Chapter 282

Bacillus amyloliquefaciens, Microbacterium resistens and *Stenotrophomonas maltophilia* in Biocontrol of *Corynespora cassiicola* in Tomato Culture

https://doi.org/10.56238/devopinterscie-282

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ABSTRACT
This article presents an approach on the use of biological control applying the action of bacteria against *Corynespora cassiicola* (Bert & Curt) Wei, as this is one of the main phytopathogen of aerial propagation that limits the productivity of solanaceous plants. The use of bacteria in the control of solanaceous diseases is a widespread methodology, since the biocontrol of phytopathogen carried out by bacteria can occur through different mechanisms such as inhibition by antibiotics, parasitism, competition for physical space or induction of plant resistance to pathogen attack. This research aimed to select, identify and evaluate in vitro and in plant antagonist bacteria with potential for the development of a bioproduct that contributes to the sustainable agricultural development of tomatoes. In plant collections and tests were carried out at the Dr. Alejo von Der Pahlen, KM 14 on road AM 010 (S: 02° 59' 45.7" and W: 60° 01' 22.3"). The in vitro tests were carried out at Embrapa Western Amazon. The results indicate that *Bacillus amyloliquefaciens* showed 75.73% of in vitro inhibition of *C. cassiicola* and 56% of target spot control when compared to *Microbacterium resistens* and *Stenotrophomonas maltophilia* in tests on tomato.

Keywords: Solanaceae, Bacteria, Biocontrol, Bioproduct.

1 INTRODUCTION

The tomato plant (*Solanum lycopersicon* L.) , belongs to the nightshade family and originates from the Andean region, comprising Peru, Bolivia and Ecuador, however, it is appreciated on all continents (CAMARGO et al., 2006). It has interesting nutritional characteristics, such as low caloric value and source of antioxidants such as lycopene and rich in vitamins A, B and C and the mineral salts potassium, iron and phosphorus (Duma et al., 2015). As well as stands out as sen do a fruit characterized as the most consumed vegetable around the globe. (Dahlke et al., 2019; FAO, 2019). The cultivation of tomato plants can be compromised by several diseases of fungal origin that can cause significant economic losses in the harvest (LOPES and ROSSATO, 2013; RODRIGUES et al. , 2018).

The target spot caused by *Corynespora cassiicola* (Bert & Curt) Wei is one of the major fungal diseases of aerial disthat infercts nightshade crops. For this reason, research involving the use of antagonistic microorganisms has intensified because prospectively indicates to have potential as to its use for the control of phytodiseases of economic importance (LOPES et al. , 2015).

The biological control of phytopathogens performed by bacteria can occur by different mechanisms, such as by antibiosis, parasitism, competition for physical space or the induction of plant resistance due to the attack of pathogens (OLIVEIRA et al. , 2010; TOYOTA et al., 2015). According to Oliveira et al. (2014) oneof the main groups used in biocontrol are actinobacteria. Vergnes et al. (2020) found that actinobacteria can develop on the surface of plant leaves and expose their potential for disease management, such as inducing defense and protection responses against foliar fungal pathogens.

Yamunarani and Pandiyan (2019) emphasize the importance of monitoring and the execution of new research using actinobacteria with potential for the development of new biofertilizers and use as biocontrol.

The monitoring of the action of biological agents in the control of phytopathogenic diseases is mostly carried out by direct and integral human work at the place of cultivation. However, the automation of the monitoring of the biocontrol action in agricultural crops can easily be performed by optical sensor in real time, because in the studies of Askraba et al. (2016) found that plant differentiation sensors based on the images capitated with the proximal spectral reflectance of the leaves allows to differentiate the cultivated plant from the weed with an accuracy of 90%.

The automated monitoring of the harmful action by phytopathogenic mycorgnisms is also already a reality in the process of intelligent agriculture, since in the studies of Udutalapally et al. (2021) it was found that the Agricultural Cyber-Physical System (A-CPS) using the worldwide web communication network through the Internet-of-Agro-Things (IoAT) performing the comparison between images of healthy leaves with leaves under phytopathogenic attack reached the results that This innovative crop technology not only improves the quality and productivity of agricultural crops, but by continuous sensing and intelligent automation achieves an accuracy of 99.24% in predicting or maximizing leaf disease control in tomato.

