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Evaluación de la actividad antimicrobiana de cepas de *Streptomyces* y *Penicillium* contra bacteria y hongos fitopatógenos de importacia en México

Evaluation of the antimicrobial activity of *Streptomyces* and *Penicillium* strains against phytopathogenic bacteria and fungi of importance in Mexico

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Resumen

Los pesticidas químicos constituyen la herramienta más utilizada para el control de plagas, sin embargo, su uso inadecuado y excesivo ha causado problemas para la salud humana y ambiental. Los géneros *Streptomyces* y *Penicillium* tiene un amplio potencial biotecnológico, siendo candidatos prometedores para su uso en el control biológico. El objetivo fue evaluar el efecto antimicrobiano de diferentes cepas de *Streptomyces* sp y una cepa de *Penicillium* frente a bacterias y hongos fitopatógenos de importancia económica en México. Se probaron cuatro cepas de *Streptomyces* y *Pencillium chrysogenum* R1 autóctonas de la región de Sonora contra seis cepas de bacterias fitopatógenas y cuatro cepas de hongos fitopatógenos. La actividad antagonista de *Streptomyces* sp frente a las cepas fitopatógenas bacterianas se evaluó por el método de doble capa. Mientras que el efecto antagónico del crecimiento de hongos fitopatógenos frente a *P. chrysogenum* R1 se evaluó por confrontación. Las cepas *S. hirsutus* SB03 y *S. nogalater* SB24 mostraron inhibición del crecimiento bacteriano frente a *E. amylovora* AT3 y *X. campestri* Xhcp AT6, siendo la inhibición más significativa frente a esta última (5 y 3 mm, respectivamente). En el ensayo antifúngico, las 4 cepas de *Streptomyces* mostraron inhibición en el crecimiento de *F. oxysporum* Fsox C11 y *R. solani* Rzsn C31. En el caso de *P. chrysogenum* R1 se observaron áreas claras de inhibición del crecimiento radial de los 5 hongos fitopatógenos. El efecto antagónico más significativo (81%) se observó en el caso de *Fusarium oxysporum*. Comparando los resultados obtenidos para las cepas de diferentes géneros y especies, se observó que la mejor actividad antimicrobiana de amplio espectro contra hongos fitopatógenos de alto impacto económico la presentó *P. chrysogenum* R1.

Abstract

Chemical pesticides are the most used tool for pest control. However, their inappropriate and excessive use has caused problems for human and environmental

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health. The genera *Streptomyces* and *Penicillium* have broad biotechnological potential, being promising candidates for use in biological control. The objective was to evaluate the antimicrobial effect of different strains of *Streptomyces* sp and a strain of *Penicillium* against phytopathogenic bacteria and fungi and their economic importance in Mexico. Four strains of *Streptomyces* and *Penicillium chrysogenum* R1 native to the Sonora region were tested against six phytopathogenic bacteria strains and four phytopathogenic fungi strains. The antagonistic activity of *Streptomyces* sp against bacterial phytopathogenic strains was evaluated by the double-layer method. The antagonistic effect of the growth of phytopathogenic fungi against *P. chrysogenum* R1 is evaluated by confrontation. The strains *S. hirsutus* SB03 and S. nogalater SB24 showed inhibition of bacterial growth against *E. amylovora* AT3 and *X. campestri* Xhcp AT6, with the most significant inhibition against the latter (5 and 3 mm, respectively). In the antifungal assay, the 4 *Streptomyces* strains showed inhibition in the growth of *F. oxysporum* Fsox C11 and *R. solani* Rzsn C31. In the case of *P. chrysogenum* R1, clear areas of inhibition of radial growth of the five phytopathogenic fungi were observed. The most significant antagonistic effect (81%) was demonstrated in the case of *Fusarium oxysporum*. Comparing the results obtained for the strains of different genera and species, it was shown that the best broad-spectrum antimicrobial activity against phytopathogenic fungi of high economic impact was presented by *P. chrysogenum* R1.

