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A strain of *Chryseobacterium* sp. isolated from necrotic leaf tissue of chayote (*Sechium edule* Jacq)

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Abstract

There are few reports of bacterial diseases in chayote because these are usually attributed to other pathogens, mainly fungi. However, from two Sechium edule Jaqc Sw plants showing necrosis of the leaves, the presumptive pathogen was isolated from symptomatic leaves on yeast-dextrose-calcium carbonate agar yielding yellow-orange color colonies that developed after 36 hours. This study was proposed to identify the microorganism associated with this pathology and to evaluate its aptitude to induce necrotic damage in four varieties in S. edule. The bacterium identification was carried out by examining the characteristics of the colonies and cells, the biochemical characteristics and a sequence of the gene fragment coding 16S rRNA. It was found that leaf necrosis of chayote may be caused by Chryseobacterium sp. Similarly, a phylogenetic analysis was performed with 26 species of this genus together with the strain under study, finding more similarity to C. indologenes and C. gleum. Significant differences were found in the expression of the symptoms of chlorosis and necrosis amongst the varieties of chayote constantly appearing throughout our study. Chryseobacterium was described in 2010 for the first time as a pathogen associated with Soft Rot of the Calla Lily in Poland. This genus has been found in different environments, and therefore it is considered appropriate to investigate its ecological role or any other association it may have with plants.

Keywords: Flavobacteriaceae, *Chryseobacterium* ecological role, plant-bacterium association, 16S rRNA.

Introduction

The genus of *Chryseobacterium* was described by Vandamme *et al.*[1] to classify some of the species that were found within *Flavobacterium*, due to the heterogenity of the group. The bacteria of this genus are characterized by their bacillary form and by their gram negative type. They form circular colonies, many with a sweet smell, which produce flexirubin, a yellow pigment that is insoluble in water[2-4].

Herzog *et al.*[5] mention that *Chryseobacterium* represent a genus that has had the highest growth in the number of species registered since 2003. In that year, the species *C. defluvii*[6] and *C. joostei*[7] were described and classified. At the moment, there are some environments and new species proposed for this genus[3,8-17], which proves that the genus comprises a group of organisms ubiquitous in nature[5], due to its ample genetic plasticity[18].

Species of this genus are often found in soil and water environments[10,15,19-21]. Other species such as *C. gleum*, *C. meningosepticum* and *C. hominis*, are human and animal pathogens[16,22,23], some others are part of the psicrotolerant and proteolitic bacteria community that cause a variety of defects in food industry products[5,8,14].

With regards to their interactions in the plant kingdom, Park *et al.*[10] found that *Chryseobacterium* sp. is a typical group of cultivable microorganisms found in the roots and rhizosphere of two plant species found in sand-dunes in the coastal areas of Tae-an in Korea. These authors, together with Bernardet *et al.*[24] and McSpadden-Gardener and Weller[25], point to the fact that it is not easy to speculate about the ecological significance of the vegetation studied, due to the lack of information about the possible rôle of the bacteria belonging to this genus associated with plants.

Mikincinski *et al.*[26] reported for the first time the presence of strains of *Chryseobacterium* associated with soft rot of the calla lily (*Zantedeschia* spp., family *Araceae*) tubers in Poland. However, based on some physiological tests, they indicated that it is possible that the calla lily was not the primary host and that the infection (or colonization) observed could have happened only occasionally.

Despite the fact that the Chayote (*Sechium edule* Jacq SW) is affected by various plagues and diseases, the most common and studied reports are mainly focused on fungi and viruses[27]. However, in 2007, Chayote plants were found in the gardens of the University of Veracruz in Xalapa, Veracruz, México, which

had wet spot on the leaves which evolved into necrosis. The bacteria colonies isolated from these caused similar symptoms when they were infiltrated on healthy leaves. Preliminary results indicated that it was a bacterium of the family Flavobacteriaceae, a group not commonly reported as phytopathogenic.

The aims of this study were the identification of the microorganism and the evaluation of its capacity to induce necrotic damage in four varieties of *Sechium edule* Jaqc Sw., in order to generate information permitting the determination of the ecological rôle of this bacterial group as its potential activity as a phytopathogen of the Chayote.

