Characterization and variability of strains of *Xanthomonas axonopodis* pv. *passiflorae* from the state of Pará, Brazil

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ABSTRACT

Bacterial spot, caused by *Xanthomonas axonopodis* pv. *passiflorae*, is a disease that has limited the cultivation of passionfruit in various orchards in Brazil. The objective of this work is to characterize and evaluate the variability of 29 strains of *X. axonopodis* pv. *passiflorae* from different municipalities producing yellow passionfruit in the state of Pará. The characterization was performed by the biochemical methods of KOH solubility, oxidase and Bactray, and the molecular methods of Xapas-F, Xapas-Ri and Xapas-Ro primers. The variability was evaluated by pathogenicity test and RAPD (randomly amplified polymorphic DNA). The strains of *X. axonopodis* pv. *passiflorae* were Gram-negative and oxidase-negative, and tests with the Bactray kit showed no relation between the collection municipality and group composition. The Xapas-F/Ri/Ro primers were specific for the strains. The primers used amplified 118 polymorphic bands in the RAPD reactions and the highest genetic similarity was between the strains PA15 and PA16. As the pathogenicity test evidenced a pathogenic variability, the strains PA2.1, PA4.5, PA14, PA4.2, PA4.1, PA4.6, PA3.4 and PA4.3 present the highest severity values for the disease. The strains of *X. axonopodis* pv. *passiflorae* show characteristics typical of the species, and genetic and pathogenic variability among them.

Keywords: bacterial spot; *Passiflora edulis* f. *flavicarpa*; biochemical tests; detection; diversity.

INTRODUCTION

Bacterial spot is an important passionfruit disease (*Passiflora edulis*) caused by the bacterium *Xanthomonas axonopodis* pv. *passiflorae* (Pereira) Gonçalves and Rosato. The state of Pará is the eleventh largest passionfruit producer in Brazil (IBGE, 2019). Its production focus mainly on yellow passionfruit (*Passiflora edulis* f. *flavicarpa*), the most appreciated variety in Brazil. However, bacterial spot has limited the cultivation of several passionfruit orchards in Brazil mainly because it is a difficult-to-control disease with a severity aggravated in regions with warmer and wetter climatic conditions (Junqueira & Junqueira, 2007).

Xanthomonas axonopodis pv. *passiflorae* is a bacterium found specifically in the genus *Passiflora* (Liberato, 2002). The infection occurs through lesions and natural openings in the plant, causing a typical symptomatology in leaves characterized by necrosis, immersion and irregularly shaped oily lesions surrounded by chlorotic areas. In fruits, there is the appearance of hard oily spots, making them unsuitable for consumption and industrial processing (Gonçalves & Rosato, 2000).

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The genus *Xanthomona*s (Xanthomonadaceae) is represented by obligatory aerobic bacteria. Its mobile cells have a single polar flagellum (Catara *et al.*, 2021). Species of the genus are producers of xanthan gum, an exopolysaccharide of high industrial interest, mainly for the food, pharmaceutical, agrochemical and petrochemical industries (Silva *et al.*, 2020). Another typical feature of bacteria of this genus is the production of a carotenoid-like group of pigments called xanthomonadine, a yellow pigment associated exclusively with the outer membrane of the bacterial cell wall (Wang *et al.*, 2017).

Some studies have demonstrated the high variability of *X. axonopodis* pv. *passiflorae* strains (Nakatani *et al.*, 2009; Gonçalves & Rosato, 2000; Munhoz *et al.*, 2011). These studies expand the knowledge about pathogen dissemination and are important for the development of plant disease control methods (Munhoz *et al.*, 2011). In addition to a variety of biochemical tests available, molecular biology offers pathogen detection techniques that has been increasingly studied because of its great accuracy in characterizing organisms. The polymerase chain reaction (PCR) based assays are rapid, sensitive, and they offer high flexibility and utility (Mesa *et al.*, 2020). The PCR-based randomly amplified polymorphic DNA (RAPD) technique is widely used to distinguish genetic variations in populations at an individual-level, produce genetic mapping and identify markers linked to desired traits (Dilipan *et al.*, 2017). The objective of this work is to characterize and evaluate the variability of 29 strains of *X. axonopodis* pv. *passiflorae* from different municipalities producing yellow passionfruit in the state of Pará.