Keywords: *Penicillium*, antimicrobial, biological control, *Streptomyces*, antagonist.

Introduction

Chemical pesticides are the most common pest management tool; however, their inappropriate and excessive use has caused problems for human and environmental health. The use of microorganisms and their metabolites is an alternative to agrochemicals for disease control, sothey have received particular attention (Syed Ab Rahman et al., 2018).

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Streptomyces and *Penicillium* are potent antagonists of phytopathogenic bacteria and fungi (Anitha & Rabeeth, 2009; Bajaj et al., 2014; Bolívar-Anillo et al., 2020; Manigundan, K., Joseph, J., Ayswarya, S., Vignesh, A., Vijayalakshmi, G., Soytong, K., Gopikrishnan, V.1* and Radhakrishnan, 2020). Some strains of *Streptomyces* have been reported as biocontrollers of phytopathogenic bacteria and fungi, such as *Erwinia carotovora* (Doolotkeldieva et al., 2016), *Ralstonia solanacearum* (Wattana-Amorn et al., 2016), *Colletotrichum dematium* (Song et al., 2020) and *Pyricularia oryzae* (Law et al., 2017).

Fungi of the genus *Penicillium* produce several biologically active compounds, such as antibiotics and hydrolytic enzymes, and have a wide range of fungicidal actions (Karpova et al., 2021). Other authors reported the biocontrol potential of *Penicillium* strains against phytopathogens of economic importance, such as *Fusarium graminearum* (Karpova et al., 2021), *F. oxysporum* f. sp. *lycopersici* (Sabuquillo et al., 2006), and the lepidopteran *Helicoverpa armigera* (Patil & Jadhav, 2015).

The study aimed to evaluate the antimicrobial activity of four strains of *Streptomyces* sp. and one of *Penicillium* sp. against phytopathogenic bacteria and fungi of economic importance in Mexico**.**

Materials and methods

Microorganisms: Culture media and growth conditions

The strains used were *Streptomyces violaceoruber* SA02, *Streptomyces hirsutus* SB03, *Streptomyces chartreusis* SA04, *Streptomyces nogalater* SB24, and *Penicillium chrysogenum* R1, from the microorganism collection of the Research and Technical Development Department of Greencorp, Mexico. Isolates of *Streptomyces* strains were routinely cultured on Trypticasein Soy Agar (ATS) pH 7 and incubated at 28 °C in the dark for 7 days. Bacterial pathogens were regularly grown in ATS with pH 7 and incubated at 28 °C for 3 days in the dark. Phytopathogenic fungi were

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routinely cultured on Potato Dextrose Agar (APD) with pH 7 and incubated at 28 °C for 7 days.

Antagonistic activity against phytopathogenic bacteria

The double-layer method was used to evaluate the antagonistic activity against strains of phytopathogenic bacteria with slight modifications (Ubillus et al., 2015). A micro drop (10 µL) of cell suspension (10^6 cells/mL) from *Streptomyces* strain was previously placed in ATS and incubated at 28 °C for 7 days. The agar overlay (top layer) was prepared by mixing 400 µL of the phytobacterial culture (10^6 cells/mL) in 4 mL of soft ATS (0.6% agar) and poured onto the Petri dishes containing the *Streptomyces* strains. . The same procedure was performed for the control group in Petri dishes with ATS without actinobacteria. All cultures were incubated for 24h at 28 °C. Once the second layer of agar had solidified, the plates were incubated for 24 hours at 28 °C. Then, the corresponding readings and measurement of the size of the halos of antagonistic activity were carried out. The antagonistic activity was determined by measuring the radius of the zone of inhibition of the phytopathogenic bacteria from the center of the disk of each actinobacteria to the edge of inhibition. Data were expressed as the diameter of the zone of inhibition. All actinobacterial strains and the control were performed in triplicate.