Material and Methods

Bacteria isolation: The bacterial strain was isolated from leaves showing symptoms of foliar necrosis in two chayote plants (*S. edule*) which were developed in the gardens of the Library and Information Services Department of the University of Veracruz in Xalapa, Veracruz, Mexico.

The chayote leaves were washed with soapy water and were superficially disinfected by immersion in a commercial sodium hypochlorite (CLOROX) solution 2% for one minute. Immediately afterwards, they were rinsed with sterilized distilled water. A fragment of approximately 1 cm^2 of diseased vegetable tissue from the edge of the necrotic damage was macerated in a porcelain mortar with 500 µL of sterilized distilled water. Samples of the solution were inoculated on a medium of the Nutritive-Glucose Agar (NGA), nutrient-broth yeast extract agar (NBY) and in Yeast-Dextrose-Calcium carbonate Agar (YDC). The cultures were incubated at 27ºC ± 1, and the different types of colonies which developed were observed after 48 hours. Re-seeding was carried out for every type of colony, until pure cultures were obtained.

Pathogenecity Test: Stage one: From the observation of the most common colonies produced, suspensions of bacteria (150 x 10^6 $CFU.ml^{-1}$) were prepared. Approximately 100 µL of this suspension was infiltrated with a 500 µL syringe on the second leaf of every *Sechium edule* var. *virens levis* plant (fruit light green in color and unarmed) which was 25 days old (plants with 6 to 7 leaves), with the aim of inducing the symptoms originally observed. 15 chayote plants were inoculated with the bacterial solution and 10 plants with sterilized distilled water as the control sample.

The plants were sown in sterilized soil at 121ºC/15 pounds of pressure/20 min in plastic pots with 2 kg capacity. Every plant inoculated was labeled with information of the infiltrated bacteria. The inoculated plants were maintained under nursery conditions during the months of June to July of 2008, with a daytime temperature that oscillated around an average of 22 ± 2 °C and with a relative humidity of 74%. The plants were irrigated with drinking water every three days.

Stage two: From tissues with symptoms, we returned to isolate the strain of bacteria and carry out the evaluation of the development of the symptoms in four varieties of *S. edule*, that, according to the Cadena-Iñiguez and Arevalo-Galarza[28] descriptors were: 1) Var. *virens levis*, whose fruits range from 9.3 to 18.3 cm in length, 6.0 to 11.4 cm wide, and 5.4 to 9.6 cm thick, light green pear-shaped form with five ribs not very marked, and a not very deep basal cleft, peduncle light green with very low pubescence; 2) Var. *xalapensis nigrum*, dark green pear fruit, 5.5 to 26.6 cm long, 4.4 to 18 cm in width and from 4.0 to 10.7 cm thick, which has five ribs not very marked and a very marked basal cleft and moderately pubescent peduncle; 3) Var. *nigrum spinosum*, pyriform fruits with a light green to dark green coloration, with dimensions of 5.8 to 17.1 cm in length, from 5.0 to 12.2 cm wide and 3.6 to 9.7 cm thick, with dense spines, five ribs not very marked and a marked based slit and low pubescence on peduncle; 4) Var. *albus spinosum*, yellow fruit of medium size, pyriform from 5.8 to 17.1 cm long, 5.0 to 12.2 cm wide, 3.6 to 9.7 cm thick, with a pronounced basal cleavage, the presence of spines of medium to low density, without the presence of ribs in appearance.

For the development of the plants, healthy fruit were grown in sterilized soil at 121° C/15 pounds pressure for 20 min in 2 kg capacity plastic pots. To perform the test, 40 plants of each variety were used with a measure range average of 150 cm in length, with 6 to 7 developed leaves. Each plant was injected on the foliar lamina of the second basal leaf with 500 µL of an acuous suspension of *Chryseobacterium* sp. of 150x10^6 $CFU.ml^{-1}$. As the control, 10 samples of each variety of chayote were inoculated with 500 µL of sterilized destilled water. The samples were maintained at an average relative humidity of 87% and a temperature of 22°C. They were watered every other day. 7, 14, 21 and 30 days after inoculation the foliar area showing chlorosis and necrosis was measured and photographed.