MATERIAL AND METHODS

The characterization and variability study were performed with 29 strains of *X. axonopodis* pv. *passiflorae* obtained from leaf samples from different municipalities producing yellow passionfruit in the state of Pará and one strain of *X. campestris* pv. *campestris* as a negative control (Table 1) at the Laboratório de Fitopatologia of the Embrapa Amazônia Oriental, Belém, Pará. All strains belong to the collection of the Laboratório de Fitopatologia of the Embrapa Amazônia Oriental.

Table 1: Strains of *Xanthomonas axonopodis* pv. *passiflorae* and *Xanthomonas campestris* pv. *campestris* used in the study according to the strain code and origin

Species	Strain	Origin
X. campestris pv. *campestris*	Xcc	Ananindeua
X. axonopodis pv. *passiflorae*	PA1, PA2.1, PA3.1, PA3.2, PA3.3, PA3.4, PA14, PA16, PA17	Igarapé-Açu
	PA4.1, PA4.2, PA4.3, PA4.4, PA4.5, PA4.6, PA4.7	Castanhal
	PA5.1, PA5.2, PA5.3, PA5.4, PA5.5	Maracanã
	PA7.2	Belém
	PA8.4, PA8.5	Ipixuna
	PA10	Santo Antônio do Tauá
	PA12	São Domingos do Capim
	PA15	Capitão Poço
	PA18	São Francisco do Pará
	PA20	Tomé-Açu

Biochemical tests

The biochemical characterization of strains was performed by KOH solubility test, oxidase test and the commercial kits Bactray I and II. For KOH solubility test, a bacterial colony was grown in the medium 523 (Kado & Heskett, 1970) for 48 h at 28 ºC, and was then transferred to a glass slide homogenized with a drop of 3% KOH solution by lifting the handle several times. Evaluation was performed by verifying whether a viscous strip formed in the mixture. When there is formation of a strip, the reaction is considered positive, indicating that the bacteria are Gram-negative. When there is no formation of the strip, the reaction is negative and the bacteria are Gram-positive.

In the oxidase test, commercial oxidase strips (Laborclin) were used. A smear was performed on the strip of a bacterial colony grown in the medium 523 for 24 h at 28 °C. After two minutes, the change or not of the strip color was evaluated. The development of a violet coloration characterizes a positive oxidase test and the non-change of color characterizes a negative oxidase test.

The commercial kits Bactray I and II (Laborclin) were used for Gram-negative and oxidase-negative bacteria. They are composed of the media o-nitrophenol beta-d-galactate pyranoside (ONPG), arginine dehydrolase (ADH), lysine decarboxylase (LCD), ornithine decarboxylase (ODC), hydrogen sulfide (H_2S), urease (URE), acetoin glucose production (VP), L-phenylalanine (PD), indole (IND), sodium citrate (CIT), malonate (MAL), rhamnose (RHA), adonitol (ADO), salicin (SAL), arabinose (ARA), inositol (INO), sorbitol (SOR), sucrose (SAC), mannitol (MAN), and rafinose (RAF). Bacterial colonies growing on the medium 523 for 48 h at 28 °C were scraped using Drigalski handles in sterile distilled water. After standardization on a magnetic stirrer, the absorbance of the bacterial suspension was measured on a UV-visible spectrophotometer SP-2000UV (Spectrum) at 600 nm and adjusted to a concentration of 0.3 absorbance units.mL^{-1} (10^8 colony forming units.mL^{-1}). 1 ml of the bacterial suspension was transferred to the reaction set. The mixture was homogenized and incubated for 24 h at 28 °C. The evaluation was performed from the developed staining following the instructions of the manufacturer›s identification system.

Primer specificity

The bacterial DNA extraction was performed according to the protocol of Ausubel *et al.* (2003). The DNA concentration was estimated in a 0.5X TBE buffer and GelRed (Biotium) stained 1.0% agarose gel by comparison with Low Mass DNA Ladder (Invitrogen) marker bands. Amplified bands were visualized under UV light and photographed on a photocumenter L-PixChemi (Loccus Biotechnology) using the L-PixImage program. The quantification of bands was performed using the LabImage 1D program.