Given this context, this research aimed to identify antagonistic compounds that inhibit phytopathogens, providing a protective effect of tomatoes (*S. lycopersium*), serve as a basis for the development of bioinputs that contribute to the sustainable agricultural development of nightshades and that in the future the action of inhibition bacterial against foliar phytodiseases are carried out by the use of an optical image sensor captured in real time by means of intelligent devicesthat use 5G/6G and IoT.

2 MATERIAL AND METHODS

2.1 OBTAINING THE PHYTOPATHOGEN

The phytopathogen C. *cassiicola* was isolated from tomato leaves presenting characteristic symptoms of target spot. The samples were collected in the tomato growing area at the Dr. Alejo von Der Pahlen Vegetable Experimental Station, KM 14 of the AM 010 road (S: 02° 59' 45.7" and W: 60° 01' 22.3"). The conidia of the fungi associated with the lesions were transferred to a petri dish containing Potato Dextrose Agar (BDA) medium (200 g potato, 20 g dextrose, 15 g agar and 1 L water). The plates were maintained in BOD at 25 °C for five to seven days. The hyphae developed were pricked to plates containing BDA and kept in BOD at 25 °C for ten days and stored in a refrigerator at 5 °C. The isolates selected for the tests in this research were confirmed by molecular biology techniquesas described in item 2.4.

2.2 SELECTION OF BACTERIA AGAINST TOMATO PATHOGENS

The antagonist bacteria were selected based on the paired culture method for antagonistic activity against the tomato plant pathogen *C. cassiicola* (INPA 2671). We evaluated 60 bacteria from the collection of microorganisms of the Molecular Biology Laboratory of Embrapa Western Amazon (SISGEN nº AB6B14F), isolated from Paullina cupana roots and sediments from the Solimões River. These isolates were reactivated in BDA culture medium and maintained in a BOD growth chamber at 28 ± 2 °C under a 12 h light regime. The screenings were performed individually in 90 x 15 mm Petri dishes with BDA medium in the antagonistic activity against *C. cassiicola.* The evaluations were performed by the analysis of paired culture according to Bastos (1997), in order to identify the three best antagonists to proceed to the next stage of evaluation. Each experiment was conducted in a completely randomized design (DIC) with only one sample unit per treatment. Of the 60 bacterial soles, the three were selected: PcA1, S ol 194 and S ol 195 that showed potential to inhibit the growth of Cand *C. cassiicola* invitro.

2.3 ANALYSIS OF IN VITRO INHIBITION OF BACTERIA AGAINST TOMATO PATHOGENS

The antagonism analysis occurred in such a way that a disc of 3 mm in diameter of the fungus was removed after 72 h of culture and transferred to the center of the Petri dish containing BDA medium and at approximately 2 cm of distance two drags were made with the bacteria approximately 2 mm wide by 3 cm long (one on each side). The control consisted of the cultivation of the phytopathogen in the absence of the antagonist. The plates were kept in a BOD growth chamber at 28 ± 2 °C under a 12 h light regime. The evaluations were performed every seven days until the twenty-first day (PI $_{7d}$, PI $_{14d}$, PI$_{21d}$). From the measurements was calculated the percentage of mycelial growth inhibition of pathogenic fungi according to the formula (LEE et al., 2014):

$$Inhibition\ (\%) = \left[\frac{(R - r)}{R} * 100\right]$$

Where:
R = radial growth of the pathogen in the control plate;
r = radial growth of the pathogen in paired cultures.

2.4 MOLECULAR IDENTIFICATION OF ANTAGONISTS AND PATHOGENS

The three selected antagonistbacteria PcA1, SOL 194 and SOL 195 were cultured in liquid ISP2 medium (3 g malt extract, 3 g yeast extract, 10 g dextrose, 1 L distilled water, pH 6.2) for 48 hours at 37 ºC. DNA extraction was performed using the Wizard Genomic DNA Purification (Promega) purification kit. The extraction protocol was Doyle's CTAB (1991) in micro extraction with silica for bacteria and in macro extraction with liquid nitrogen for the fungus.

The molecular identification of the bacteria was conducted by the amplification of fragments of ribosomal RNA 16S (16S rRNA), using the primer P027F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'- GGTTACCTTGTTACGACTT -3') (WHITE et al., 1990; KUMARAE AND SOUSA, 2002). .