The plants of chayote were accommodated in random blocks. Every plant of every variety was considered to be an experimental unit. The percentage of the area with chlorosis and necrosis in the inoculated leaf was determined. For the analysis, data were transformed to arch bosom square root of $x+1$[29]. It was used a multiple analysis of variance (MANOVA) for a split-plot design (range = large plot, days = small plot), followed by univariate analysis (ANOVA). Comparisons were performed by Tukey-Kramer paired to determine the differences among the varieties of chayote.

Morphological, physiological and cultural characterization: After detecting the bacteria type that caused the symptoms observed in the field, the morphological characteristics of the bacterial colonies and their capacity to develop in different cultural mediums were observed in NGA, YDC, NBY and Medium B of King (KB). The flexirubin-type pigments were observed by flooding the plates with 20% (w/v) potassium hydroxide[30]. Additionally, tests of hyper-sensibility were carried

out with tobacco and basic biochemical reactions, as well as some complementary tests for phyto-pathogen bacteria genus and species indicated by Holt *et al.*[31] and Schaad *et al.*[32]. The morphology of the bacteria cell is determined using a scanning electron microscope (Geol JSM-5600) from the Institute of Ecology in Xalapa, Veracruz, Mexico.

Amplification and sequencing of 16S rRNA gene: The molecular characterization of the strain was performed by PCR: 16S rRNA (sub-unit 16S of the ribosomal RNA gene). Genomic DNA was isolated by the method described by Madigan and Martinko[33] using buffer lysis (Tris-HCl 10 mM, pH 8; EDTA 1 mM, SDS 1%) reported by Flamm *et al.*[34]. The integrity of the genomic DNA obtained was detected by electrophoresis in 0.9 % agarose gel stained with ethidium bromide. The 16S rRNA region was amplified with 8f (5'-CACGGATCCAGACTTTGA TYMTGGCTCAG-3') and 1512r (5'-GTGAAGCTT ACGGYTAGCTTGTTACGACTT-3') bacterial universal primer pairs[35]. The reaction mixture for PCR amplification was prepared according to Luna *et al.*[36]. The amplifications were performed in a Master-cycler personal Eppendorf thermo-cycler. The PCR reaction details were as follows: an initial denaturing at 94 ºC for 2 min; 35 cycles of denaturing, annealing and elongation of 94ºC for 10 seg, 59ºC for 20 seg, 72 ºC for 2 min and a final elongation at 72 ºC for 4 min.

The amplified product of the16S rRNA gene was purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and sequencing of the 16S rRNA gene was carried out by Macrogen USA Corp. using 8f, 1512r and 968f (5´-AACGCGAAGAACCTTAC-3´) primers. The sequences were analyzed with the BioEdit version 7.0.0 (Isis Pharmaceuticals, Inc.) and they were blasted with database from the GenBank of the NCBI (National Center for Biotechnology Information). Sequences of representative *Chryseobacterium* species were searched in the GenBank database. MEGA 5.0 software programme which were started with a set of aligned sequences using Clustal W, and searches for phylogenetic trees according to Neighbor Joining (NJ) and Maximum Parsimony (MP) algorithms[37-39]. Subsequently, the sequences were discharged in the GenBank and the accession numbers were assigned.

Results and Discussion

Isolation of the bacteria: physiological and cultural characterization: Of all the different types of bacteria developed and inoculated for Koch's postulates, only one produced the symptoms observed in the field. The bacteria in question presented an adequate colonial development in the mediums of YDC, NGA and NBY, with the following characteristics: Yellow-orange color, shiny, convex, circular, with a smooth and complete border, of a semi-mucoid consistency, of 1 mm of diameter after 36 hours of incubation at 27ºC ± 2, and 2 mm after 48 h. In KB the lowest colonies showed a less intense yellow color with semi-translucent borders.

The results of the physiological tests of the strain under study are summarized in table 1. Additionally, it showed positive results to the tolerance to 3% NaCl and the production of flexirubin; so much so that they were negative for the production of a fluorescent pigment diffusible in the KB medium and did not have a pectolytic capacity, as they did not degrade tissue of the tubers of the potato nor did they produce holes when cultivated in the CVP medium (Crystal violet polipectate).