The PCR reactions were conducted using the primers Xapas-F (5 ‘ATCCCGACAGGCTCCACC 3’), Xapas-Ri (5 ‘CATATCGACGACCTTCGCTT 3’) Xapas-Ro (5 ‘CGAAATCTTTTGTTAGACCTT 3’) (Munhoz *et al.*, 2011) in a total volume of 25.0 µL containing 1 X Green Go Taq® buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µM of each primer, 0.03 U of *Taq* DNA polymerase (Invitrogen) and 40.0 ng of DNA. The DNA amplification was performed in a thermocycler (Biocycler) under the following conditions: 3 min at 94 °C for initial denaturation, 1 min at 94 °C for denaturation, 1 min at 58 °C for annealing, 1 min at 72 °C for extension with 35 cycles, and 5 min at 72 °C for final extension. Amplification products were evaluated in a 0.5X TBE buffer and GelRed-stained 0.8% agarose gel using the 1 kb DNA Ladder molecular weight marker (Promega). Amplified bands were visualized under UV light and photographed on a photocumenter using the L-Pix Image program.

RAPD

The primers for RAPD reactions with the strains of *X. axonopodis* pv. *passiflorae* were previously tested to select the primers with the best amplification and polymorphism.

The RAPD reactions were conducted in a total volume of 14.10 µL containing 1.17 X Green Go Taq® buffer, 2.13 mM $MgCl_2$, 0.07 mM dNTPs, 0.74 mg BSA, 4.96 µM of each primer, 0.07 U of *Taq* DNA polymerase, and 35.0 ng of DNA. The DNA amplification was performed in a thermocycler (Biocycler) under the following conditions: 2 min at 92 °C for initial denaturation, 1 min at 92 °C for denaturation, 2 min at 37 °C for annealing, 2 min at 72 °C for extension with 40 cycles, and 3 min at 72 °C for final extension. Amplification products were evaluated in 5X TBE buffer and GelRed stained 1.5% agarose gel using the 1 kb Plus DNA Ladder molecular weight marker (Invitrogen). Amplified bands were visualized under UV light and photographed on a photocumenter using the L-Pix Image program.

The RAPD reaction gels were evaluated by designating one to represent fragment amplification and null for no am-

plification. With this data, a binary matrix was constructed. Genetic similarity between samples was estimated using the PAST v.1.34 program (Hammer *et al.*, 2001) according to the Jaccard similarity coefficient. The dendrogram was constructed based on the unweighted pair group method with arithmetic averages (UPGMA) to determine the genetic relation between isolates. An analysis of *bootstrap* using 10,000 resamples was performed to verify the reliability of the generated dendrogram.

Pathogenicity test

The virulence variability of the strains was evaluated through a pathogenicity test performed using the bacterial suspension inoculation methodology described by Oliveira *et al.* (2018). Yellow passionfruit seeds of the 'Gigante Amarelo' cultivar were sown in 3-kg pots containing soil, sand and manure (2:1:1). After the plants presented one to two true leaves, thinning was performed, leaving only two plants per pot. Inoculation was performed by cutting with a scissor previously immersed in the bacterial suspension prepared with tap water at a concentration of 10^8 CFU.mL^{-1}. Two leaves were inoculated per plant. After inoculation, the plants were kept for 24 h in a humid chamber. Control plants were cut with scissors previously immersed in tap water. The experimental design was randomized blocks with four replications (four plants/plot), totaling 16 plants per treatment. Bacterial spot severity was assessed at 3, 6, 9, 12, 15 and 18 days after pathogen inoculation using a diagrammatic scale with five levels of severity: 2, 5, 11, 26 and 59% (Miranda, 2004; Laranjeira, 2005). With the obtained values, the area under the disease progress curve (AUDPC) proposed by Shaner & Finney (1977) was calculated. An analysis of variance was performed, and means were compared by Scott & Knott test (1974) at 5% probability.

RESULTS AND DISCUSSION

Biochemical tests

In the biochemical characterization by KOH solubility test and oxidase test, all 29 strains of *X. axonopodis* pv. *passiflorae* and the strain of *X. campestris* pv. *campestris* were Gram-negative and oxidase-negative.

In the biochemical tests with the Bactray I and II, there was no evident relation between the collection municipality and the composition of the formed groups. There were differences between strains in L-phenylalanine (PD), sodium citrate (CIT), malonate (MAL) and sucrose (SAC) media (Table 2). Nine groups formed; three groups for strains that differed from the other strains in only one biochemical medium: PA1, PA14, PA20 (SAC), PA2.1 (PD), PA 4.3, PA17, PA18 (MAL), four groups for strains that differed in two media: PA3.1 (CIT and MAL), PA3.3, PA15 (MAL and SAC), PA3.4 (PD and CIT), PA4.1 (CIT and SAC); one group for the strain PA3.2 (CIT, MAL and SAC), which differed by three media; and one group for the strains PA4.2, PA4.4, PA4.5, PA4.6, PA4.7, PA5.1, PA5.2, PA5.3, PA5.4, PA5.5, PA7.2, PA8.4, PA8.5, PA10, PA12 and PA16, which did not differ among the evaluated media. The strain of *X. campestris* pv. *campestris* differed in ornithine decarboxylase (ODC), acetoin-glucose (VP), sodium citrate (CIT) and sucrose (SAC) media.

