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Search for biological activity of proteolytic enzymes isolates from native and cultivated Bromeliaceae in Corrientes on phytopathogenic microorganisms

Búsqueda de actividad biológica de aislados de fitoproteasas de Bromeliaceae nativas y cultivadas en Corrientes sobre microorganismos fitopatógenos

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Summary: The aim of this work was to evaluate the biological activity of protein isolates from the native *Bromelia serra* (BS) and cultivated bromeliad *Ananas comosus* (AC) on phytopathogenic microorganisms. Samples of AC were obtained from the experimental station of the Universidad Nacional del Nordeste in Corrientes, Argentina and of BS from natural forests also in Corrientes, Argentina. Extracts of stem and leaves of AC and leaves of BS were processed through a precipitation with acetone, the pellet was re-suspended in autoclaved buffers and called protein isolates. The antimicrobial activity was evaluated by the disc diffusion technique, using phytopathogenic bacteria. However, an inhibition zone was not observed in any of the treatments with protein isolates, unlike the positive control with *Streptomycin*. The minimum inhibitory concentration of bacterial growth was determined by broth microdilution technique. The results showed that the protein isolates did not inhibit bacterial growth. Furthermore, antifungal activity against *Fusarium oxysporum* was evaluated by MTT method, but no significant inhibition was observed either. Although the protein extracts did not show biological activity against the microorganisms evaluated, future experiments will continue the evaluation by using other microorganisms. In addition, after a purification scheme will be established, different operational conditions and tests will be used with pure enzymes instead of their extracts.

Key words: *Ananas comosus*, *Bromelia serra*, natural control.

Resumen: El objetivo de este estudio es evaluar la actividad biológica de aislados proteicos de la planta nativa *Bromelia serra* (BS) y cultivada *Ananas comosus* (AC) sobre microorganismos fitopatógenos. Se obtuvieron muestras de bromeliáceas cultivadas (AC) del Campo Experimental de la Facultad de Ciencias Agrarias (UNNE) y de la bromeliácea nativa BS de montes naturales de la región. Se prepararon extractos de tallo y hojas de AC y hojas de BS, los cuales después de ser precipitados con acetona y obtener pellets, fueron resuspendidos en *buffer* estéril, los cuales se denominaron aislados proteicos. Se evaluó la actividad antimicrobiana frente a bacterias fitopatógenas mediante la técnica de difusión en disco. Sin embargo, no se observó la formación de un halo de inhibición en ninguno de los tratamientos con aislados proteicos, a diferencia del control positivo con estreptomicina. Se realizó la concentración inhibitoria mínima de crecimiento bacteriano por la técnica de microdilución en caldo. Los resultados mostraron

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que los aislados proteicos no inhibieron el crecimiento bacteriano. Además, se evaluó la actividad antifúngica contra *Fusarium oxysporum* mediante la técnica del MTT, pero tampoco se observó inhibición. Aunque los extractos de proteínas no mostraron actividad biológica contra los microorganismos evaluados, futuros experimentos continuarán la evaluación utilizando otros microorganismos. Además, una vez que se establezca un esquema de purificación, se utilizarán diferentes condiciones operativas y pruebas con enzimas puras en lugar de sus extractos.

Palabras clave: *Ananas comosus, Bromelia serra*, control natural.

Introduction

The existence of natural compounds and other natural products has many effects that depend on the application and properties of the compounds themselves (e.g. their use as bio-pesticides). The use of agrochemicals is commonly used to control fungal or microbial diseases in crops; however, stands out the relevance that studies are having in the science of agroecology on products with biological activity or biopesticides of natural origin that could replace agrochemicals that impact the environment, in certain cases in an excessive manner. More than 6,000 botanical species have been identified that have the potential to eliminate pests and are also used as natural controllers for crop diseases (Meena & Mishra, 2020). Plant-derived pesticides show minimal residual activity and affect a minimal number of non-target insect species. Botanical biopesticides show compatibility with integrated pest management (IPM) programs (Xu *et al.,* 2011).