Antagonistic activity against phytopathogenic fungi

The antagonistic effect on the strain of the phytopathogenic fungus was the confrontation using the method proposed by Loc et al. (2020) with slight modifications. First, the 4 strains of *Streptomyces* spp and the *P. chrysogenum* R1 strain were inoculated on PDA plates. Then, a 3-day-old mycelium explant of each phytopathogenic fungus was placed on each PDA plate. The plates were incubated for 7 days at 28°C. The antagonistic activity was determined by measuring the growth of the phytopathogenic fungus. All experiments were carried out in triplicate.

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The percentage of inhibition of the phytopathogen was determined based on the controls' growth of the mycelial radius (RI). The RI will be calculated using the following equation (1):

%I =100 * (Rc-Ri)/Rc……..(1)

Where: Ri is the mycelial growth radius of the pathogen, measured in the direction of the inoculum towards the antagonist. Rc is the radius of the mycelial growth of the pathogen, measured in the direction of the maximum radius without the presence of the antagonist.

Experimental design and statistical analysis

A completely randomized experimental design was used where each strain corresponded to a treatment. Response variables of diameter and radius of inhibition were analyzed by one-way analysis of variance (ANOVA) and a comparison of means with Tukey using Statistica 7.

Results and discussions

Antagonistic activity of the studied strains

Table 1 shows the growth response of phytopathogenic bacteria and fungi in the presence of the strains subjected to the antagonistic activity study.

Table 1: Qualitative response in the growth of phytopathogenic bacteria in the presence of the strains subjected to the antagonistic activity study: "+" - evidence of inhibition, "-" - without inhibitory effect. (The radius of the growth inhibition zone is presented in parentheses).

	***S. hirsutus* SB03**	***S. nogalater* SB24**	***S. violaceoruber* SA02**	***S. chartreusis* SA04**	***P. chrysogenum* R1**

C. michiganensis Cvmhm AT2	-	-	-	-	-
E. amylovora Ewam AT3	+	+ (2 mm)	-	+ (2 mm)	-
P. syringae Pssy AT4	-	-	-	-	-
X. axonopodis Xhao AT5	-	-	-	-	-
X. campestri Xhcp AT6	+	+ (5 mm)	-	+ (3 mm)	-
P. cerotovorum Pecv AT7	-	-	-	-	-
F. oxysporum Fsox C11	+ (6.19 mm)	+ (40.76 mm)	+ (8.65 mm)	+ (9.87 mm)	+ (81.12 mm)
R. solani Rzsn C31	+ (8.12mm)	+ (22.60 mm)	+ (24.16 mm)	+ (11.25 mm)	+ (25.73 mm)
Phytophthora sp. NSN Phy	-	-	-	-	+ (75.67 mm)
B. cinerea Bter GM3	-	-	-	-	+ (56.82 mm)
C. gloeosporioides Clge C09	-	-	-	-	+ (59.25mm)

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Only the *S. hirsutus* SB03 and *S. nogalater* SB24 strains showed inhibition of bacterial growth against *E. amylovora* AT3 and *X. campestri* Xhcp AT6, with the most significant inhibition against the latter (5 and 3 mm, respectively). *S. violaceoruber* SA02 and *S. chartreusis* SA04 did not show antibacterial activity (**Table 1**).

Streptomyces produces a broad spectrum of antibiotics and volatile organic compounds (VOCs), which act against pathogens by interrupting communication between bacterial cells (quorum sensing) and a variety of enzymes that degrade the fungal cell wall (Song et al., 2020; Wonglom et al., 2019).