Bacteria of the genus *Xanthomonas* have characteristics known to be Gram-negative, oxidase-negative, and non-urease (URE) and -indol (IND), producing unrealized acid production from carbohydrates such as rhamnose (RHA), adonitol (ADO), inositol (INO) and sorbitol (SOR) (Vauterin *et al.*, 1995), in agreement with the negative results for the biochemical tests of all strains in this study. These results corroborate those obtained by Malavolta Junior *et al.* (2001), who characterized a strain of *X. axonopodis* pv. *passiflorae* from the state of São Paulo. The authors found that it had Gram-negative cells and a negative result for oxidase, urease and indole. Tassa & Duarte (2002), for the first report of *X. axonopodis* pv. *passiflorae* as a causal agent of passionfruit leaf lesions in Mato Grosso state, characterized it as Gram-negative and oxidase-negative. Halfeld-Vieira & Nechet (2006) found that a strain of *X. axonopodis* pv. *passiflorae* in yellow passionfruit leaves collected in Roraima state were Gram-negative and oxidase-negative, and negative for urease production.

Primer specificity

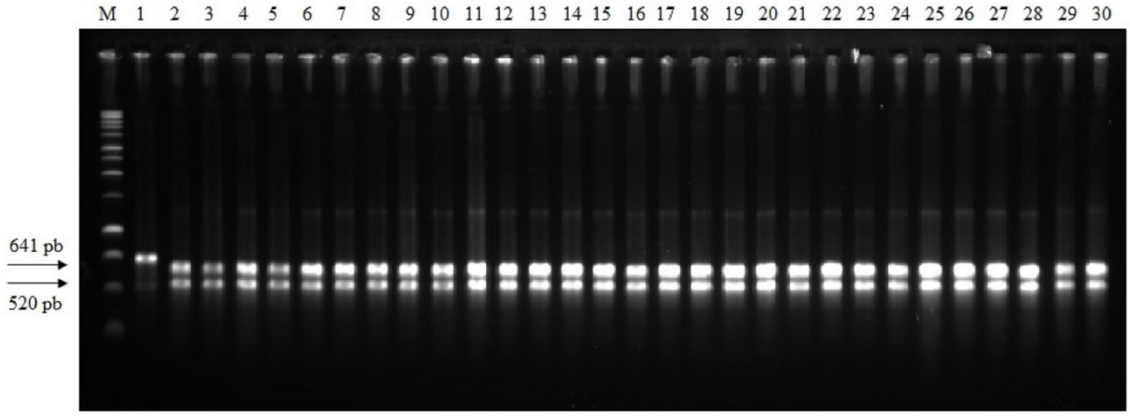
Upon detecting 29 strains of *X. axonopodis* pv. *passiflorae*, the Xapas-F/Ri/Ro primers amplified two fragments of approximately 641 and 520 pb (Figure 1), in agreement with the results obtained by Munhoz *et al.* (2011) and Silva (2012). For the strain of *X. campestris* pv. *campestris*, the Xapas-F/Ri/Ro primers amplified only one fragment of

approximately 690 pb, demonstrating a high specificity for detection of the species *X. axonopodis* pv. *passiflorae.* The 641 pb amplified fragment is common to all strains of the genus *Xanthomonas* because the Xapas-F and Xapas-Ro primers were designed to anneal conserved sequences. Although the strain of *X. campestris* pv. *campestris* amplified one fragment of approximately 690 pb instead of the 641 pb fragment, it has a small size variation between different species, although this fragment is common to the genus *Xanthomonas*. The 520 pb fragment was amplified by the Xapas-Ri primer and was designed to be specific for the pathovar *passiflorae* (Munhoz *et al.*, 2011).