Morphology of the bacteria cells: The bacteria cell showed a short form of bacillus (1 µm long) (figure 1). These did not develop spores and were determined to belong to Gram-negative type.

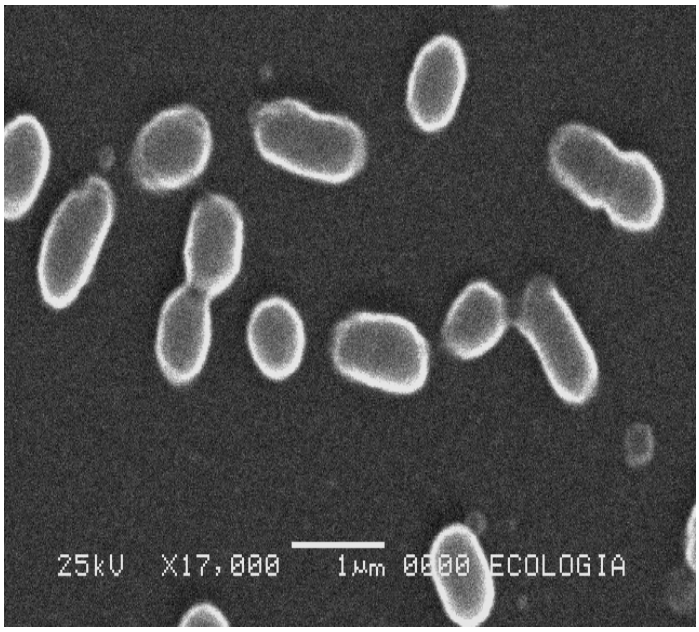


Figure-1
Morphology of the cells of *Chryseobacterium* sp. under a scanning electron microscope (Geol JSM-5600). Zoom X 17,000

Analysis of the 16S rRNA gen: The sequences were registered in GenBank as KC237770, KC247671 and KC309689. Blast analysis indicated that the strain showed an increased similarity with species of the genus *Chryseobacterium.* The strain was more related to *C. indologenes* LMG8337 (NR_042507.1) and *C. gleum* CCUG14555 (NR_042506.1). However, it was noted that the isolates of this strain formed a single group with 100% similarity between each of them and 41% with the aforementioned species (figure 2). The species of *Flavobacterium* employed as an external group were grouped in an independent clade.

Table-1
Differential characteristics for *Chryseobacterium* sp. (DQ530122.1), *C. indologenes* (AY050493.1), *C. gleum* and *Chryseobacterium* sp. (KC237770, KC247671 and KC309689 associated with necrosis of *Sechium edule*).

Test	*Chryseobacterium* sp (KC237770, KC247671 and KC309689)	*Chryseobacterium* sp (DQ530122.1)	*C. indologenes* (AY050493.1)	*C. gleum* (AB680759.1)
Habitat		Me, S	Me, S	S, P
Color of the colony	yellow/orange	yellow/orange	orange	yellow/orange
Type of respiration	A	A	A	A
HR tobacco	(+)	(-)	(-)	
Motility	(-)	(-)	(-)	D
Catalase	(+)	(+)	(+)	(+)
Oxidase	(+)	(+)	(+)	(+)
Levan	(-)	(-)	(-)	
Indole	(-)		(+)	(+)
Hydrolysis of lipids	(+)		(+)	(+)
Liquifiying of jelly	(+)	(+)	(+)	(+)
Pectolysis of the potato	(-)	(+)	(+)	
Starch degradation	(+)	D	(+)	(+)
Growth at 25º C	(+)	(+)	(+)	(+)
Growth at 37º C	(+)		(+)	(+)
Growth at 41 °C	(-)	(-)	(-)	(-)

Me: of free life associated to plants; S: soil; P: animal pathogens; (+): positive; (-): negative; A: aerobic; D: weak