In the fungal assay (**Table 1**), the 4 *Streptomyces* strains tested showed inhibition in the growth of *F. oxysporum* Fsox C11 and *R. solani* Rzsn C31. However, for *P. chrysogenum* R1, clear areas of inhibition of radial growth of the 5 phytopathogenic fungi were observed. The most significant antagonistic effect (81%) was observed in the case of Fusarium oxysporum with *P. chrysogenum* R1. The formation of a zone of inhibition indicates the production of antifungal substances by antagonistic fungi against the pathogen. On agar plates, *P. chrysogenum* R1 can secrete hydrolytic enzymes that affect the cell wall of plant pathogens, which plays an essential role in restricting the growth of pathogens (Zhao et al., 2021).

The genus *Penicillium* can inhabit the soil and includes many species with the potential for the biological control of plant diseases. Several investigators have previously reported the antifungal activity of *Penicillium* as an antagonist of F. oxysporum. Karpova et al. (2021) reported that *P. chrysogenum* VKPM F-1310 inhibited the radial growth of Fusarium oxysporum (55%), which is comparable to those obtained in this study. Grigorcea et al. (2021) reported an effective control of *Fusarium oxysporum* var. orthoceras with *Penicillium* chrysogenum. *Penicillium* bilaiae 47M-1 inhibited the growth of *F. oxysporum* by 81.3% (Zhao et al., 2021). *P. oxalicum* significantly reduced tomato wilt caused by *Fusarium* spp. under greenhouse conditions (Sabuquillo et al., 2006). *Penicillium* sp. PNF2 can improve the damage caused by *Fusarium* sp. 40,240 in sesame crops and is a plant growth-promoting fungus (Manigundan et al., 2020).

Comparing the results obtained for the strains of different genera and species, it was observed that the best broad-spectrum antimicrobial activity against phytopathogenic fungi of high economic impact was presented by *P. chrysogenum* R1. The evaluation of the antimicrobial activity of four *Streptomyces* sp. and one of *P. chrysogenum* R1 against phytopathogenic bacteria and fungi showed that microorganisms even belonging to the same genus differ in antimicrobial activity, and some lack it.

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Reference

Anitha, A. & Rabeeth, M. (2009). Control of *Fusarium* wilt of tomato by bioformulation of *Streptomyces griseus* in green house condition. *African Journal of Basic & Applied Sciences*, *1*(2): 9–14.

Bajaj, I., Veiga, T., Van Dissel, D., Pronk, J. T. & Daran, J. M. (2014). Functional characterization of a *Penicillium chrysogenum* mutanase gene induced upon co-cultivation with *Bacillus subtilis*. *BMC Microbiology*, *14*(1): 1–16. https://doi.org/10.1186/1471-2180-14-114

Bolívar-Anillo, H. J., Garrido, C. & Collado, I. G. (2020). Endophytic microorganisms for biocontrol of the phytopathogenic fungus *Botrytis cinerea*. *Phytochemistry Reviews*, *19*(3): 721–740. https://doi.org/10.1007/s11101-019-09603-5

Doolotkeldieva, T., Bobusheva, S. & Suleymankisi, A. (2016). Biological control of *Erwinia carotovora* ssp. carotovora by *Streptomyces* species. *Advances in Microbiology*, *06*(02): 104–114. https://doi.org/10.4236/aim.2016.62011

Karpova, N. V., Yaderets, V. V., Glagoleva, E. V., Petrova, K. S., Ovchinnikov, A. I. & Dzhavakhiya, V. V. (2021). Antifungal activity of the dry biomass of P*enicillium chrysogenum* f-24-28 and is application in combination with azoxystrobin for efficient crop protection. *Agriculture (Switzerland)*, *11*(10): 935 https://doi.org/10.3390/agriculture11100935

Law, J. W. F., Ser, H. L., Khan, T. M., Chuah, L. H., Pusparajah, P., Chan, K. G., Goh, B. H. & Lee, L. H. (2017). The potential of Streptomyces as biocontrol agents against the rice blast fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). *Frontiers in Microbiology*, *8*(JAN). https://doi.org/10.3389/fmicb.2017.00003