Table 2: Biochemical characterization of strains of *Xanthomonas axonopodis* pv. *passiflorae* by Bactray I and II

Strains	ONPG	ADH	LCD	ODC	H_2S	URE	VP	PD	IND	CIT	MAL	RHA	ADO	SAL	ARA	INO	SOR	SAC	MAN	RAF
Xcc	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-
PA1	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-
PA2.1	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
PA3.1	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-
PA3.2	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-
PA3.3	+	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-
PA3.4	+	+	+	+	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-
PA4.1	+	+	+	+	-	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-
PA4-2	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA4.3	+	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-
PA4.4	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA4.5	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA4.6	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA4.7	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA5.1	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA5.2	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA5.3	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA5.4	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA5.5	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA7.2	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA8.4	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA8.5	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA10	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA12	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA14	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-
PA15	+	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	-
PA16	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
PA17	+	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-
PA18	+	+	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-
PA20	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-

ONPG: o-nitrofenol-beta-d-galactate-pyranoside; ADH: Arginine dehydrolase; LCD: Lysine decarboxylase; ODC: Ornithine decarboxylase; H_2S: Hydrogen sulfide; URE: Urease; VP: acetoin glucose production; PD: L-phenylalanine; IND: Indole; CIT: Sodium citrate; MAL: Malonate; RHA: Rhamnose; ADO: Adonitol; SAL: Salicin; ARA: Arabinose; INO: Inositol; SOR: Sorbitol; SAC: Sucrose; MAN: Mannitol; RAF: Rafinose.



M. 1 kb DNA Ladder molecular weight marker (Promega), 1. *X. campestris* pv. *campestris*, 2. PA1, 3.PA2.1, 4. PA3.1, 5. PA3.2, 6. PA3.3, 7. PA3.4, 8. PA4.1, 9. PA4.2, 10. PA4.3, 11. PA4.4, 12. PA4.5, 13. PA4.6, 14. PA4.7, 15. PA5.1, 16. PA5.2, 17. PA5.3, 18. PA5.4, 19. PA5.5, 20. PA7.2, 21. PA8.4, 22. PA8.5, 23. PA10, 24. PA12, 25. PA14, 26. PA15, 27. PA16, 28. PA17, 29. PA18, 30. PA20.

Figure 1: DNA amplification of strains of *Xanthomonas axonopodis* pv. *passiflorae* on 0.8% agarose gel using Xapas-F, Xapas-Ri and Xapas-Ro primers.

RAPD

The RAPD reactions were initially performed with 87 primers, of which 14 were selected from the clear and polymorphic bands (Table 3). The primers used amplified 118 polymorphic bands. The primer OPA 08 had the highest number of polymorphisms, totaling 15 polymorphic bands. The primers OPR 03, OPR 04 and OPR 07 had the lowest number of polymorphisms, i.e., six polymorphic bands each.

Table 3: Primers used for RAPD analysis of strains of *Xanthomonas axonopodis* pv. *passiflorae* and number of amplified polymorphic bands

Primers	Sequence 5'-3'	Number of polymorphic bands
OPA 08	GTGACGTAGG	15
OPAZ 04	CCAGCCTCAG	11
OPF 10	GGAAGCTTGG	7
OPM 02	ACAACGCCTC	7
OPM 05	GGGAACGTGT	7
OPM 14	AGGGTCGTTC	7
OPR 03	ACACAGAGGG	6
OPR 04	CCCGTAGCAC	6
OPR 05	GACCTAGTGG	14
OPR 07	ACTGGCCTGA	6
OPR 08	CCCGTTGCCT	8
OPR 14	CAGGATTCCC	8
OPR 19	CCTCCTCATC	9
OPQ 15	GGGTAACGTG	7

In the dendrogram constructed (Figure 2), observed two major clusters of the strains. One cluster comprised two strains and a second cluster included 27 strains with a bootstrap 99% between the two groups. Similarity levels between strains ranged from 0.0425 to 0.7179. The highest genetic similarity was between the strains PA15 and PA16 (71%). On the other hand, the lowest similarity was obtained by comparing the strains PA1 and PA18 (4%). Observing the grouping of strains, there was no evident relation between the municipality of origin of the strains and the formation of groups.

