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Article

Antagonism of *Streptomyces* spp. on *Ralstonia solanacearum* causing bacterial wilt in potatoes

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Abstract

Bacterial wilt is a disease that affects the crops of potato, *Solanum tuberosum*, and is caused by *Ralstonia solanacearum*, which generates a decrease in productivity and economic losses for farmers. The objective was to evaluate the antagonistic activity of *Streptomyces* spp. against *R. solanacearum*, which causes bacterial wilt in *S. tuberosum*. A descriptive cross-sectional study was conducted, where seeding by depletion and streaking were used for bacterial isolates of tuber and soil samples. Antagonistic activity was assessed by perpendicular streaks. A total of 28 strains of *R. solanacearum* and 50 of *Streptomyces* spp. were isolated, and 22% of them inhibited the growth of the phytopathogenic bacterium. The results of the analysis of variance (Anova) showed a significant inhibitory effect of *Streptomyces* against *R. solanacearum*. For its part, Tukey's test revealed differences between treatments. Therefore, *Streptomyces* spp. has the potential to be used as biocontrol agents, aspects that should be evaluated in experiments with potato plants.

Palabras clave:

Ralstonia solanacearum, *Streptomyces* spp., antagonism, bacterial wilt.

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Introduction

Potatoes (*Solanum tuberosum* L.) are considered the third most significant crop in the world, with approximately 20 million hectares distributed in 150 countries, among which China, India, Russia, the United States of America, Poland, and Ukraine stand out (FAO, 2022). Peru ranks twelfth (MIDAGRI, 2022), with the departments with the highest production being Huánuco, Cusco, Huancavelica, Puno, Cajamarca, and Junín. The crop is exposed to several serious diseases, including bacterial wilt caused by *Ralstonia solanacearum* (Khairy *et al* ., 2021). It causes destruction in tomato (*Solanum lycopersicum*), peanut (*Arachis hypogaea*), eggplant (*Solanum melongena*), potato, and banana (*Musa paradisiaca*) crops. The pathogen infects more than 1.5 million hectares, generating a decrease in productivity and economic losses (Wang *et al* ., 2019).

Its spread is due to its easy adaptability to different climates and ability to subsist in water and soil, decreasing up to 89% of yield in Mexico (Rueda *et al* ., 2018). The elimination of *R. solanacearum* has been difficult due to the persistence of the bacteria in the soil, the use of seed without certification, and inefficient chemical control. A previous research assessed the antagonism of actinomycetes isolated from compost against potato phytopathogens, where the genus *Streptomyces* inhibited the growth of *R. solanacearum* by 23.5%, which allowed the authors to affirm that they are possible candidates to biologically control bacterial wilt (Pérez *et al* ., 2015).

The antagonistic activity of *Streptomyces* spp. is due to the production of secondary metabolites that affect the growth of pathogens, but as they are also colonizing organisms of the rhizosphere, they release inhibitory substances, such as bacteriocins, siderophores, and organic acids (Khan *et al* ., 2023). In addition, they are the largest producers of antimicrobial compounds that are used as biocontrol agents for plant diseases caused by bacteria and fungi (Kumar *et al* ., 2021). The objective of this research was to assess the antagonistic activity of *Streptomyces* spp. isolates against *R. solanacearum* causing bacterial wilt in *S. tuberosum*.

Materials and methods

Ralstonia solanacearum isolate

The potato samples were collected from crops in the communities of San José and Congona, province of Cutervo. A total of 100 tubers with symptoms were collected, which were placed in Ziploc bags and transported to the laboratory, where the recommendation by Fornos *et al* . (2021) was followed., which consisted of washing five times with distilled water and then disinfecting with 70% alcohol. Subsequently, a cut was made in the tuber with a scalpel and an aliquot of the bacterial exudate was obtained from the affected area and seeded in plates with tetrazolium chloride (TZC) agar to be incubated at 30 °C for 48 h. The colonies of *R. solanacearum* were irregularly shaped, red, with white edges. Identification was made on the basis of morphophysiological characteristics (Phondekar *et al* ., 2020) by Gram staining, tests of 3% potassium hydroxide, catalase, oxidase, citrate, triple iron sugar (TSI), lysine iron (LIA) and motility, indole and ornithine (MIO) (Figure 1).