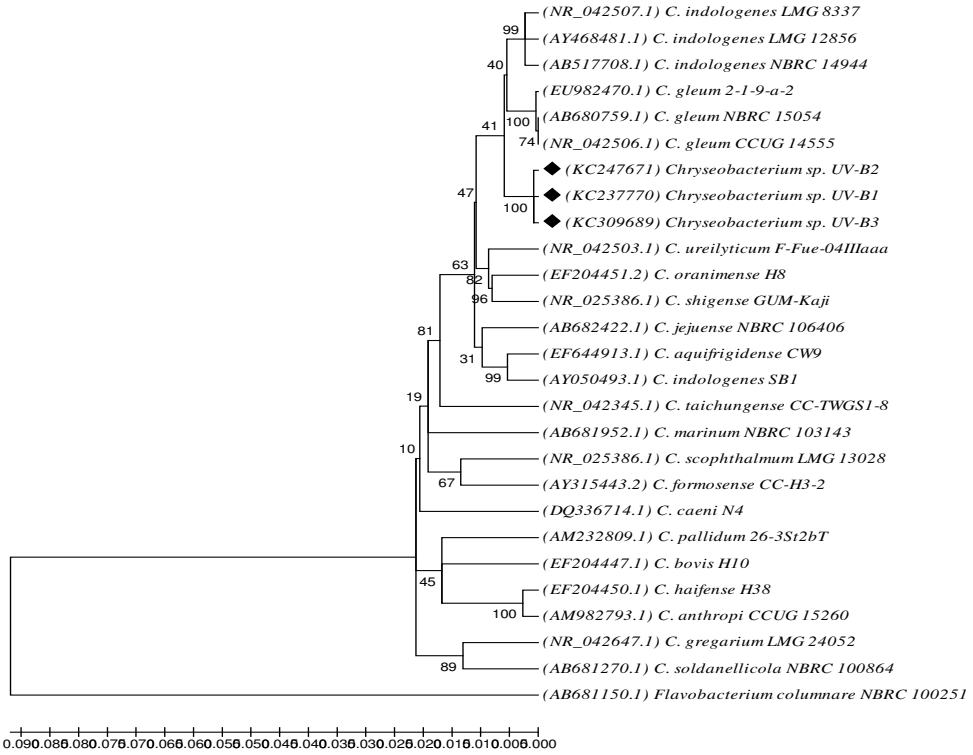


Figure-2
Phylogenetic analysis based on 16S rRNA gene sequences using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site

Pathogenicity tests: At 30 days after inoculation, the leaves of the plants of the variety *nigrum xalapensis* (VO) showed 25% chlorosis and 21% necrosis, while the plants of the variety *virens levis* (VC) showed 59% chlorosis and 49% necrosis. None of these varieties showed dead guides. Conversely, plants of varieties *nigrum spinosum* (VE) developed 79% chlorosis and 60% necrosis and death in one guide. In particular, the *albus spinosum* variety plants (AE) were the most affected, the chlorosis symptoms starting from the fifth day after inoculation, so that by day 30, it was found that all plants were affected by both chlorosis and necrosis; 12 plants had dead guides and the leaves inoculated of the rest of plants evaluated showed up to 72% necrosis and later withered. In all inoculated plants of both varieties where death appeared (*nigrum spinosum* and *albus spinosum*), symptoms of chlorosis and necrosis first appeared on the lower leaves and later on the higher leaves. None of the control plants from different varieties developed symptoms.

Differences were encountered (Lambda of Wilks = 0.396, DF= 6, 280; P < 0.0001) in the expression of chlorosis and necrosis symptoms amongst the varieties of chayote in a constant manner throughout our study. Here, the differences increased gradually in proportion throughout the treatment (Lambda of Wilks = 0.219, DF= 6, 834; P < 0.0001).

The variety *albus spinosum* (AE) differed significantly in chlorosis and necrosis from the remaining varieties 7 days after inoculation, whilst at 14 days all varieties showed differences. Finally, after 30 days the *albus spinosum* (AE) and *nigrum spinosum* (VE) varieties did not present any significant difference in the case of chlorosis and all varieties were significantly different in the case of necrosis (figure 3).