Bacterial diseases of vegetables are mainly caused by members of the genera: *Erwinia, Pectobacterium, Xanthomonas, Spiroplasma, Phytoplasma* and many others. The Green Revolution in the production of cereal crops has generated confidence in public opinion regarding the satisfaction of the future food needs of the growing population. There are several devastating diseases, such as the bacterial blight (*Pyricularia oryzae*) of rice (*Oryza sativa*), which is widely observed in tropical Asia, where approximately 60% of the world's population lives (UNDES Affairs 2011). Chitosan (poly-D-glucosamine) is recently being incorporated into biopesticides. Bacteria are hypersensitive to chitosan and its derivatives, so chitosan could be expected to protect the plant from bacterial diseases (Hassan & Chang, 2017). Chitosan exhibited antimicrobial activity against Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *Escherichia coli*, respectively; these were incorporated into biodegradable nanocapsules (Pinheiroa *et al.,* 2015).

Bromelain, derived from pineapple [*Ananas comosus* (L.) Merr.], a Bromeliaceae specie, is a crude preparation known for containing at least four well-studied peptidases: stem bromelain, fruit bromelain, comosain, and ananain (Tochi *et al.,* 2008; Arshad *et al.,* 2014). This enzyme mixture finds diverse applications, including its use in food tenderization, beverages, baking, cosmetic products, preparation of protein hydrolysates, animal feed, tooth whitening, and various sectors of the textile and pharmaceutical industries (Arshad *et al.*, 2014; Manzoor *et al.,* 2016). This trend is reflected in the rising demand for proteases as eco-friendly alternatives (Arshad *et al.,* 2014; Husain, 2018).

Bromelain has also been studied for its importance in agro-industry. It was proven that transgenic expression of the fruit bromelain gene BAA1 confers greater resistance to bacterial soft rot in Chinese cabbage (*Brassica rapa*) (Jung *et al.,* 2008). Additionally, bromelain has been proved antimicrobial potential against different phytopathogenic microorganisms (López-García *et al.,* 2012; Dutta *et al.,* 2013)

Proteolytic enzymes from different species of Bromeliaceae family have been studied before, but they have only characterized the fruit of *Bromelia serra* Griseb. (Caffini *et al.,* 1988; Salese *et al.,* 2022). Most of the proteases investigated within the Bromeliaceae family are derived from their fruits. However, the presence of these fruits depends on climatic conditions or the prevailing season

for flowering. In contrast, the availability of leaves and stems remains constant, and in the case of *B. serra* (BS), it is notably abundant. Gómez Herrera *et al.* (2022) characterized the proteolytic activity of BS leaves in crude and isolated extracts, showing interesting characteristics and properties for food industry. This positions BS leaf protein isolates as potential sources of bioactive compounds, so it is necessary to continue studying their application for different uses, as well as the application of extracts of other pineapple plant organs.

Therefore, the objective of this work is to evaluate the biological activity of protein isolates from *Bromelia serra* and *Ananas comosus* on phytopathogenic microorganisms.

Material and Methods

Plant and microbial source

Samples. The plant material of *Ananas comosus* (AC) was provided by the experimental station of the Universidad Nacional del Nordeste in Corrientes, Argentina. Leaves of *Bromelia serra* (BS), a native bromeliad from natural forests in the region, were sampled from San Cosme, Corrientes, Argentina (27°27'07"S, 58°38'16"W). The vegetal material was placed in plastic bags and kept at −20 °C until use.

Microorganisms were provided by Vegetal Physiology and Plant-microorganism Interaction Laboratory (Facultad de Ciencias Agrarias-Instituto de Botánica del Nordeste): *Xanthomonas axonopodis* pv. *manihotis* (Xm), *X. citri* subsp. *citri* (Xc), *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Ralstonia solanacearum* (Rs), *Pseudomonas syringae* pv. *tomato* (Pse), and *Fusarium oxysporum* (Fo).

Preparation of AC stem and leaf extract and BS leaves. Stems and leaves from cultivated bromeliads were washed with 0.1% hydrogen peroxide solution. Both were cut into small pieces, weighed and processed with a blender (Smart-tek) in 100 mM sodium acetate buffer pH 5.00 in a proportion of 1 g tissue/mL buffer. The homogenate was filtered through a twice folded piece of gauze to remove plant debris and centrifuged at 12,000×*g* for 15 min at 4 °C (Thermo Scientific). The buffer contained 5 mM EDTA (Biopack) and 5 mM cysteine (Sigma-Aldrich) as protective agents for the enzymes.