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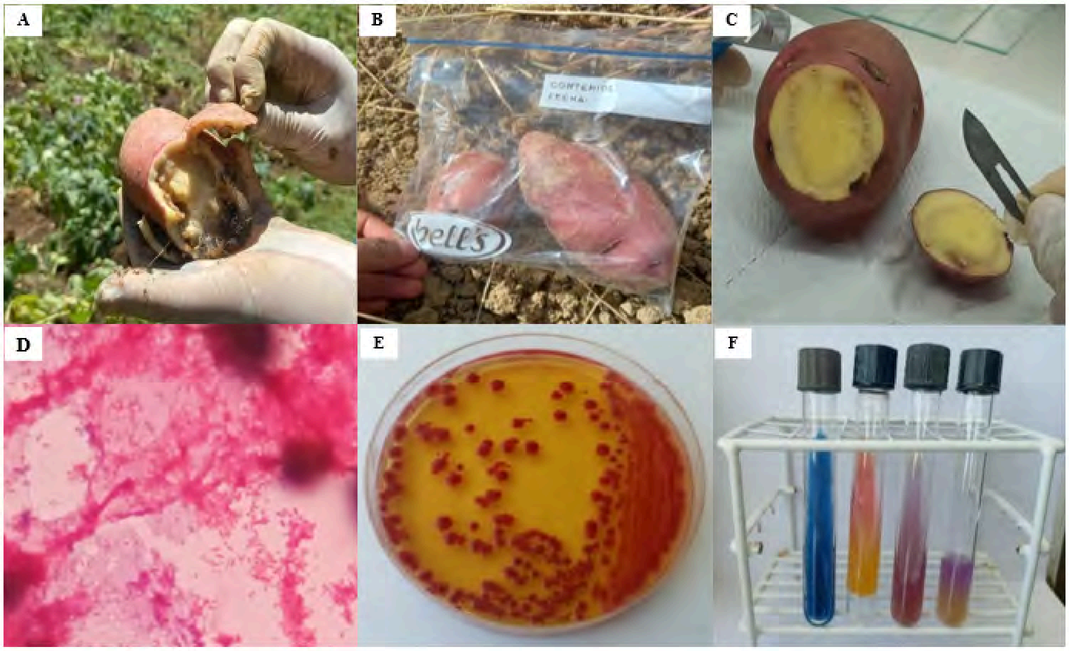
Figure 1. Isolation and identification of *R. solanacearum*. A and B) selection and collection of tubers; C) extraction of vascular ring flow; D) Gram-negative bacilli of vascular flow; E) colony-forming units in TZC and F) biochemical reaction.



Isolation of *Streptomyces* spp.

A total of 95 samples of potato cultivation soil were collected at a depth of 2 to 8 cm and deposited (5 to 10 g) in Ziploc bags. They were then processed according to the recommendations by Parada *et al* . (2017), making serial dilutions until obtaining 1x10^{-3} g ml^{-1} and pasteurization at 70 °C for 15 min. From each dilution, 100 µl was sown in Petri dishes with oat agar, and they were incubated at 30 °C for 7 days. For the identification, the following macroscopic characteristics of the colonies were considered: type, size, pigment diffusion, and aerial and vegetative mycelium coloration. In addition, Gram staining, a technique of microculture, and fermentation of sugars were performed (Figure 2).