Discussion: The results of the tests as a whole suggest that the bacteria associated with the pathology observed in the plants of *S. edule* L. coincide with the description of the organisms of the genus *Chryseobacterium* of the family Flavobacteriaceae. The genus was taxonomically established by Vandamme *et al.*[1] to reclassify some of the species that were previously found within the genus *Flavobacterium*, due to the heterogeneity of the group.

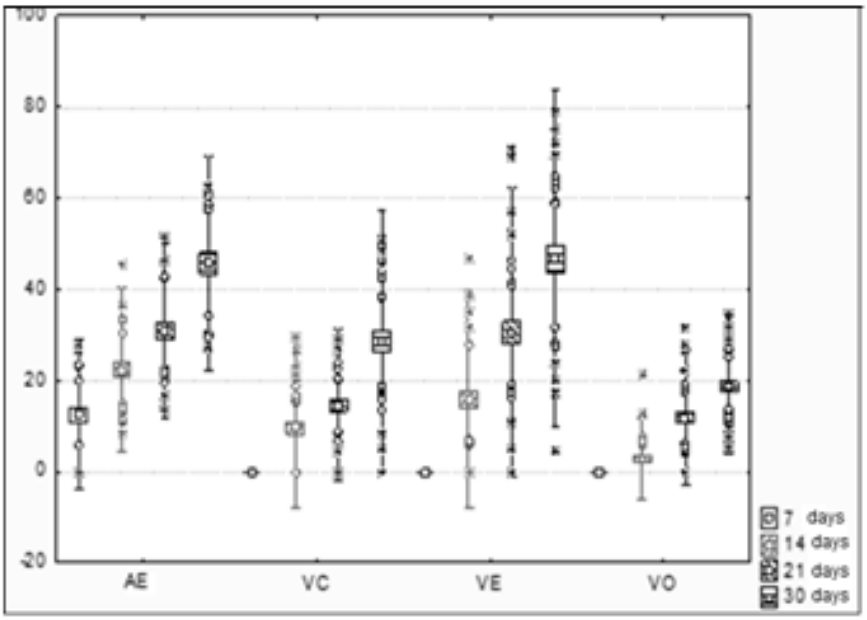
Chryseobacterium represents one of the genuses that have had the highest growth with respect to the number of species reported, as it includes group organisms ubiquitous in nature [5]. The majority of the members of the genus are found amply distributed in soil environments as well as water environments[10,14,17,19,20,21]. There are reports that species of this genus are pathogens in humans and animals, including *C. gleum* and *C. indologenes* that can cause hospital infections in humans and have also been found in diseased fish and frogs[40,41].

Its association with vegetable species is not clear due to the lack of information about the possible roles that some bacteria belonging to this genus have in ecosystems[10,24,25]. Mikincinski *et al.*[26] proposed for the first time the strains of this genus as pathogens associated with soft rot of Calla Lily (*Zantedeschia* spp.) in Poland. Soft rot is related to the activity of pectolytic enzymes of the bacteria that cause it. In this case, the authors found that the strains of *Chryseobacterium* (*Chryseobacterium* sp. DQ530122.1 and *C. indologenes* AY050493.1) showed such capacity. Our study shows that the strains of *Chryseobacterium* sp. did not have this property, highlighting the fact that the pathogens of the case study were different, which at the beginning led us to formulate a proposal that was not about strains of the same species. Additionally, the induction of a reaction of the hyper-sensibility to tobacco (table 1) showed opposite results to those observed by Mikicinski *et al.*[26].