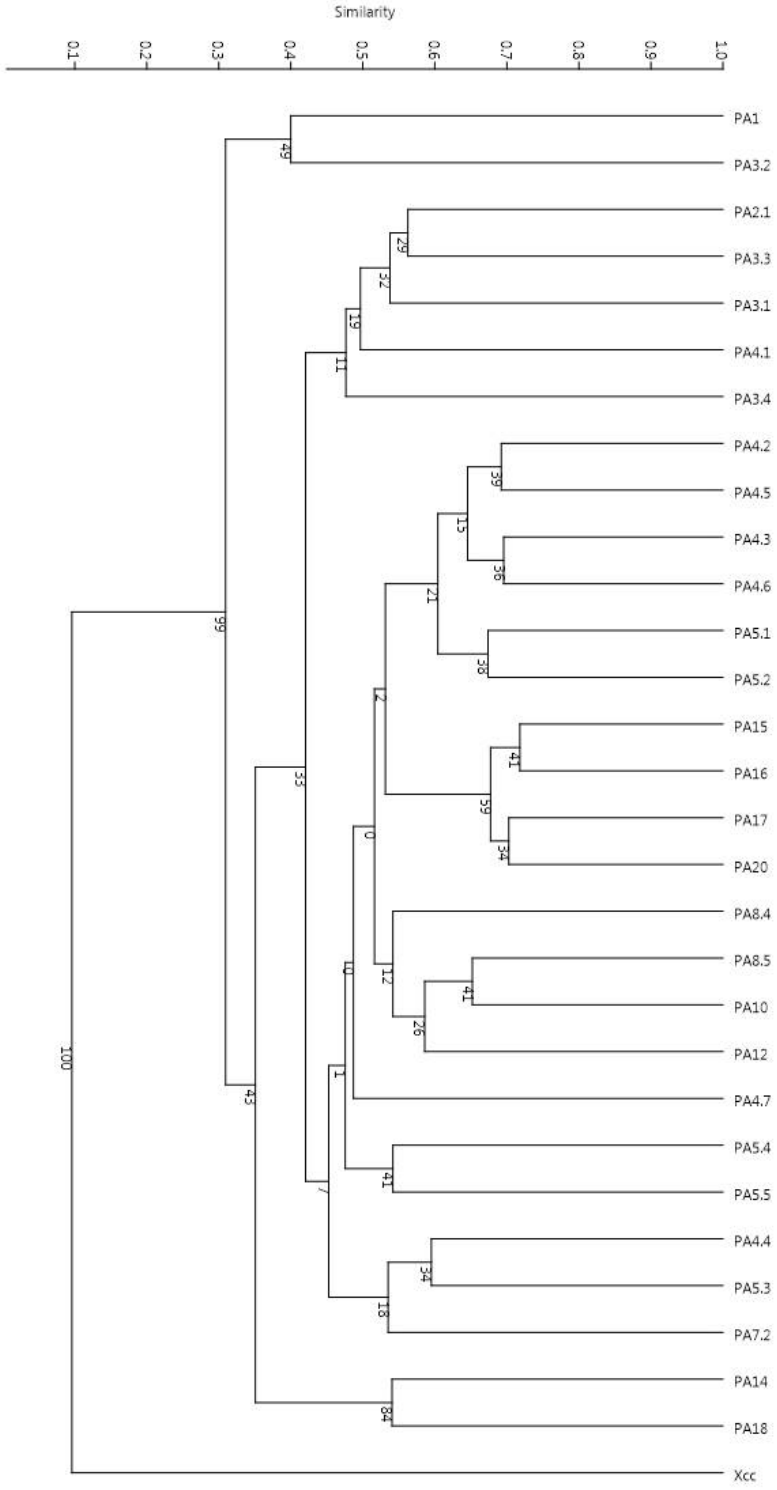
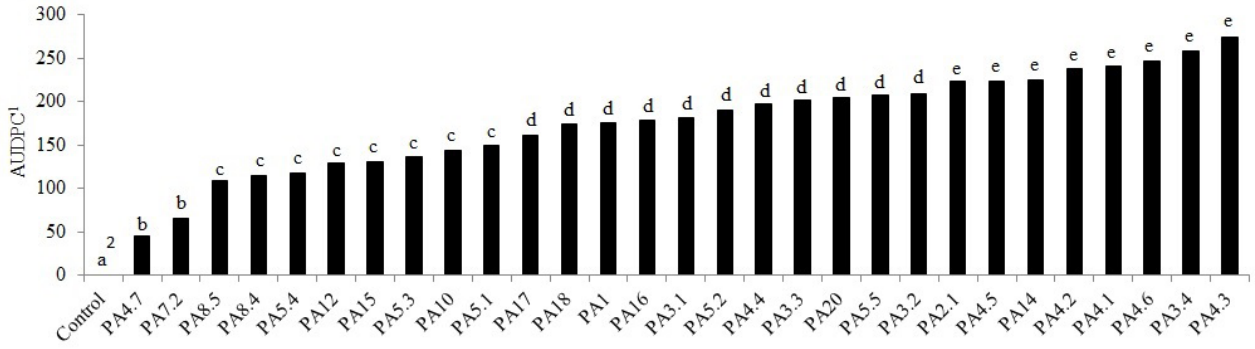