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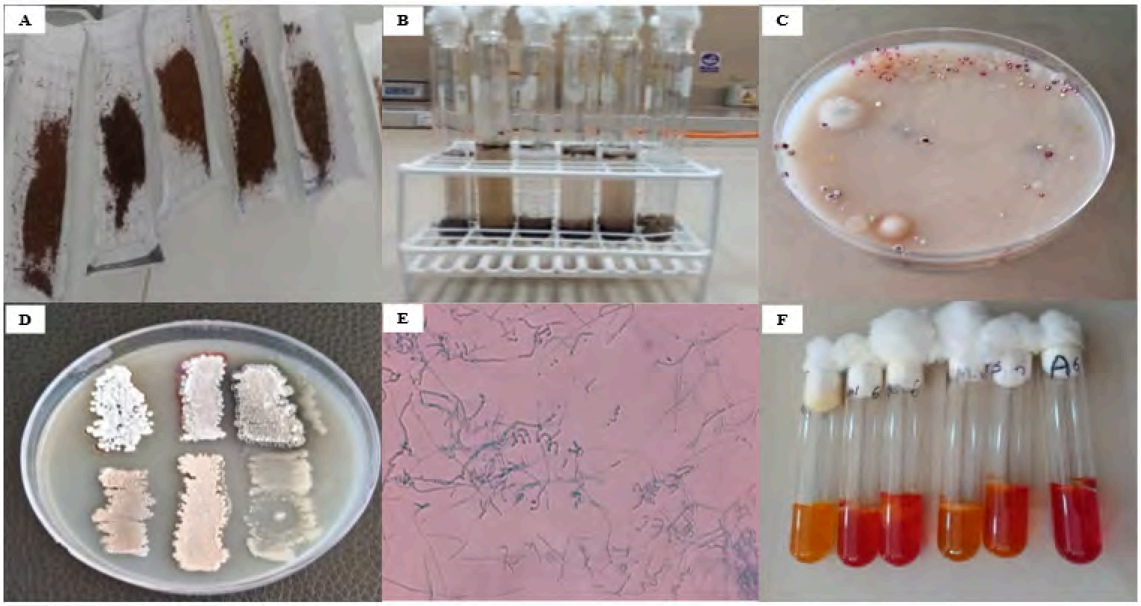
Figure 2. Isolation and identification of *Streptomyces* spp. A) soil samples; B) dilutions; C) colony-forming units in oat agar; D) macroscopic appearance; E) microscopic observation and F) fermentation of sugars.



Evaluation of antagonism

The perpendicular streak methodology described by Hossain and Rahmanll (2014) was used, which consisted of seeding by depletion and streaking the strain of *Streptomyces* spp. in the central part of a Petri dish containing Mueller-Hinton agar and incubating it at 30 °C for seven days. The *R. solanacearum* strains were then seeded perpendicularly and incubated at 30 °C for 48 h. The reading was performed by measuring the inhibition halo, considering positive antagonism when the streak of *R. solanacearum* began to grow at least 5 mm away from the streak of *Streptomyces* spp.

Experimental design and statistical analysis

The antagonistic effect was evaluated by applying an analysis of variance (Anova), adjusting it to the 50 x 28 x 3 factorial design to demonstrate the differences between the fifty isolates of *Streptomyces*, twenty-eight of *R. solanacearum*, and three replications, generating 4 200 experimental units. The statistical analysis of the differences between the inhibition means was complemented with Tukey's multiple comparisons test at 0.05% probability. To do this, the statistical software Statistica was used.

Results and discussion

In vitro isolation of *R. solanacearum*

The three potato varieties: chaucha, molinera, and unica, were obtained from the communities of San José and La Congona, province of Cutervo. Forty-five percent of isolates were Gram-negative bacilli, of which 28% were *R. solanacearum*, with most of the isolates, 47.4%, belonging to the molinera variety (Table 1).

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Table 1. Isolation characteristics of *R. solanacearum* in potato.

Variables	n	(%)
Communities		
La Congona	50	50
San José	50	50
Varieties		
Chaucha	30	30
Molinera	35	35
Única	35	35
Gram staining		
Gram-negative bacillus	45	45
Not observed	55	55
Culture		
Positive	28	28
Negative	72	72
Isolates		
Chaucha	6	15.8
Molinera	9	36.8
Única	13	47.4

There are reports of isolates of *R. solanacearum* from pseudostems of banana, eggplant, and tomato (Ling *et al* ., 2020; Kaari *et al* ., 2022). Where nightshades are predisposed to substrates such as glutamine, asparagine, amino acids and sugars, which promote bacterial growth (Baroukh *et al* ., 2022). On the other hand, Vargas *et al* . (2023) state that the presence of R genes helps to recognize pathogenic proteins. In addition, a high transpiration rate and structure of the xylem favors the spread of the bacterium (Mamani, 2015).