The fungal isolate INPA 2671 was identified with the primers ITS1 (5'TTC CGT AGG TGA ACC TGC GG 3') and NL4 (5' TCC TCC GCT TAT TGA TAT GC 3') (White et al., 1990; Kumarae and Sousa, 2002). The reactions were prepared for a final volume of 25 µl, containing: 50 ng of total DNA; 0.5 pmol of each primer; 1X reaction buffer (100 mM Tris-HCl (pH 8.8 at 25 °C), 500 mM KCl, 0.8% (v/v) Nonidet P40); 2 mM $MgCl_2$; 1 mM dNTPs and 1U Taq DNA polymerase. The thermocycler programmed for initial denaturing at 95 °C for 5 min, 35 denaturing cycles at 95 °C for 30 sec, annealing 55 °C for 60 sec, elongation at 72 °C for 1.5 min. Final elongation at 72 °C for 5 min. The fragments submitted to electrophoresis in agarose gel 1.5 % (m/v) with molecular weight marker of 1 kb (Invitrogen), developed with ethidium bromide and photographed under light UV light using the Molecular Imaging System (Loccus Biotecnologic L-Pix. Chemi). The PCR products were also purified with 20% PEG and incubated in a thermocycler for 15 minutes at 37 ºC (Applied Biosystems) to be used in the sequencing reactions reallized with the volume of 10 µl (5 µl purified PCR product, 2 µl of Bigdye v 3.1 (Thermo Fisher), 2 µl of the buffer 5 X (Applied Biosystems) and 3.2 pmol of each primer. The thermocycler programmed to 96 °C for 4 min, followed by 30 cycles at 96 °C for 10 sec, 50 °C for 5 sec and 60 °C for 4 min. The sequencing reactions were analyzed in the Genetic Biosystems 3500 sequencer (Applied Biosystems/Hitachi).

The identification of bacteria and fungi was conducted by phylogenetic analysis based on the sequences obtained together with sequences from the NCBI database (http: //www.ncbi.nlm.nih). The individual sequences were aligned with the Clustal W software (ZHU et al., 2015) for the observation of

the phylogenetic tree in the MEGA v.11 program (DONTHU et al. 2021). The topology of the tree evaluated through bootstrap analysis using 1000 resamples.

2.5 EVALUATION OF BACTERIA IN PLANT EXPERIMENTS

The evaluation of the control dand *C. cassiicola* in tomato plants using the selected bacteria was conducted in a greenhouse at the Experimental Station of Vegetables, Dr Alejo Von Der Pahlen of INPA, (Rodovia AM 010, km 14, Manaus-AM). In general, the evaluations were carried out every 5 days for a period of 35 days after the inoculation of the bacteria in the seedlings, totaling 6 evaluations. The parameter evaluated *in plant* was the severity of the disease.

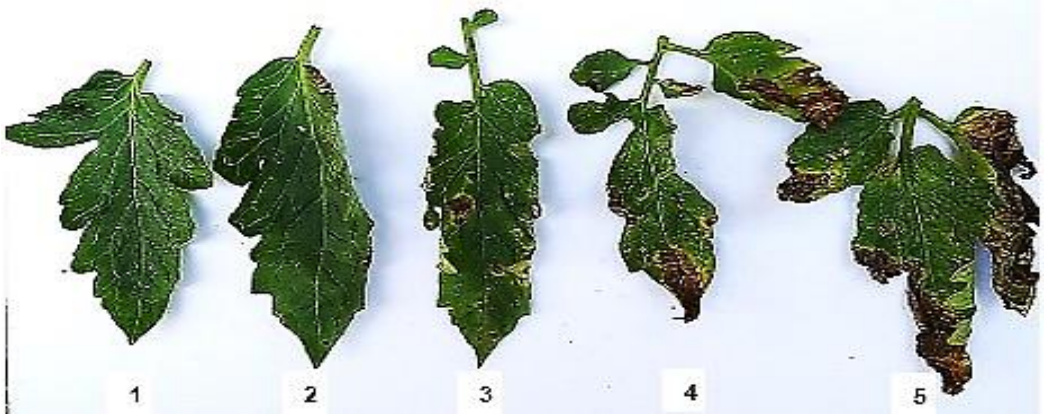
The possibility of controlling the disease was studied in three independent experiments. In the experiment to analyze the preventive effect, the putative biocontrolling bacteria were inoculated 5 days before the inoculation of the phytopathogen. In the coinoculated effect experiment, the putative biocontrolling bacteria were inoculated concomitantly with the phytopathogen. In the curative effect experiment, putative biocontrolling bacteria were inoculated when the disease was initiating symptoms in the plant. The positive control was inoculated only the pathogen without the putative biocontrolling bacteria and the negative control only sterile water. The bacterial extracts were obtained by growth in liquid ISP2 culture medium, under mechanical agitation of 200 rpm for up to 24 hours, with the optical density adjusted to A_{540}= 0.5 (approximate concentration of 10^8 cfu $^{mL-1}$). Then centrifuged at 7800 rpm for 6 minutes at 15 ºC in a centrifuge (eppendorf Centrifuge 5430 R) and the bacterial mass diluted in sterile water. (ROCHA and MOURA, 2013). The commercial substrate Tropstrato HT Hortaliça autoclaved at 120 ºC at 1 atm was used (Makishima and Carrijo, 1998).