The extracts were precipitated with 4 volumes of cold acetone (-20 °C) and then resuspended in their corresponding sterile buffer, which were sterilized to avoid contamination from them. The pellets resuspended in buffer were called protein isolates. The proteolytic activity of the protein isolates was assayed using azocasein (1%) as substrate (Sarath *et al*., 1989). One unit of activity (U) was defined as the amount of enzyme needed to produce and increase in optical density of 1.0 at 440 nm at 37 °C in 30 minutes. Also, a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done to confirm the presence of the proteins (Laemmli, 1970).

Antibacterial activity by disc diffusion technique

Cultures of Xc, Xm, Cmm, Pse and Rs were performed using TSB (Trypticase Soy Broth), and incubated for 48 h, using mechanic agitation, at room temperature. Paper filter disks (6 mm diameter) were embedded with 10 µl of the AC and BS protein isolates. The discs were dried at room temperature. The TSA (Trypticase Soy Agar) plates were inoculated with 0.1 ml of bacterial culture (10^8 cfu/ml), that was spread superficially, and then the filter paper discs soaked in the protein isolates were placed in the center of the plate. The plates were incubated at 28 ºC for 24 h and the diameter of the inhibition zone was measured (Demo *et al.*, 2005; Lambir *et al.,* 2022). Positive controls (antibiotics: *Streptomycin*) and negative controls (extract buffer) were performed (De Pooter *et al.,* 1995).

Antibacterial activity by broth microdilution technique

Determination of minimum inhibitory concentration (MIC) of bacterial growth was measured according to Prieto *et al.* (2020) with some modifications.

Determination of the initial concentration of cells: serial dilutions of a factor of 10 were made from the bacterial suspensions up to a 10^{-5}

dilution. After that, 170 µl of these dilutions were placed in sterile 96-well microplates (GenBiotech), from column 1 to 5. Then 20 µl of the diluent (sterile water) and 10 µl of a 0.01% resazurin sodium salt (Sigma-Aldrich) solution was added. The plate was incubated for 4 h at 28 ºC and the first dilution incapable of reducing resazurin (blue) was chosen as the working concentration. Visualization is based on the ability of living microorganisms, at a certain concentration (~10^{-6}), to reduce resazurin. The blue color (oxidized state) indicates a concentration of cells unable to reduce resazurin and the pink color (reduced state) indicates the adequate concentration that reduces resazurin.

Determination of the minimum inhibitory concentration (MIC): the protein isolates use for MIC were 1.07 mg/mL of, 0.44 mg/mL and 1 mg/mL from AC stem, AC leaves, and BS leaves respectively. The protein isolates were diluted at 1/2, 1/4, 1/8, 1/16 using broth as solvent and was performed in sterile eppendorf. Commercial bromelain (0.1 mg/ml) was also tested (Sigma-Aldrich) with dilutions of 1, 1/2, 1/4, 1/8 and 1/16. 42.5 µl of the inoculum and 400 µl of the isolates and their dilutions were placed in eppendorfs. 250 µl of each mixture was transferred into a microtiter plate and incubated for 22 h at 28 °C and then 10 µl of a 0.01% resazurin solution was added to each well. Again, the microtiter was incubated for 2 h at 28 ºC, and then the inhibition was assessed visually (blue color).

Antifungal activity by MTT method

The antifungal activity of the protein isolates against Fo strains was evaluated by microdilution colorimetric technique. This assay uses the redox indicator Thiazolyl Blue Tetrazolium Bromide MTT (GoldBio), which is pale yellow in its oxidized state, but has a dark purple color in its reduced form (MTT-formazan). A stock solution of MTT was prepared dissolving 5 mg of MTT in 1 mL of PBS. This reaction is carried out by the action of mitochondrial dehydrogenases active in living cells (Kuhn *et al*., 2003). The technique was carried out according to the methodology proposed by Meletiadis *et al*. (2000). The fungus grew for 10 days on a potato agar medium. Subsequently, a count was carried out in a Neubauer chamber by sowing 10 µl of the fungus, reaching a conidia concentration of $1x10^{6}$. mL^{-1}. Subsequently, the fungus was incubated for 48 h at 28 °C with the protein isolates from AC leaf and stem, BS leaf, and commercial bromelain. Blank controls were also made from potato broth, isolates, and fungus suspension. Subsequently, 25 µL of MTT solution was added to each tube and incubated in the dark for 24 h. Once the incubation was completed, the tubes were centrifuged at 13,000 rpm for 5 min to obtain the MTT-formazan as a precipitate. The dye is released from inside the cells and dissolved by adding 100 µL of DMSO, followed by vortex shaking. The absorbance at 550 nm of the solutions was measured in a spectrophotometer. The results of the treatments and controls are affected by the absorbance of the blank (non-inoculated tubes). The percentage of mycelial growth inhibition (PIC), which corresponds to the amount of MTT not converted into MTT-formazan, was calculated according to the formula:

$$PIC= [(AB-AT)/AB] \times 100$$

where AB corresponds to the average (n= 3) of the absorbance for the blank control, while AT indicates the average (n= 3) obtained for the absorbance of the treatments.

Results and Discussion

The proteolytic activity using azocasein as substrate showed that the protein isolates had 2.6, 10.65, 13.18, and 20.22 U/mg for commercial bromelain, BS leaves isolate, AC leaves isolate, and AC stem isolate, respectively. The highest activity was shown by the AC stem protein isolate and the BS leaves isolate showed similar activity from Gómez Herrera *et al.,* 2022, with the same specie (BS), using the same substrate. The protein profile of the BS isolates (Fig. 1) detected the presence of the same protein bands (22, 43, and 62 kDa) reported by Gómez Herrera *et al.* (2022). The AC leaves isolate showed proteins with 27, 24, 22, 20, 17, and 14

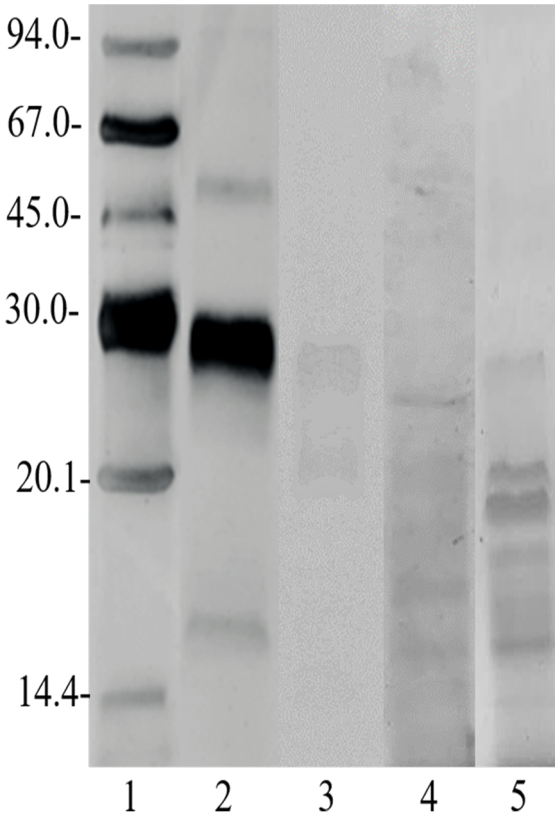

Fig 1. SDS-polyacrylamide gel electrophoresis (PAGE). Lane 1: standard protein markers, lane 2: protein isolate from *Bromelia serra* leaves, lane 3: commercial bromelain, lane 4: protein isolate from *Ananas comosus* leaves, and lane 5: protein isolate from *Ananas comosus* stem.

kDa; while the AC stem isolate protein profile showed more protein bands (43, 40, 25, 21, 18, 16, 15, 14, and 13 kDa).

The antimicrobial activity by plate disc diffusion technique (Table 1) was repeated three times for each treatment (BS leaves, AC stem and AC leaves isolates). The growth of the tested bacterial strains (Pse*,* Xm*,* Xc*,* Cmm and Rs) was not inhibited by any of the treatments. Abdulrahman *et al.* (2015) found that AC extracts from the pineapple fruit did not inhibit *Bacillus subtilis*, *Streptococcus pyogenes* and *Escherichia coli*; however, it did show growth inhibition in *Proteus spp. and Corynebacterium spp.* at 24 °C incubation, showing that the inhibition of these extracts depends on the susceptibility of each bacterial species. Abdulrahman *et al.* (2015) also tested the antibacterial activity of AC extracts at several incubation temperatures (24 °C, 37 °C,) and pH media (7 and 10), showing different responses from bacterial growth inhibition, suggesting that culture conditions may alter bacterial susceptibility to extracts, or that extracts may change their bioactivity at different pH and temperature conditions. On the other hand, there are no previous reports of antimicrobial activity of extracts of *Bromelia serra* performed in plate disc diffusion technique against the bacterial strains tested in this study.