Loc, N. H., Huy, N. D., Quang, H. T., Lan, T. T. & Thu Ha, T. T. (2020). Characterisation and antifungal activity of extracellular chitinase from a biocontrol fungus, *Trichoderma asperellum* PQ34. *Mycology*, *11*(1): 38–48. https://doi.org/10.1080/21501203.2019.1703839

Manigundan, K., Joseph, J., Ayswarya, S., Vignesh, A., Vijayalakshmi, G., Soytong, K., Gopikrishnan, V. & Radhakrishnan, M. (2020). Identification of biostimulant and microbicide compounds from *Streptomyces* sp . UC1A-3 for plant growth promotion and disease control M aterials and m ethods Sample collection Rhizosphere soils were collected from *Capsicum annum* (Chilli) agricultural fi. *International Journal of Agricultural Technology*, *16*: 1125–1144.

Patil, N. S. & Jadhav, J. P. (2015). Significance of *Penicillium ochrochloron* chitinase as a biocontrol agent against pest Helicoverpa armigera. *Chemosphere*, *128*: 231–235. https://doi.org/10.1016/j.chemosphere.2015.01.038

Sabuquillo, P., Cal, A. D. & Melgarejo, P. (2006). Biocontrol of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. *Biological Control*, *37*(3): 256-265. https://doi.org/10.1016/j.biocontrol.2006.02.009

Grigorcea, S., Nedbaliuc, B., Chiriac, E., Aluchi ,N. & Grigorcea, D. (2021). Determination of the particularities of growth and development in different temperature conditions of micromicets *Alternaria* spp., *Fusarium* spp. and Botrytis spp. in dual culture with *Penicillium* spp. *Acta et Commentationes Exact and Natural Sciences*, *1*(11): 138–145. https://doi.org/10.36120/2587-3644.v11i1.138-145

Song, L., Jiang, N., Wei, S., Lan, Z. & Pan, L. (2020). Isolation, screening, and identification of actinomycetes with antifungal and enzyme activity assays against *Colletotrichum dematium* of *Sarcandra glabra. Mycobiology*, *48*(1): 37–43. https://doi.org/10.1080/12298093.2020.1716604

Syed Ab Rahman, S. F., Singh, E., Pieterse, C. M. J. & Schenk, P. M. (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Science*, *267*: 102–111. https://doi.org/10.1016/j.plantsci.2017.11.012

Ubillus, J. A., Quispe, J. L., Durán, R. R., Trujillo, S. M. & Salazar, L. L. (2015). Actividad antimicrobiana y sinérgica de metabolitos producidos por. *Revista de La Sociedad Venezolana de Microbiología*, *35*: 13–19.

Wattana-Amorn, P., Charoenwongsa, W., Williams, C., Crump, M. P. & Apichaisataienchote, B. (2016). Antibacterial activity of cyclo(L-Pro-L-Tyr) and cyclo(D-Pro-L-Tyr) from Streptomyces sp. strain 22-4 against phytopathogenic bacteria. *Natural Product Research*, *30*(17): 1980–1983. https://doi.org/10.1080/14786419.2015.1095747

Wonglom, P., Suwannarach, N., Lumyong, S., Ito, S., Matsui, K. & Sunpapao, A.

(2019). Streptomyces angustmyceticus NR8-2 as a potential microorganism for the biological control of leaf spots of Brassica rapa subsp. pekinensis caused by *Colletotrichum* sp. and *Curvularia lunata*. *Biological Control*, *138*(July): 104046. https://doi.org/10.1016/j.biocontrol.2019.104046

Zhao, X., Ni, Y., Zhao, H., Liu, X., He, B., Shi, B., Ma, Q. & Liu, H. (2021). Plant growth-promoting ability and control efficacy of *Penicillium aurantiogriseum* 44M-3 against sesame *Fusarium* wilt disease. *Biocontrol Science and Technology*, *31*(12): 1314–1329. https://doi.org/10.1080/09583157.2021.1946011