The Santa Cruz tomato grow crops was sown directly in 500 mL plastic cups containing the substrate. A fertigation with 50 mL of N-P-K (Plantafol 20:20:20) at a concentration of 2 g $^{L-1}$ was performed 72 hours before sowing.

Two mechanized and automated sprinkler irrigations were performed per day for a period of 15 minutes each. From the thirtieth day after sowing, two manual irrigations with 50 mL of direct water were also performed in the experimental unit. Every seven days the experimental units were fertigated (CARRIJO et al. , 2005) with 50 mL of Plantafol 20:20:20 diluted in natural water and obeying the amount of 2 g $^{L-1}$.

The quantification of the severity of the target spot caused by the fungus *C. cassiicola* was made every five days, based on the diagrammatic scale of notes for analysis of the severity of the target spot proposed by Costa (2012) which establishes the following notes: 1 = healthy leaflet; 2 = low severity, up to 10% of the injured tissue; 3 = mean severity, 11 to 20% of the injured tissue; 4 = medium-high severity, 21 to 40% of the injured tissue; 5 = high severity, above 40% with large necrotic areas (Figure 1).

Figure 1 – Diagrammatic scale of notes for analysis of the severity of *Corynespora cassiicola* in tomato leaflets.



Source: Costa (2012) with adaptations by the author of this research, 2022.

The experiments were conducted in a randomized block design (DBC) with five replicates per treatment. To verify the difference in the treatments of all experiments, analysis of variance (ANOVA) was used and Tukey's test was subsequently performed at 5% probability ($P \leq 0.05$). The analyses were conducted using the SISVAR software version 5.6 (FERREIRA, 2019).

The experiments consisted of five treatments, being: treatment 1: positive control (with C. cassiicola and without antagonistic bacteria); treatment 2: isolate PcA1 and C. cassiicola; treatment 3: isolate Sol 194 and C. cassiicola; treatment 4: isolate Sol 195 and C. cassiicola and treatment 5: negative control (without both *C. cassiicola* and antagonist bacteria) only sterile water.

Inoculations of C. cassiicola in tomato plants were performed when the plants had four true leaves. The fungus was inoculated by spraying 10 mL of the solution containing a suspension calibrated in 10^5 conidia $^{mL-1}$ (Neubauer chamber) in the leaf region of each experimental unit designed to receive the phytopathogen (TERAMOTO et al., 2013). In each experimental unit, five leaflets from each of the last three leaves composed of the apical region received five bites with a sterile needle to facilitate the entry of the pathogen into the plant tissue. This same technique was used for the inoculation of biocontrolling agents.

3 RESULTS

3.1 IN *VITRO* EVALUATION OF BACTERIA AGAINST *TOMATO C. CASSIICOLA*

The screening performed with 60 bacteria indicated that the three isolates: PcA1, S ol 194 and Sol 195, showed potential to inhibit the growth of the phytopathogen *C. cassiicola* (isolate INPA 2671). The antagonists selected fors and the fungus were submitted to molecular identification and the resultsare presented in Table 2.

In *vitro* evaluations performed against *C. cassiicola* showed inhibition efficiency of 75.73%.

The isolate Sol 194 presented 66.54% of inhibition contra *C. cassiicola* after 7 days. Followed by Pc A1 with a mean percentage of 60.74% and Sol 195 with 58.69% inhibition. In the analyses of the percentage of antagonism of 14 days, Pc A1 presented the highest percentage of inhibition with 70.20%,

followed by the isolate Sol 194 with 65% of inhibition. At 21 days, it was also the Pc A1 that remained progressively, with the highest percentage of inhibition, 75.73% (Table 1) (Figure 2).

Table 1 – Mean percentages of *Corynespora cassiicola* inhibition between 7 and 21 days of evaluation.