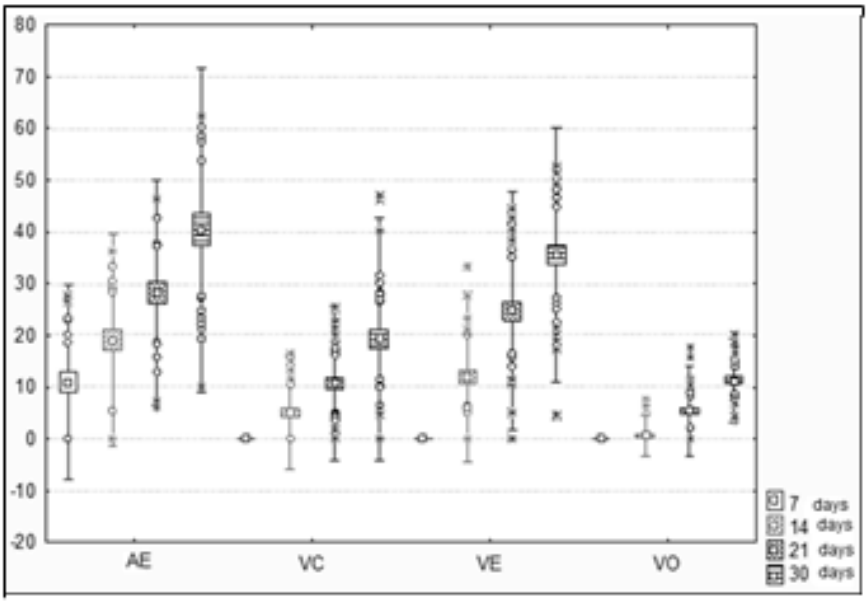
The analysis of the sequence of the 16S rRNA gen located the three isolations (KC237770, KC247671 and KC309689) of the strain of the study, next to *C. gleum* and *C. indologenes* (figure 2). Specifically, we found higher nucleotide similarity (%) between strains of *C. indologenes* and *C. gleum* than between *Chryseobacterium* sp. with any strains of the genus (Table 2), however the percentage difference observed between the strains of *Chryseobacterium* sp. (KC237770, KC247671 and KC309689) and *C. indologenes* (AB517708.1 and LMG8337) and *C. gleum* (AB680759.1, EU982470.1 and NR042506.1) ranges from 1.1 to 2 and that observed only between *C. indologenes* and *C. gleum* is 1.6 to 1.7.

Table-2
Nucleotide similarity (%) found among strains of *Chryseobacterium* sp. (KC237770, KC247671 and KC309689) vs. *C. gleum* (AB680759.1, EU982470.1 y NR_042506.1) and *C. indologenes* (AB517708.1 y LMG8337) resulting from the analysis of a 1404 nucleotide sequence of 16S rRNA gene

		% similarity							
		1	2	3	4	5	6	7	8
1	UV-B1 KC237770		9.9	99.7	98.4	95.2	98.4	98.2	90.2
2	UV-B2 KC247671	0		99.7	98.3	95.2	98.4	98.3	90.5
3	UV-B3 KC309689	0.3	0.3		96.3	95.2	97.1	98.1	90.7
4	*C.gleum AB680759.1*	1.4	1.4	1.7		97	99.2	98.3	92.4
5	*C.gleum EU982470.1*	1.7	1.7	2	0.2		97	95.6	90
6	*C.gleum NR_042506.1*	1.4	1.4	1.7	0	0.3		98.3	91.1
7	*C.indologenes AB517708.1*	1.1.	1.1	1.4	1	1.1	1.1		91.7
8	*C.indologenes LMG8337*	1.7	1.7	2	1.6	1.7	1.7	0.6	
		1	2	3	4	5	6	7	8
		% Divergence							



a)



b)

Figure-3

Percentage of chlorosis a) and necrosis b) developed in the varieties of de *Sechium edule* Jacq Sw (BE - *albus spinosum,* VE - *nigrum spinosum,* VC - *virens levis* y VO - *nigrum xalapensis*) inoculated with *Chryseobacterium* sp. Chlorosis (F3,423=45.8; P<=.0001), Necrosis (F3,423=66.7; P<=.0001)

It proved interesting to observe a difference of the isolations of bacteria obtained in this study with the species *C. indologenes* and *C. gleum*, which, like the majority of the species of this genus, synthesize indole from tryptophan, which our isolations did not do. Currently, only the *C. scophthalmum* species[13] and *C. indologenes* isolated from soft rot of the calla Lily are reported as negative for this test. However, the phylogenetic analysis based on 16S rRNA gene sequences grouped this species in a clade completely different from our strain in study.

