of inoculation, which extended to sizes of 10 cm after ~8 days, whereas no symptoms were observed in the uninoculated control (Fig. 5A and B). The plants

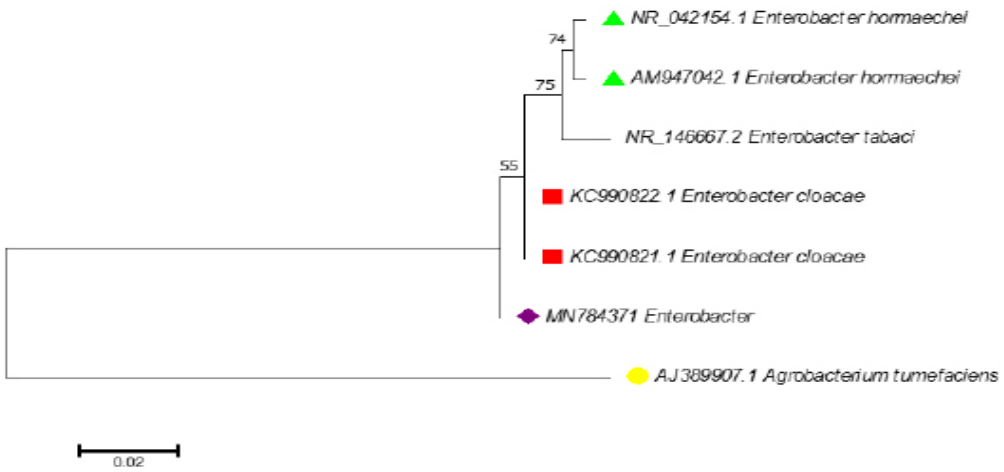

Figure 4 Phylogenetic relationship between the sequences of bacteria obtained from *H. undatus* with other nearby species of the Enterobacter genus.

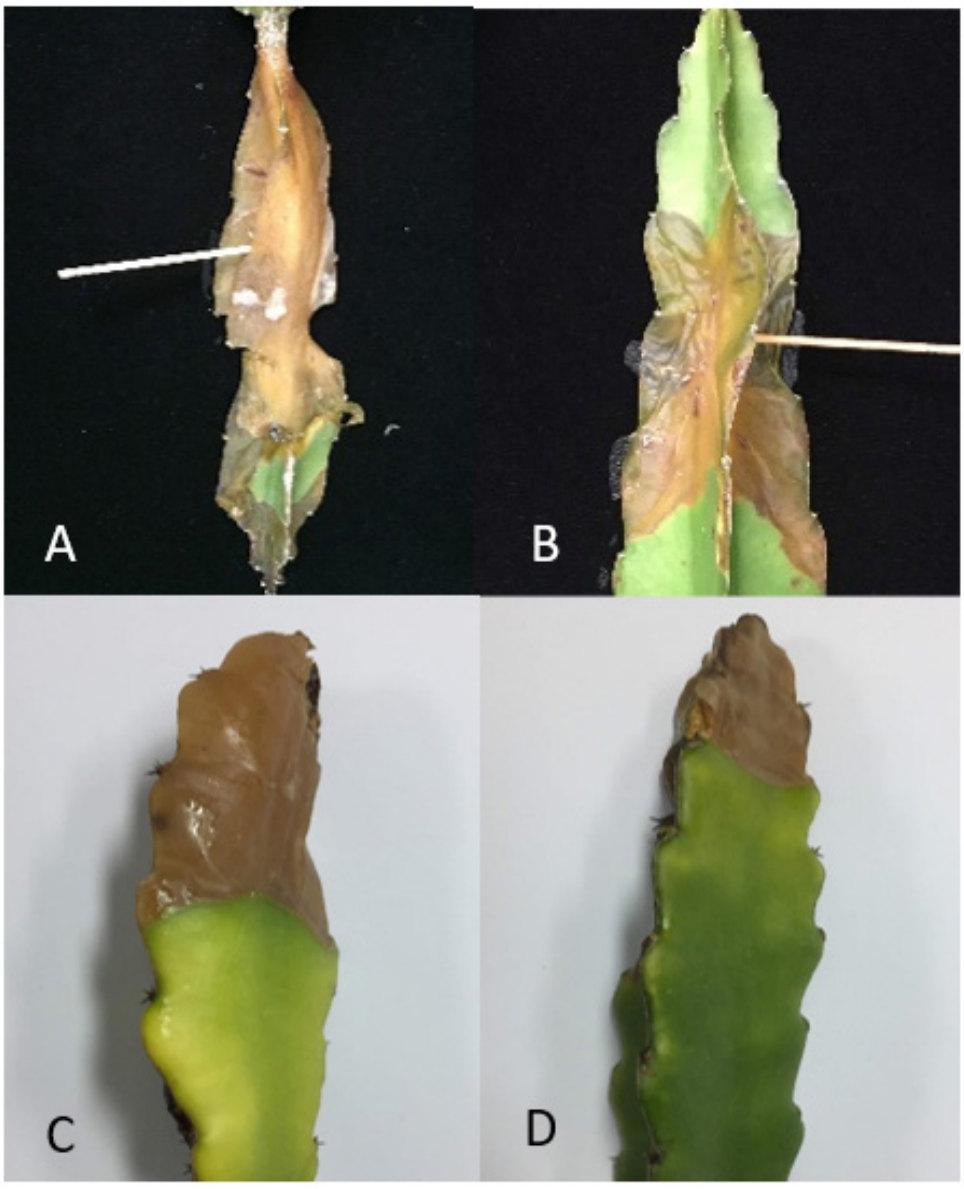

Figura 5. Pathogenicity test. A and B stem inculation in wet chamber, C and D stems incised in stems in greenhouse

inoculated in the greenhouse also developed symptoms of soft rot with the same characteristics observed in stems in the humidity chambers (Fig. 5C and D)