Isolated bacterial	%-Inhibition 7 days	%-Inhibition 14 days	%-Inhibition 21 days
Sol 195	58.69 b	63.35 b	61.47 b
Pc A1	60.74 b	70.20 b	75.73 b
Sol 194	66.54 b	65.00 b	59.25 b
CV (%)	9,91	10,46	16,25
DMS	15,38	17,33	20,88

Measures of treatments followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability ($P \leq 0.05$). CV: Coefficient of variation. DSM: Mean deviation of significance.

Figure 2 – Evaluation in paired culture for 21 days of *C. cassiicola* against *Bacillus amyloliquefaciens, Stenotrophomonas maltophilia* and *Microbacterium*.

Source**:** Monteiro, 2022.

3.2 MOLECULAR IDENTIFICATION OF ANTAGONISTS AND PATHOGENS USED IN THIS STUDY

The molecular analysis based on the complete genome of the isolate PcA1 and region 16 of the other bacteria, revealed that PcA1 belongs to species *Bacillus amyloliquefaciens* based on dDDH above 70%, the isolate Sol 194 presented 87.99% of identity with *Microbacterium resistens* and the isolate Sol 195 with 96.44% as *Stenotrophomonas maltophilia* (Table 2).

Table 2 – Molecular identification of the isolates used in this study.

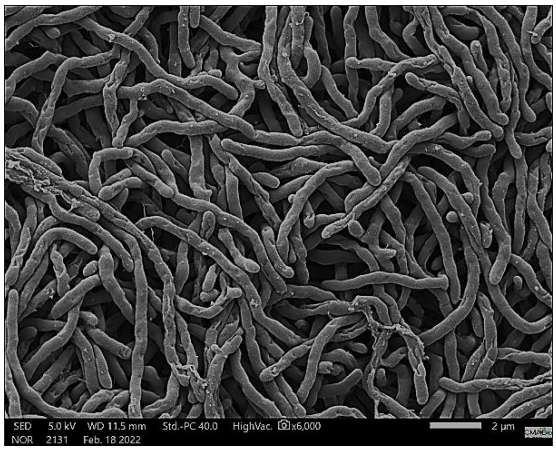
Isolated	Barcode	Id(%)	Access N	Identification
PcA1	16S rDNA	96,54	OM541330.1	*Bacillus amyloliquefaciens* strain GRSB-21
SOL 194	16S rDNA	87,99	KF703723.1	*Microbacterium resistens* strain EAAG97
SOL 195	16S rDNA	96,44	KX350046.1	*Stenotrophomonas maltophilia* strain F3-2-41
C. cassiicola **(INPA 2671)**	ITS	99,58	MT470606.1	*Corynespora cassiicola* strain HEV244L

Source: NCBI (http: //www.ncbi.nlm.nih)

3.2.1 Scanning Electron Microscopy – SEM

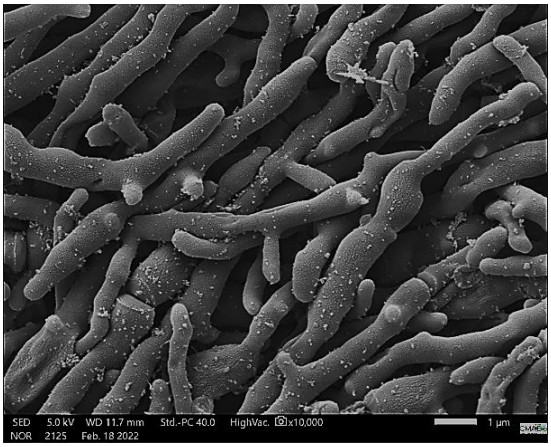
The disposition of the bacteria at the time of cell reproduction, with mycelial reproductive development behavior (Figures 3 and 4) and the structure of their colony similar to filamentous fungi (Figure 5) (Oliveira et al. 2014) indicate that two of the bacterial isolates, *Microbacterium resistens* (Sol 194) and *Stenotrophomonas maltophilia* (Sun 195) these are actinobactria.

Figure 3 – Colony structure of M. resistens (SOL 194) similar to filamentous fungi in scanning electron microscopy (SEM). Scale bars: 2 µm.