As can be seen in Fig. 2, as an example of some treatments against Pst, none of the AC and BS protein isolates could achieve an

Table 1. Antimicrobial activity by disc diffusion technique.

Bacterial strains	Treatments				
	BS leave isolate	AC leave isolate	AC stem isolate	*Streptomycin* (mm)	Extraction *buffer*
Pseudomonas syringae pv. *tomato*	NI	NI	NI	I (2.26 ± 0.12)	NI
Xanthomonas axonopodis pv. *manihotis*	NI	NI	NI	I (1.12 ± 0.09)	NI
Xanthomonas citri subsp. *citri*	NI	NI	NI	I (0.94 ± 0.07)	NI
Clavibacter michiganensis subsp. *michiganensis*	NI	NI	NI	I (1.73 ± 0.24)	NI
Ralstonia solanacearum	NI	NI	NI	I (0.74 ± 0.07)	NI

Abbreviations: AC: *Ananas comosus*, BS: *Bromelia serra*, NI: No inhibition, I: Inhibition (halo diameter in milimeters).

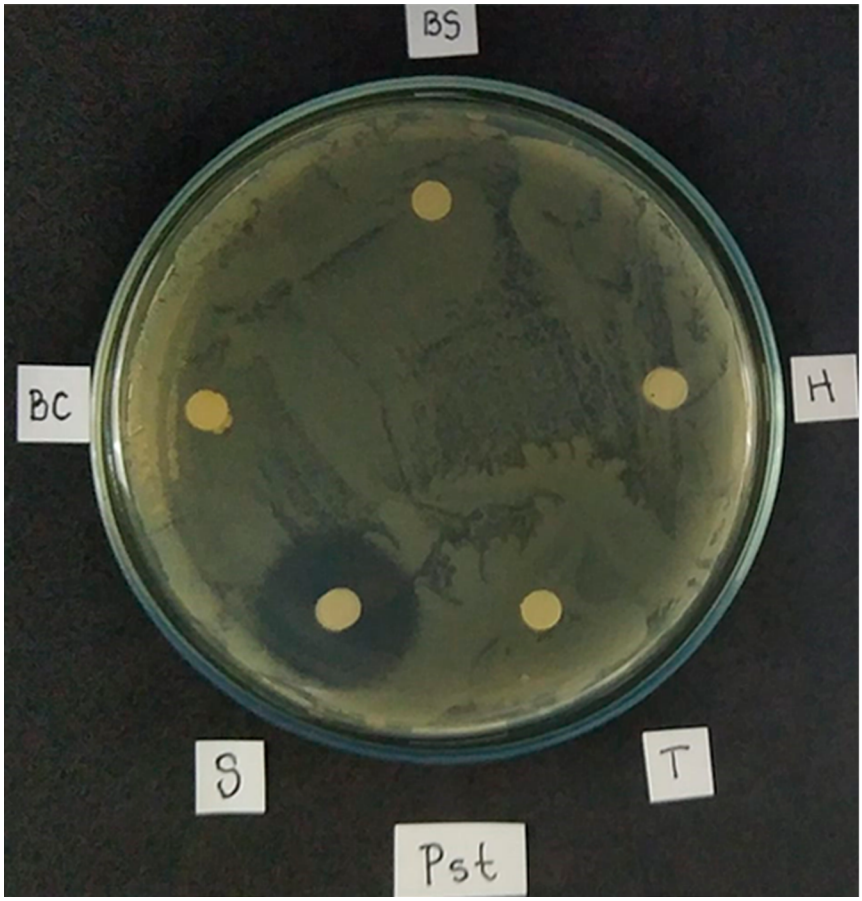

Fig. 2. Antibacterial activity by disc diffusion technique inoculated with *Pseudomonas syringae* pv. *tomato* (Pst). The filter paper discs are soaked with the protein isolates of *Ananas comosus* stem (T), *Ananas comosus* leaves (H), *Bromelia serra* leaves (BS), Commercial bromelain (BC), and *Streptomycin* (S).