Additionally, it is important to take into account the place where the *S. edule* plants were developed. The diseased plants were

close to a drainage channel where waste water flows from the town, as well as at the season that evidence was found, when the temperature and relative humidity of the zone were elevated, causing an increase in plant diseases. All this, as well as the lack of reports of similar cases of this culture and for these bacteria species with reference to other vegetable species, brings us to point out that there is a possibility that the infection (colonization) of these plants could have been fortuitous as was previously referred by Mikincinski *et al.*[26].

However, the data produced from the pathogenicity tests on the different varieties of *S. edule* allow us to boldly put forward the proposal that it is possible to establish a pathogenic host relationship amongst these species and that the amount of damage found depends on the variety of *S. edule* in question. Nevertheless, we cannot overlook the fact that the method of inoculation employed facilitated entrance of bacteria into the vegetative tissue overcoming the plants' structural defenses against pathogens, such as, for example, the thickness of the cuticle and the amount of wax present within it, the trichomes and estomes whose function has been demonstrated in several studies[42]. On the other hand, the differences in the degree of pathogenicity of *Chryseobacterium* sp. found in the different varieties of chayote could possibly be attributed to differences in defense elements of a chemical nature, such as the cucurbitacins terpens of the cucurbitaceae species[43].

The wild varieties of *S. edule* (with dark green bitter fruits) characteristically have a greater concentration of cucurbitacins, while the yellow or *¨albus¨* varieties have a lesser quantity of these terpens as well as chlorophyll a and chlorophyll b, but have a greater quantity of carotenoids[43] which has been attributed to a possible adjustment in the route of the mevalonic acid (AMV)[44].

The hypothesis that the differences in the pathogenic activity of *Chrysobacterium* sp. in the different varieties of *S. edule* may be due to the amount of cucurbitacins present is strengthened with the information produced by the studies of Cadena-Iñiguez et al.[43,45] where it was found that the fruits of the variety *nigum xalapensis* indicate a greater concentration of cucurbitacins (0.0195 $G100g^{-1}$), when compared with samples of the three remaining varieties included in this study, while *albus spinnosum*, which showed the greatest sensibility to this bacteria, produced approximately one quarter (0.0051 $G100g^{-1}$) of the concentration of cucurbitacins found in *nigrum xalapensis*. However, in the case of *nigrum spinosum* values of 0.0190 $G100g^{-1}$ were found, which were much nearer to the variety *nigrum xalapensis*, and even then, this variety showed higher levels of pathogenicity when compared with *nigrum xalapensis* and *virens levis*, which leads us to propose another hypothesis which involves the presence of spines on the fruits as an informative characteristic of their sensitivity.

According to studies of morphological and biochemical similarity and the migratory routes of *S. edule*, the varieties *albus spinosum* and *nigrum spinosum* are more remote from their wild ancestor when compared with *virens levis* and *nigrum xalapensis*[43,45]. Contrary to what one would assume, the presence of spines in the varieties of *S. edule* does not necessarily constitute a distinctive characteristic of the wild examples, but these are the result of an adaptive clinal variation within the adaptive characters of the species *Sechium* sp. and can be related to environmental gradients and manipulation for domestication[46]. This represents an additional element in explaining the greater damage observed in the varieties *albus spinosum* and *nigrum spinosum*. Several authors have shown evidence that the wild examples of a taxon show greater tolerance or resistance to phyto-pathogens since the wild patho-system is much more flexible and possess a greater genetic variability and their populations respond to the pressures of selection[47,48].

We consider It essential to continue investigating the ecological role of the species of the genus *Chrysobacterium*, whether as a pathogen or regarding any other type of association they may have with plants or other organisms, since *Chryseobacterum* is a widely extensive group[10,15,17,19,20,21], and which, among other aspects has been proposed as plant growth promoting rhizobacteria[49,50].

Conclusion

As was previously mentioned, the results indicate that it is possible to establish a pathogenic host relationship amongst the species and differences were encountered in the expression of chlorosis and necrosis symptoms amongst the varieties of chayote. However, we consider it necessary to implement diagnosis programs in established chayote crops and to continue to develop studies that confirm *Chryseobacterium* sp. as a pathogen of *S. edule*, or alternatively conclude that this is only due to an isolated case owing to the great genetic plasticity that this bacteria presents and that *S. edule* is not a primary host of *Chryseobacterium sp*.

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