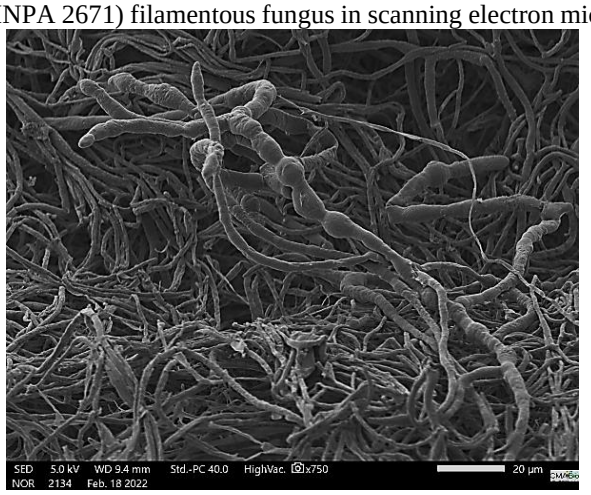
Source: Author (2022).

Figure 4 – Structure of the colony of S. maltophilia (SOL 195) similar to filamentous fungi in scanning electron microscopy (SEM). Scale bars: 1 µm.



Source: Author (2022).

Figure 5 – *Corynespora cassiicola* (INPA 2671) filamentous fungus in scanning electron microscopy (SEM). Scale bars: 20 µm.



Source: Author (2022).

3.1 IN *PLANT* EVALUATION OF BACTERIA AGAINST *TOMATO C. CASSIICOLA*

In plant n the preventive effect, both *Stenotrophomonas maltophilia* (Sol 195) and *Bacillus amyloliquefaciens* (PcA1), maintained the lowest means of severity of the target spot disease with a score of 2.6, with no statistical differences between them. Followed by *Microbacterium resistens* (Sol 194) with the highest mean (3.40), presenting the lowest efficiency in the control of the target spot disease (Table 3) (Figure 5).

When evaluated by co-inoculation, *Microbacterium resistens* (Sol 194) presented the lowest mean (2.6), but without statistical difference when compared to the others. In the evaluation of the curative effect, *Bacillus amyloliquefaciens* (PcA1) maintained the lowest mean severity of the target spot disease (2.8), remaining without statistical distinction at the level of 5%. *Stenotrophomonas maltophilia* (Sol 195) had a mean severity of 3.4 target disease. Followed by the bacterium Sol 194 with a score of 4 of mean severity of the target spot disease (Table 3) (Figure 5).

The isolate PcA1remained in the three tests the lowest mean severity of this disease in tomato. Both in the co-inoculated effect and in the curative effect, there was no change in the value of the note in relation to the control of the phytopathogenic action of the fungus C. cassiicola to the tomato, being considered the most efficient in the control of the fungus *C. cassiicola* in the tomato plant in a greenhouse (Table 3) (Figure 5).

Table 3 – Mean C. *cassiicola* severities evaluated in tomato.

Treatment	**Average Severity**		
	Preventive effect	**Coinoculation**	**Healing effect**
Sol 195	2.60 to	3.00 to	3.40 to b
Pc A1	2.60 to	2.80 to	2.80 to
Sol 194	3.40 to b	2.60 to	4.00 b c
Negative control	3.80 to b	4.80 b	4.80 c
Positive control	4.80 to b	5.00 b	4.60 c
CV (%)	29,93	16,02	16,13
DMS	1,94	1,10	1,19

Measures of treatments followed by the same letter in the column do not differ from each other by Tukey's Test at 5% probability. CV: Coefficient of variation. DSM: Mean deviation of significance.