inhibition zone against the studied bacterial species, while the inhibition of *Streptomycin* can be clearly observed (positive control), showing inhibition halos ranging from 0.68 mm to 2.34 mm diameter. However, despite that no inhibition was observed for different treatments, we decided to evaluate the antibacterial activity also using the broth microdilution method. This was due to that, in exceptional cases, some compounds could have unreliable results using disc diffusion method (Dickert *et al.,* 1981; Hood *et al.,* 2003). It is known that the result obtained by the disc diffusion technique depends not only by the antibacterial activity of the tested compound but also by its diffusion capacity on culture media (among others). In this sense, the utilization of a technique that allows to directly exposing the microorganism to the tested substance, as the microdilution technique, could be a way to avoid a result of false resistance to that substance. Antimicrobial activity of BS and AC isolates against the tested bacterial and fungus species were analyzed using the broth microdilution and MTT techniques, respectively. These techniques allow to assess the cellular viability and cell culture proliferation in each treatment, through colorimetric assays. These techniques are based on the enzymatic reduction of the compounds MTT and resazurin by dehydrogenases that utilize

NADH/NADPH as co-substrates (Präbst *et al.,* 2017). The measurement of NADH and NADPH content is closely linked to cellular viability and metabolic activity, but direct measurement of these pyridine nucleotides although is possible, might not be the most optimal indicator of metabolic activity given the significance of their turnover rate. Then, the measurement of MTT/resazurin reduction allows to measure indirectly NADH/NADPH content in an easy way, given that the resulting colored product from the reduced form of these compounds can be quantified using basic spectroscopic methods. These colorimetric assays, utilizing compounds like MTT and resazurin, prove to be more sensitive and reliable than alternative methods for testing cellular viability (Präbst *et al.,* 2017).

In this context, the broth microdilution technique was used to evaluate the minimum inhibitory concentration (MIC) for each isolate (leaves of BS, leaves of AC and stem of AC) against different phytopathogenic bacteria. First of all, the initial cell concentration (~10^5 cell) was determined using a colorimetric method as the first dilution unable to reduce resazurin solution (0.01%), remaining blue color (Fig. 3). This cell concentration was determined for each repetition of the treatments. For example, in Fig. 3, the selection of the initial cell concentration for Rs was dilution number 2 (1/10 of the initial culture concentration) and for Cmm, Pse, Xc and Xm was dilution 3 (1/100 of the initial culture concentration) regarding cell concentration. These differences could be related to different amounts of inoculum as starter for each bacterial culture, and to different growth rates between bacterial species.

The results of the broth microdilution technique (Table 2) showed no growth inhibition of any of the tested bacterial species exposed to the different protein isolates or commercial bromelain (Pink, mauve colors), at all the tested concentrations (BS leaf isolate: 0.88, 0.44, 0.22, 0.11 and 0.06 mg/mL; AC leaf isolate: 0.39, 0.19, 0.10, 0.05 and 0.02 mg/mL; AC stem isolate: 0.95, 0.47, 0.24, 0.12 and 0.06 mg/mL; commercial bromelain: 0.088, 0.044, 0.022, 0.011, 0.006 and 0.003 mg/mL). Only the negative control using resazurin and buffer remained blue (Fig. 4). These results denote that in the present work all tested bacterial species were not susceptible to bromelain, since the different isolates potentially containing this enzyme, and the commercial bromelain, were unable to inhibit their growth. By contrast, Dutta *et al.* (2013) found that the leaves extract from pineapple fruit crowns inhibited the microbial growth in 70-95% using turbidity measurements with MIC range of 1.65–4.95 mg/mL for *Bacillus subtilis* and *Candida albicans*. For other Bromeliaceae species, Ávalos-Flores *et al.* (2022) showed that the partial-purified protease from *Bromelia karatas* inhibited the growth of *Salmonella typhimurium* at a concentration of 3.0 mg/mL and displayed an equivalent effect in *Listeria monocytogenes*.