Figure 2: Dendogram generated by UPGMA method using Jaccard similarity coefficient for 29 strains of *Xanthomonas axonopodis* pv. *passiflorae*, characterized by RAPD.

Nakatani *et al.* (2009) found similar results. The authors evaluated the variability of 50 strains of *X. axonopodis* pv. *passiflorae* from four sites in the state of São Paulo using 15 primers. They found that there was no evident relation between collection site and group composition. However, the similarity levels ranged from 0.6887 to 0.9688. Gonçalves & Rosato (2000), evaluating the genetic diversity of 55 strains of *X. axonopodis* pv. *passiflorae* from seven Brazilian states using four previously selected primers, did not find a relation between the collection region and the genetic similarity of the strains. The groups formed according to the collection region. The authors also identified 15 groups with a similarity of up to 0.7; three main groupings included 94% of the strains. Munhoz *et al.* (2011), using the AFLP technique (amplified fragment length polymorphism) to measure the genetic diversity of 87 strains of *X. axonopodis* pv. *passiflorae* from different regions of Brazil, estimated a genetic similarity from 0.16 to 1.0.

Pathogenicity test

From the analysis of the pathogenicity test, the virulence variability of the strains was verified. The strains PA2.1, PA4.5, PA14, PA4.2, PA4.1, PA4.6, PA3.4 and PA4.3 from the municipalities of Igarapé-Açu and Castanhal presented the highest values of disease severity, followed by the strains PA17, PA18, PA1, PA16, PA3.1, PA5.2, PA4.4, PA3.3, PA20, PA5.5 and PA3.2 from the municipalities of Igarapé-Açu, São Francisco do Pará, Maracanã, Castanhal and Tomé-Açu (Figure 3). And the strains PA4.7 and PA7.2 from the municipalities of Castanhal and Belém, respectively, were highlighted with the lowest values of disease severity.



[1]AUDPC: Area under the disease progress curve.

Figure 3: Yellow passionfruit reaction to strains of *Xanthomonas axonopodis* pv. *passiflorae*.

In the first evaluation, at 3 days after inoculation, no bacteria presented symptoms of the disease. At 6 days, only the strains PA4.3, PA17, PA18, PA20, PA15, PA14, PA16, PA3.4, PA4.2 presented symptoms with averages between 0.07 and 0.29%, which correspond to values below the lowest severity level assessed. At 9 days, the strains PA14, PA16, PA20 and PA4.5 presented averages of 5.38 to 5.79%, values close to the intermediate level of severity of 5%. At 12 days, the strains PA4.1, PA5.2, PA4.6, PA4.3, PA5.5, PA3.4, PA4.2 and PA4.5 presented averages between 12.25 and 17.25%, values between the intermediate levels of 11 and 26%. At 15 and 18 days, it was already possible to observe averages above the highest level of severity of the diagrammatic scale for strains PA3.4, PA4.3, PA.4.1, PA4.2, PA4.6 and PA2.1, considering value 100% severity when the leaf fell off the plant.

Nakatani *et al.* (2009), evaluating the aggressiveness of 5 strains of *X. axonopodis* pv. *passiflorae* collected from four different sites in the state of São Paulo, also verified the existence of variability in the aggressiveness of the strains. The strain SMA6 produced the highest value of injured leaf area (LFA), not differing statistically from the LFA values obtained for the strains SPD10 and HOL8, but statistically higher than the strains VCR1 and SMD1.

CONCLUSIONS

The strains of *X. axonopodis* pv. *passiflorae* from the state of Pará present a biochemical characteristic typical for the genus.

The primers Xapas-F/Ri/Ro have been shown to be specific for detecting strains of *X. axonopodis* pv. *passiflorae*.

The analysis by RAPD reveals the existence of genetic

variability among the strains of *X. axonopodis* pv. *passiflorae*.

The pathogenicity test demonstrates the variability regarding the virulence of the strains of *X. axonopodis* pv. *passiflorae*.

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