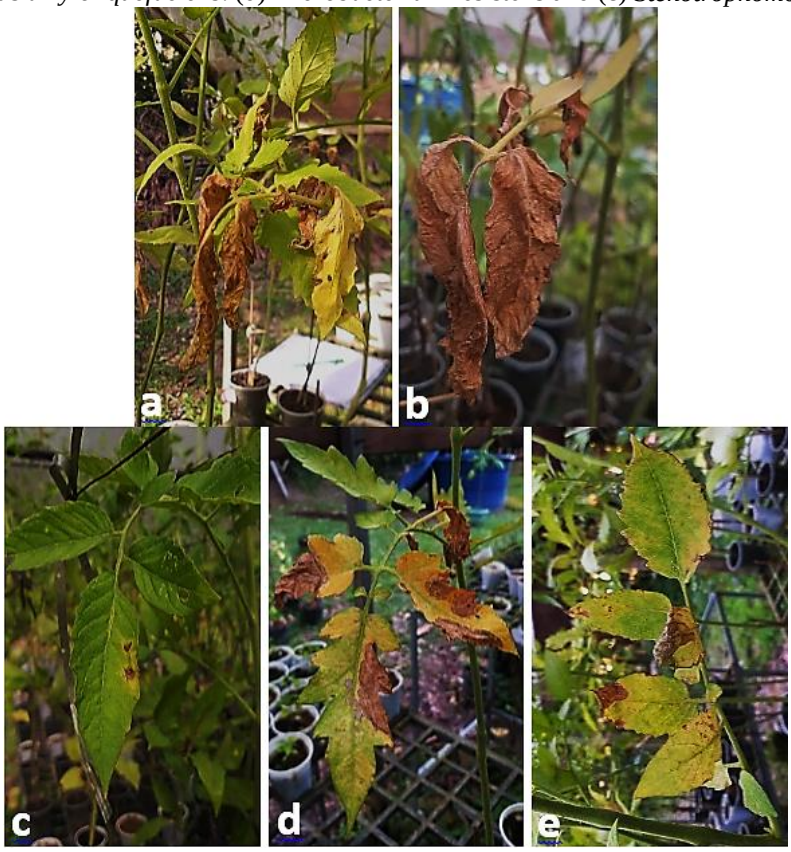
Figure 6 – Evaluation of the severity of the target spot by C. cassiicola in preventive effect for 35 days: (a) Negative control. (b) Positive control. (c) *Bacillus amyloliquefaciens*. (d) *Microbacterium resistens* and (e) *Stenotrophomonas maltophilia*.

Source: Author (2022).

Figure 7 – Evaluation of the severity of the target spot by C. cassiicola in co-inoculated effect for 35 days: (a) Negative control. (b) Positive control. (c) *Bacillus amyloliquefaciens*. (d) *Microbacterium resistens* and (e) *Stenotrophomonas maltophilia*.

Source: Author (2022).

Figure 8 – Evaluation of the severity of the target spot by C. cassiicola in curative effect for 35 days: (a) Negative control. (b) Positive control. (c) *Bacillus amyloliquefaciens*. (d) *Microbacterium resistens* and (e) *Stenotrophomonas maltophilia*.



Source: Author (2022).

4 DISCUSSIONS

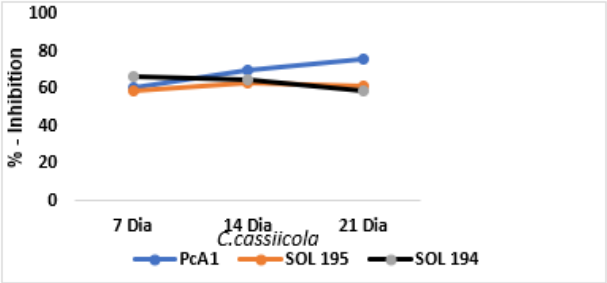
The tests in the period of seven days of *in vitro* antagonism (Graph 1) against the fungus C. cassiicola indicated that *Microbacterium resistens* and *Stenotrophomonas maltophilia*, even without statistical distinctions, but with numerical differences in relation to the bacteria evaluated, obtained percentage of inhibition of *C. cassiicola;* of 66.54% and 58.69% respectively. This result contributes to the work of López *et al.* (2021), since in *their in vitro* studies two isolates of the genus *Microbacterium* and two isolates of the genus *Stenotrophomonas* reduced the growth of *C. cassiicola* by more than 25%.

Bacillus amyloliquefaciens showed 60.74% growth inhibition of *C. cassiicola* in the seven-day in *vitro* tests. And it continued progressively, assuming the first place regarding the percentage of inhibition of this fungus in the other evaluations up to 21 days (70.20% and 75.73%, respectively). The results of the percentage of inhibition of *C. cassiicola* promoted by *Bacillus amyloliquefaciens* in this study were higher than the results achieved with the studies of Riddech *et al*. (2017) because in his studies by the double culture method performed for seven days the *B. amyloliquefaciens* showed only 47.63% inhibition of the fungus *C. cassiicola.*

The results of control of the fungus *C. cassiicola* isolated from tomatiro by the boldness of *Bacillus* presenting high percentages may be a promising indicator to be used in the reduction of the incidence of agricultural diseases of tropical areas (DOS SANTOS et al., 2022). As well as collaborating

with the studies of... regarding the use of the genus bacillus as a growth antagonist of phytopathogenic fungi (GABARDO et al., 2020)

Graph 1 – Analysis of inhibition in paired culture from 7 to 21 days of *C. cassiicola* against: *Bacillus amyloliquefaciens* (Pc A1); *Microbacterium resistens* (Sol 194) and *Stenotrophomonas maltophilia* (Sol 195).