Discussion

The samples were collected from two different agroecological zones, the first, in the district of Independencia in Pisco, is located along the Peruvian coast, which is a desert, agricultural area dependent on irrigation for the growth and development of crops. From July to September, relative humidity is high, and, considering that the sampling was carried out in the middle of September, the high relative humidity of the early hours and heat in the afternoons favoured the appearance of bacterial diseases in the cultivation of pitahaya. The district of Naranjos de Rioja is located in a jungle region, where rainfall is abundant from the months of September to March and the crops are mostly grown in dry land. The high temperature of this area, along with rainfall, favours the presence of bacterial diseases during these times. The sampling in Naranjos was carried out in the month of January. Other factors that favour the presence of diseases caused by *Enterobacter* in pitahaya are management practices that include pruning and pest damage of the epidermis that acts as an entry point for bacteria and other organisms (Zimmerman & Granata, 2002).

The symptoms occurring at both sampling sites are quite similar, making it difficult to describe any site-specific characteristics. When the progress of the *Enterobacter* rot symptoms was restricted, it was likely due to a defence response of the plant generated, thanks to the high-calcium content, which the plant is able to store in the form of oxalate in its cells (Franceschi & Horner 1980; Webb,1999; Faheed, Mazen, & Abd, 2012). In the presence of a phytopathogen, the oxalate calcium migrates to the middle lamina of the tissue cells, binds with the pectins, and gives rise to the calcium polypectates, which are more difficult to degrade by the pectic enzymes of the bacteria. Depending on the calcium content, a plant can restrict disease progression and, in the case of pitahayas, cause the detachment and separation of the infected tissue from the healthy tissues as observed.

The nine gram-negative bacterial colonies were selected for traits, including the ability to degrade pectin compounds, which was determined by the formation of holes in the CVP medium. However, these holes were not as deep as those produced by the *Pectobacterium* genus (Schaad, 2001), which could indicate that the isolated and selected bacteria have less capacity to produce pectinolytic enzymes than those of the genus *Pectobacterium.* The nine bacterial colonies also caused symptoms of soft rot in the inoculated potato slices, which would corroborate the secretion of pectinolytic enzymes, and these results are consistent with those published by other researchers who report that *E. cloacae* also causes soft rot in potato tubers (Abd, AlKhazindar, & Sayed, 2018). Some enterobacteria that cause soft rot are characterised within the entire group of phytopathogens by their high pectinolytic activity, which gives them the property of producing the symptoms of soft rot in reserve organs (Husain & Kelman, 1956). It has also been documented that many of the *Enterobacter* species have a strong glucose dehydrogenase enzymatic activity, which oxidises D-glucose to D-gluconate without the presence of pyrroloquinoline quinone (Grimont & Grimont, 2006).

The results of the identification of the isolated and selected bacterial colonies showed that *E. cloacae* has a high percentage of identity, but the literature mentions an *Enterobacter cloacae* complex, which is composed of up to six *Enterobacter* species: *E. asburiae*, *E. cloacae, E. hormaechei, E. kobei, E. ludwigii*, and *E. nimipressuralis*. These species share close genetic relationships and belong to conserved regions; however, polymorphisms allow for them to be molecularly differentiated. Phenotypically, they can be distinguished by biochemical tests, such as the Biolog, Vitek, and TSI, among others (O'Hara et al., 1989; Brenner, McWhorter, Kai, Steigerwalt, & Farmer,1986; Hoffmann & Roggenkamp, 2003).

The 16s rDNA region was used to construct phylogenetic relationships because it is a highly repeated genome fragment that has remained fairly uniform throughout evolution. Genetic differences in the sequences of the rDNA of *E. cloacae* in the 16s region do not allow for the formation of a homogeneous taxonomic group, demonstrated by an irregular phylogenetic tree in which the strains of *E. cloacae* are strongly interrelated with other species of this genus, such as *E. hormaechei, E. aerogenes*, and other enterobacteria, including *Escherichia coli*. This illustrates the genetic heterogeneity of the species, which can hinder their systematic identification (Tang et al., 1998; Hoffmann & Roggenkamp, 2003).

It is important to mention that *E. cloacae* is a commensalistic bacterium that inhabits water, sewage, soil, and meat and is present in hospital environments and the digestive tract of humans and animals (Grimont & Grimont, 2006). There are also several reports of these enterobacteria affecting various plant species, such as pitahayas in Costa Rica by *E. hormaechei* (Retana et al., 2019) and in Malaysia by *E. cloacae* (Masyahit et al., 2009); papaya plants (*Carica papaya* L.) in Hawaii; onion bulbs (*Allium cepa* L.) in California and Colorado, USA (Bishop & Davis 1990); and ginger (*Zingiber officinale*) in Hawaii (Nishijima et al., 2004). These reports indicate that *E. cloacae* has a polyphagous character, causing diseases in different plant species.

This is the first official report of *E. cloacae* in pitahaya in Peru. The pathogenicity test, which was performed by reproducing the same symptoms observed in the fields that were sampled and fulfilling Koch's postulates, revealed that *E. cloacae* is a causative agent of soft rot disease of pitahaya in the districts of Independencia, Pisco, and

Naranjos, Rioja. However, other species of enterobacteria cause similar symptomatology in other parts of the world. Continued sampling in other production areas of this crop should be performed to determine if there are other species associated with this disease.

Conclusion

According to the results of this research work it is concluded that *Enterobacter cloacae* is the causative agent of the soft rot of pitahaya cladioli in the districts of Independencia (in Pisco) and Naranjos (in Rioja); being this the first official report of this pathogen in this crop in Peru.

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