The soil microbiota, as long as it is in balance, will prevent pathogens from reaching levels sufficient to cause diseases (Zhang *et al* ., 2022). However, the use of pesticides, nitrogen fertilizers, and phosphorus and potassium deficiency reduce beneficial microorganisms (Cao *et al* ., 2022). *Ralstonia*, on the other hand, spreads rapidly in ferralitic soils at pH between 5 and 7 and at temperatures between 25 and 35 °C. In addition, it uses virulence factors, such as type III secretion system (T3SS), motility, biofilms, and exopolysaccharides (EPS) to infect plants and obstruct xylem (Vailleau and Genin, 2023). Thus, it manipulates the metabolism of the host plant, where galacturonic acid and salicylic acid, released by the cell walls of the plants, are used for nutrition. For its part, L-glutamic acid promotes the production of virulence factors (Shen *et al* ., 2020).

Isolation and *in vitro* evaluation of the antagonism of *Streptomyces* spp.

Of 95 soil samples, 50 strains of *Streptomyces* spp. were isolated, 34% were pigmented and 64% did not present this characteristic; in addition, 22% showed antagonism (Table 2). Figure 3 shows the positive antagonism of strain 10 of *Streptomyces* spp., which managed to inhibit strains 16, 17, 19 and 26 of *R. solanacearum*.

Table 2. Characteristics of the isolate of *Streptomyces* spp. from soil of potato crops.

Variables	n	(%)
Communities		
La Congona	45	47.4
San José	50	52.6
Macroscopic characteristics		

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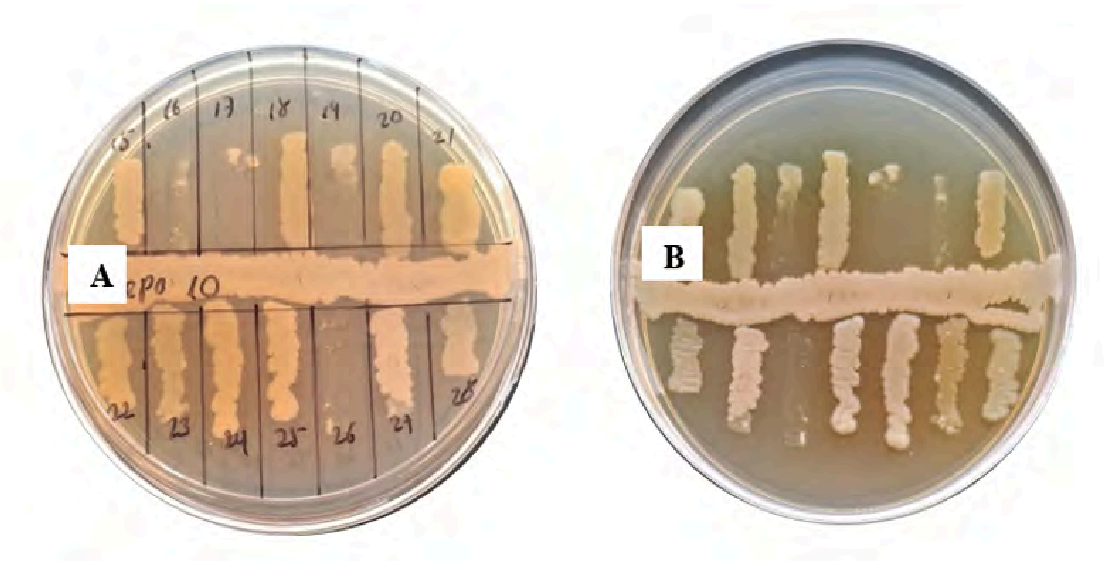
Variables	n	(%)
Positive	50	52.6
Negative	45	47.4
Positive isolates		
With pigment	17	34
Without pigment	33	66
Antagonistic activity		
Positive	11	22
Negative	39	78

Figure 3. Positive and negative antagonism of strain 10 of *Streptomyces* spp. against *R. solanacearum* by perpendicular streaks on Mueller-Hinton agar: A) reverse view of the culture and B) obverse view of the culture.