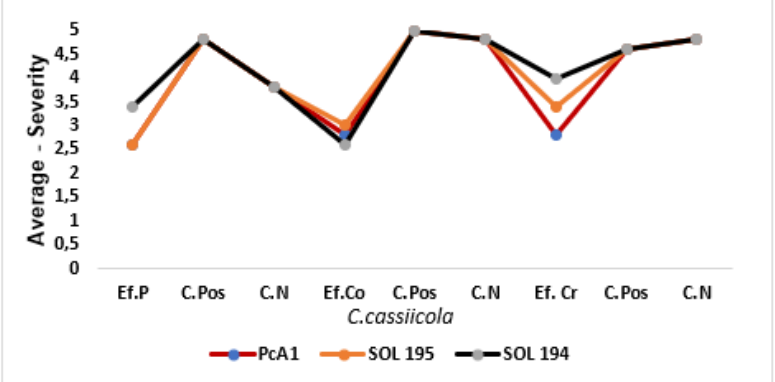


Source: Author (2022).

The *in plant* tests (Figure 6) indicated important situations, such as all experimental units and especially the negative control where plants that were not inoculated with the pathogen during the experiments were infected with *C. cassiicola* and present the symptoms of the target spot disease with greater severity than those treated with the putative biocontrolling bacteria. These results confirm the veracity of the studies of Pernezny and Simone (1999) and the research of MacKenzie *et al*. (2018) which state that the primary inoculation of *C. cassiicola* occurs by airborne conidia of the fungus. Another interesting result was that *B. amyloliquefaciens* maintained the lowest mean severity of the target spot when compared to the other bacteria evaluated in the three experiments and reduced by 56% the severity of the target spot disease in the tomato.

The results evaluated in *plant in* the antagonistic action against *C. cassiicola* besides being superior to those evaluated by Imran *et al*. (2022), contribute to the research of these authors because as the form of infection and the manifestation of the disease of the fungus *Alternaria solani* are similar to the fungus *Corynespora cassiicola*, in the studies of these authors the *B. amyloliquefaciens* reduced by 36.58% the severity of the disease caused by *Alternaria solani* and in this study it is emphasized that the efficiency of control of the target spot performed by *B. amyloliquefaciens* was 56%.

Figure 10 – Analysis of the severity of the target spot in *plant* caused by *C. cassiicola* for 35 days in greenhouse against bacterial isolates: *Bacillus amyloliquefaciens* (Pc A1); *Microbacterium resistens* (Sol 194) and *Stenotrophomonas maltophilia* (Sol 195).



Source: Author (2022).

7 CONCLUSIONS

1- *In* vitro, *Bacillus amyloliquefaciens* maintained a progressive increase in growth inhibition of C. cassiicola until the last day of analysis, with 75.73% inhibition. Showing to be the most efficient in antagonistic tests against *C. cassiicola.*

2- *Stenotrophomonas maltophilia* and *Bacillus amyloliquefaciens* were the most efficient in reducing the severity of the target spot in tomato plants, when used preventively. The isolate SOL 194 showed greater efficiency in the control of the target mancha, when evaluated in the co-inoculation. And the PcA1 isolate was also the most efficient in reducing the severity of the disease when inoculated after the disease established in the plant (curative effect).

3- How is the severity of the target spot evaluated by the development of the necrotic halo in the leaflet of the plant and for this it is important to use images, so thereis a real need to implement a digital monitoring system with real-time image and that this system is interconnected to the world communication network so that not only the producer Be able to manage with greater knowledge of the agents interfering with your cultivation as you can have greater precision for decision making regarding the proper use of biocontrols.

Therefore, the results obtained indicate that *Bacillus amyloliquefaciens* (Pc A1) is efficient to count *C. cassiicola* and has potential for biocontrol ofthe target spot. However, noresearch needs to be carried out over a longer period than these studies. However, there are prospects of elucidating the formulation of a bioproduct from metabolites from this *Bacillus*, as well as the propossibility of developing a biological product that satisfactorily meets the control of the target spot so that nightshade farmers can succeed in their productions without the need for the use of pesticides.

ACKNOWLEDGMENT

The authors thank the Foundation for Research Support of the State of Amazonas – FAPEAM, the Coordination of Superior Level Staff Improvement – CAPES, the National Council for Scientific and Technological Development – CNPQ, Embrapa Western Amazon, the National Institute of Research of the Amazon, the State Secretariat of Health of Amazonas. All for the immeasurable support and commitment to carry out this scientific research.

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