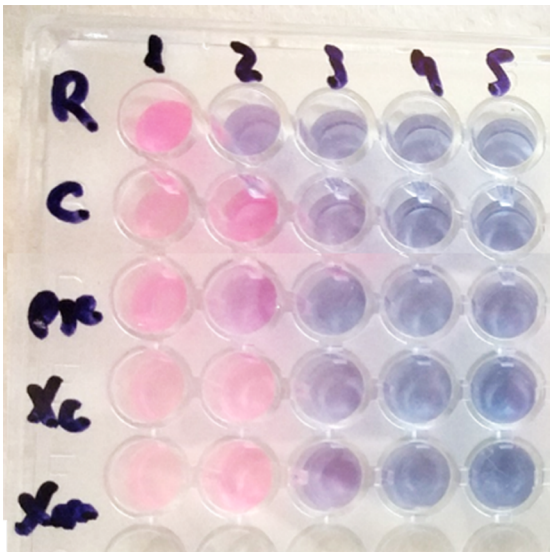

Fig. 3. Selection of the initial cell concentration using 0.01% resazurin solution. *Ralstonia solanacearum* (R), *Clavibacter michiganensis* subsp. *michiganensis* (C), *Pseudomonas syringae* pv. *tomato* (Pse), *Xanthomonas citri* subsp. *citri* (Xc) and *Xanthomonas axonopodis* pv. *manihotis* (Xm). Numbers 1 to 5 represent the concentration of a ten-fold dilution of bacteria.

The antifungal activity of the protein isolates (BS leaves, AC stem and leaves)

Table 2. Determination of the minimum inhibitory concentration by broth microdilution technique.

Bacterial strains	Treatments				
	BS leaf isolate*	AC leaf isolate*	AC stem isolate*	Commercial bromelain*	Negative control
Pseudomonas syringae pv. *tomato*	Pink	Pink	Pink	Pink	Blue
Xanthomonas axonopodis pv. *manihotis*	Pink	Pink	Pink	Pink	Blue
Xanthomonas citri subsp. *citri*	Pink	Pink	Pink	Pink	Blue
Clavibacter michiganensis subsp. *michiganensis*	Pink	Pink	Pink	Pink	Blue
Ralstonia solanacearum	Pink	Pink	Pink	Pink	Blue

Abbreviations: AC.: *Ananas comosus*, BS: *Bromelia serra*, *Treatments had no growth inhibition against all tested bacteria. Concentration tested: BS leaf isolate (0.88, 0.44, 0.22, 0.11, and 0.06 mg/mL); AC leaf isolate (0.39, 0.19, 0.10, 0.05, and 0.02 mg/mL); AC stem isolate (0.95, 0.47, 0.24, 0.12, and 0.06 mg/mL); commercial bromelain (0.088, 0.044, 0.022, 0.011, 0.006, and 0.003 mg/mL).

