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ANTIFUNGAL ACTIVITY OF VOLATILE ORGANIC COMPOUNDS FROM SOIL-DERIVED ACTINOMYCETE ISOLATES AGAINST *FUSARIUM OXYSPORUM* AND *RHIZOCTONIA SOLANI*

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Introduction

Phytopathogenic fungi, in particular the wilt and root rot diseases caused by representatives of the genera *Fusarium* and *Rhizoctonia*, are inflicting substantial economic damage on agricultural crops worldwide. Since the use of chemical fungicides against these pathogens gives rise to environmental problems and leads to the development of resistance in the pathogens, interest in developing biological control agents based on soil microorganisms, and in particular on actinomycetes (especially members of the genus *Streptomyces*), has been growing in recent years.

Actinomycetes are known as a rich source of secondary metabolites, among which volatile organic compounds (VOCs) are of particular importance. Certain VOCs produced by actinomycetes possess the ability to inhibit the mycelial growth and development of phytopathogenic fungi.

The aim of the study was to evaluate the antifungal activity of volatile organic compounds produced by soil-derived actinomycete isolates against *Fusarium oxysporum* and *Rhizoctonia solani*.

Materials and methods

For this study, four isolates that had exhibited the highest activity — *Streptomyces* sp. 2/1, *Streptomyces* sp. 3, *Streptomyces* sp. 7 and *Streptomyces* sp. S4 — were selected from among soil actinomycetes whose antagonistic properties towards phytopathogenic fungi had been assessed in earlier experiments. In the experiments carried out for this selection, the actinomycete isolates were streaked onto the nutrient medium and agar–mycelium blocks of the phytopathogenic fungi were placed opposite them, and antagonistic activity was assessed on the basis of the degree of inhibition of the pathogens' mycelial growth. The strains *Fusarium oxysporum* 21 and *Rhizoctonia solani* 38, isolated in our previous study from diseased potato tubers during the storage period, were used as test pathogens.

The antifungal activity of the volatile organic compounds (VOCs) of the actinomycete isolates was evaluated by the double dish (sealed plate) method. Potato dextrose agar (PDA) medium was poured into both Petri dishes. An agar–mycelium disc 5 mm in diameter, taken from an actively growing culture of the pathogen, was placed in the centre of one dish, while the actinomycete isolate was streaked onto the second dish. The lids were then removed and the bases of the dishes were placed face to face and sealed tightly with parafilm, yet without coming into direct contact with each other. The paired dishes were incubated at 25 °C for 7 days. In the control variant, the dish inoculated with the pathogen was paired with a dish containing only sterile PDA medium, without any actinomycete.

Inoculation of the actinomycete was performed according to two schemes: (1) simultaneous inoculation — the actinomycete and the pathogen were inoculated at the same time and the dishes

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were paired immediately (isolate *Streptomyces* sp. 3 was tested under this scheme); (2) pre-incubation — the actinomycete was first grown separately for 2 days and then paired with a freshly inoculated pathogen dish (isolates *Streptomyces* sp. 2/1, S4 and 7 were tested under this scheme). Isolate *Streptomyces* sp. 2/1 was additionally tested under the simultaneous inoculation scheme as well; in this case the growth of strain *R. solani* 38 was measured on day 3 (when the control had reached 35 mm).

After 7 days of incubation (on day 3 for the simultaneous variant of *Streptomyces* sp. 2/1), the diameter of the pathogen colony was measured and compared with that of the control variant. The degree of inhibition of mycelial growth was calculated using the following formula:

$$Inhibition\ (\%) = [(Dc - Dt) / Dc] \times 100$$

where: Dc — diameter of the pathogen in the control dish (mm); Dt — diameter of the pathogen in the experimental dish (mm).

Results

After seven days of incubation, the colony diameter of strain *F. oxysporum* 21 in the control variant was 35 mm. Under the influence of isolate *Streptomyces* sp. 7, the pathogen colony diameter decreased to 20 mm, and 42.86% inhibition was recorded. Under the influence of isolates *Streptomyces* sp. 3 and 2/1, the colony diameter was 25 mm, and 28.57% antifungal activity was observed for both isolates. Under the influence of isolate *Streptomyces* sp. S4, however, no inhibition of the growth of strain *F. oxysporum* 21 was observed.

In the experiment conducted with strain *R. solani* 38, the diameter of the control colony was 45 mm. Under the influence of *Streptomyces* sp. 3, the colony diameter decreased to 35 mm and 22.22% inhibition was determined. Under the influence of isolates *Streptomyces* sp. 2/1 and *Streptomyces* sp. 7, which had been pre-grown for two days, the colony diameter of strain *R. solani* 38 was 40 mm, and 11.11% inhibition was recorded. In the case of isolate *Streptomyces* sp. S4, the growth of the pathogen colony did not differ from the control and no antifungal activity was observed.

The activity of isolate *Streptomyces* sp. 2/1 against *R. solani* 38 was also evaluated under conditions of simultaneous inoculation. While on day 3 of the experiment the pathogen colony diameter in the control variant had reached 35 mm, under the influence of the VOCs of isolate *Streptomyces* sp. 2/1 its diameter was 15 mm, and 57.14% inhibition was recorded.

Conclusion

The results obtained showed that the volatile organic compounds produced by soil-derived actinomycete isolates possess the ability to inhibit the mycelial growth of strains *F. oxysporum* 21 and *R. solani* 38 under non-contact conditions. The highest activity against *F. oxysporum* 21 was exhibited by isolate *Streptomyces* sp. 7 (42.86%), and against *R. solani* 38 by isolate *Streptomyces* sp. 2/1 under simultaneous inoculation (57.14%). The results demonstrate that the VOC-mediated antifungal effect differs depending on the actinomycete isolate, the phytopathogen species and the inoculation conditions.

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