In the research, it was possible to obtain up to five different isolates of *Streptomyces* spp. in a single sample, and in others, no strain was obtained. Nevertheless, Espinosa *et al* . (2021) indicate that the presence and diversity of the bacterium depend on nutrients, soil pH, microbial competitors, and biological and chemical activity of the soil. On the other hand, the production of diffusible pigment was variable, similar to the report by Antido and Climacosa (2022), where 14 strains were gray, yellow and pink, which is attributed to synthesized pigments, such as melanin, actinorhodin, prodigiosin, carotenoids and polyketide compounds, whose production depends on environmental conditions, metabolism, and bacterial genetics (Abraham and Chauhan, 2018).

Twenty-two percent of *Streptomyces* spp. demonstrated antimicrobial activity, with strains 21, 10 and 16 standing out for their greater antagonism. Similar findings were reported by Zaki *et al* . (2022); Kaari *et al* . (2022), they obtained 9 and 4 antagonistic strains, respectively. Nonetheless, there is a difference in the radius of action as it is smaller than those reported by Ling *et al* . (2020), who obtained an inhibition zone of 30.5 and 32.8 mm. This difference depends on the interaction of factors, including genetic diversity, synthesis of peroxidase that degrades cell wall components and antibiotic production.

The antagonistic activity was evaluated through an analysis of variance, Anova, finding statistically significant differences between the treatments of the 50 strains of *Streptomyces* and 28 of *R. solanacearum*, as well as their interactions, where the *p*-value (0) demonstrates that there is antagonistic ability (Table 3).

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Table 3. Analysis of variance (Anova) of the antagonistic activity of *Streptomyces* spp. on *R. solanacearum*.

Categories	Sum of squares	Degrees of freedom	Mean square	F	p
Streptomyces	3 190.46	49	65.111	403.5	0
R. solanacearum	540.72	27	20.027	124.1	0
Streptomyces * *Ralstonia*	16 670.56	1 323	12.601	78.09	0
Error	451.83	2 800	0.161		

According to Tukey's discriminative test performed on the different treatments, it was shown that the antagonism averages of the strains S24, S47, S20, S39, S30, S33, S43, S18, S10, S16, and S21 of *Streptomyces* spp. showed different antagonistic activity. Strains of *Streptomyces* sp. (S24 and S47) showed a lower inhibition spectrum compared to strain 21 of *Streptomyces* sp. (Table 4).

Table 4. Tukey's multiple comparison test (0.05) for the variable of *Streptomyces* spp. with positive antagonism.

Strains of *Streptomyces* spp.	Inhibition	Significance						
S24	1.488095	b						
S47	1.636905	b						
S20	1.928571		c					
S39	2.083333		c	d				
S30	2.202381			d	e			
S33	2.380952				e			
S43	2.684524					f		
S18	3.071429						g	
S10	3.095238						g	
S16	3.273810						g	
S21	5.690476							h

The fact that *R. solanacearum* strains showed sensitivity to *Streptomyces* species indicates significant potential for biocontrol. However, it provided new information on strains with antagonistic spectrum with certain limitations, such as the variable effectiveness of the isolates. Therefore, more research is needed to understand the mechanisms of interaction and application in agricultural practices, generating ecological, sustainable, and productive agriculture.

Conclusions

We identified 28 strains of *R. solanacearum* from tuber samples and 50 *Streptomyces* spp. isolated from soil with crops of potato *S. tuberosum*. Twenty-two percent of *Streptomyces* spp. demonstrated antimicrobial activity, with the strains S21, S10, and S16 showing the greatest antagonism, which highlights their potential as biocontrol agents for pathogens, such as *R. solanacearum*. In this context, the basic scientific knowledge available on this phytopathogen has also been expanded as there are strains that are more sensitive to *Streptomyces* spp. under *in vitro* conditions.

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