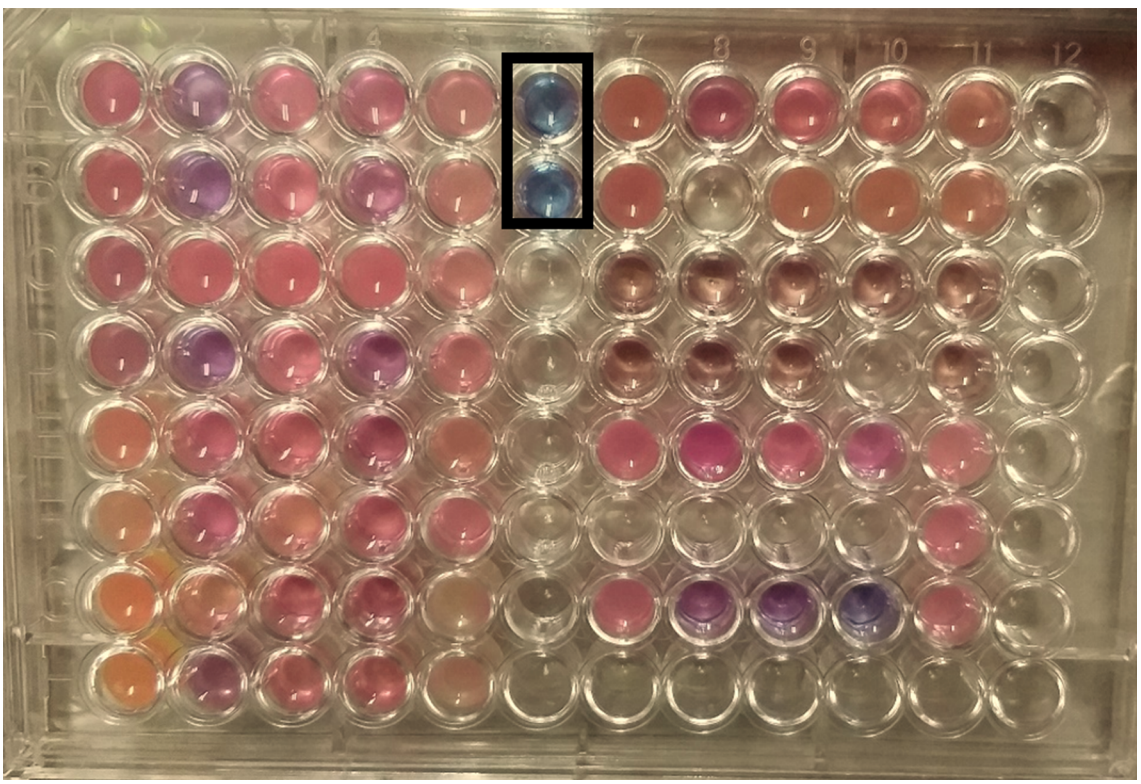

Fig. 4. Broth microdilution technique. The black rectangular border shows the negative control in the microtiter (6A and 6B). Columns 1 and 7 are inoculated with *Ralstonia solanacearum,* 2 and 8 with *Pseudomonas syringae* pv. *tomato,* 3 and 9 with *Xanthomonas citri* subsp. *citri*, 4 and 10 with *Xanthomonas axonopodis* pv. *manihotis*. Microtiter cells: protein isolates from *Ananas comosus* stem (1A to 5A and 1B to 5B), protein isolates from *Ananas comosus* leaves (1C to 5C and 1D to 5D), protein isolates from *Bromelia serra* leaves (1E to 5E and 1F to 5F), Commercial bromelain (7C to 11C and 7D to 11D). The rest of the remaining cells are repetitions from the protein isolates.

and commercial bromelain against *Fusarium oxysporum* was evaluated by MTT method. The obtained results are shown in the Table 3. It is noteworthy that the inhibition percentages were negative. This is probably due to the presence of the protein non related to enzymes in the isolate that resulted in greater fungus growth than in their absence. This indicates that the protein isolates evaluated had a stimulatory effect on fungus growth, which could also be caused by the autoproteolysis of the proteases providing peptide substrate (Mazorra-Manzano *et al.,* 2018) to *Fusarium oxysporum*. All protein isolates tested in this study showed the same results (as shown in Table 3). However, it is worth noting that López-García *et al.* (2012) found that commercial bromelain had a fungicidal effect on *Fusarium oxysporum*. These results differ from the ones found in our study, they could be caused for different reasons as, enzyme extraction, the technique sensitivity or resistance of the *Fusarium oxysporum* strain isolated in this region that could respond differently to commercial bromelain treatment (Ghannoum *et al.,* 1999).

In addition to the possible reasons why the antibacterial and antifungal activities did not yield positive results, like exhibiting some inhibition by the isolates, it is still a result that should be published. Negative results are also results since they contribute knowledge and can prevent other researchers from repeating the trial, leading to time loss (Culebras & Franco-López, 2016).

Table 3. Percentage of mycelium growth inhibition of *F. oxysporum* for different protein isolates and commercial bromelain.

Treatments	Percentage of Inhibition (%)
Commercial Bromelain	NI (-59.72)
AC stem	NI (-105.66)
AC leaves	NI (-116.33)
BS leaves	NI (-230.19)

Abbreviations: AC: *Ananas comosus*, BS: *Bromelia serra,* NI: (no inhibition).

Conclusion

Although none of the protein extracts could biologically control the microorganisms examined, it is necessary to continue with the purification of the protein extracts to obtain pure proteolytic enzymes, since in this present work only tests were carried out with protein isolates from *Bromelia serra* and *Ananas comosus*. We also performed biological activity of the protein isolates using cellular activity techniques for phytopathogenic microorganisms. In further studies, the enzymes from *Bromelia serra* and *Ananas comosus* will be purified in order to test fungal or bacterial inhibition using the same strains; and also test the purified enzymes in other phytopathogenic microorganisms.

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