EFFECT OF ACTINOMYCETES OF STREPTOMYCES GENUS ISOLATED FROM POTATO CULTIVATION ON THE PATHOGENIC FUNGUS RHIZOCTONIA SOLANI

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Abstract

The investigations indicated strong antagonistic properties of the tested strains of Streptomyces spp. in relation to the Rhizoctonia solani. Actinomycetes isolated from potato cultivated under ecological farming system turned out to be particularly active. 60% of strains (tested on the ground with pH 7) isolated from this farming system inhibited the growth of the spawn of R. solani very strongly. Ecological system of potato farming was more favourable for the development of antagonistic actinomycetes in comparison with conventional farming system.

Key words: antagonism, Streptomyces spp., Rhizoctonia solani, potato, biological plant protection

Introduction

Rhizoctonia solani Kühn is considered the most serious of all fungi causing potato diseases, except for Phytophthora infestans (Mont.) de Bary (Walczak et al. 1999). This pathogen is especially arduous due to its poliphagic character and ability of surviving on organic wastes in the absence of a host. Rhizoctonia solani causes various potato ailments (tubers black scurf, sprout rot and base of stem decay) that can appear during the whole period of vegetation. The main sources of infection are soil and black scurf tubers, where the fungi survive as sclerotium which is a form of their resting spore (Banville et al. 1996, Kapsa 1996). Losses caused by rhizoctoniosis, depending on conditions favouring its development, range from several to a dozen or so percent (Kochman and Węgorek 1997) and even 50% (Häni et al. 1998).
Plant protection against *R. solani* is extremely difficult in ecological farms, where the farmers cannot use chemical agents against pathogens and potato pests. Using biological methods, as well as methods relating to the crop rotation, seems to be an interesting alternative in the plant protection against this pathogen.

There are potentially antagonistic microorganisms in soil which inhibit the growth of soil pathogens. Appearance of diseases is caused by the lack of proper conditions for their activity (Kwaśna 1987). Actinomycetes are one of the most important biotic groups of soil microflora. Their great importance results mainly from production of antibiotics and multiple enzymatic activity, confirmed by numerous investigations (Hunter-Cevera and Eveleigh 1990, Marcinowska and Bis 1998).

The aim of this experiment was to study antagonistic properties of actinomycetes of *Streptomyces* genus isolated from potato grown in the ecological and conventional systems in relation to *R. solani*. Strains of *Streptomyces* spp. coming from two investigated facilities – an ecological and conventional farm – were compared in respect with their inhibiting impact on the potential pathogen of potato.

**Material and methods**

Actinomycetes of the genus *Streptomyces* were taken from soil beyond roots as well as rhizosphere and rhizoplane of potato grown in two farming systems. Ecological and conventional systems differ in the way of protection of plants against diseases and fertilization used. Detailed characterization of agrotechny treatments applied in the compared farming systems was presented in the study of Breza-Boruta and Paluszak (2003). Potato ‘Aster’ was cultivated on lessive soils of the good rye soil agricultural suitability complex. 75 isolates of actinomycetes were isolated during vegetation period from each farming system: 30 strains from soil, 30 from rhizosphere and 15 from rhizoplane were isolated on Williams and Davies selected medium (1965). Totally 150 strains were used for testing. Diagnostic of actinomycetes was performed on the base of macro- and microscopic features according to Holt et al. (1994) and Hunter-Cevera and Eveleigh (1990).

The tested species of *R. solani* was isolated from infected roots of potato coming from the ecological farm. Taxonomic determination of the fungus was performed on the base of Gilman (1971) and Domsch and Gams (1972).

The method of simultaneous two cultures was used to estimate antagonistic features of investigated actinomycetes in relation to the tested fungus. Five-day-old cultures of *Streptomyces* spp. were used in the studies. They were inoculated with the method of line inoculation – with a line of 40 mm on King B medium. After seven days’ incubation a 72-hour-old culture of *R. solani* was added to each actinomycete. The mycelium was cut off from the edge with a sterilized corkborer in the shape of 8 mm diameter circles. Control plates without actinomycetes were inoculated in the same way. The plates were incubated in temperature 19–20°C. All tests were repeated three times with two pH values: 6 and 7.
The growth inhibition rate of *R. solani* was estimated after 72 and 120 h of incubation. The measure of inhibition rate was the difference between the radius of the fungal spawn growing on control plates and the radius of the fungal spawn cultivated with the presence of actinomycetes, taking in millimetres.

The evaluation of antagonistic features of tested population of *Streptomyces* spp. in relation to *R. solani* was performed using modified method of Cooper Chilton (Johnson et al. 1960). The strength of strains effect was characterized in a scale of five degrees, where the length of inhibition area of the fungal spawn was estimated proportionally, in relation to the control: 0% – lack of effect (impact), 1–25% – weak effect, 26–50% – medium effect, 51–75% – strong effect, 76–100% – very strong effect.

The results were calculated statistically with the use of variance analysis. The differences of mean inhibition areas were evaluated with Tukey’s test with $\alpha = 0.05$. The measurement of the fungal spawn radius length after five days of cultivation was used to calculations.

**Results**

Investigated group of actinomycetes showed strong antagonistic features in relation to *R. solani*. The results of antagonistic strains of *Streptomyces* spp. were presented in Figures 1–3. On the base of the analysis with Student’s t-test, no significant differences have been found in the size of inhibition area of the fungal spawn grown on the medium with pH 6, and on the medium with pH 7.

All tested strains of actinomycetes isolated from soil inhibited the growth of *R. solani* (Fig. 1). Only 10% of strains (isolated from the conventional farming) showed weak inhibitory effect – in the tests on the medium with pH 6. Actinomycetes strongly inhibiting the fungal growth were the most numerous and made 63% on the medium with pH 6 and 57% on the medium with pH 7 (strains isolated from ecological farming) and 60% and 50% (pH 6 and pH 7, respectively) of isolates from the conventional system. The strongest inhibitory properties showed 43% of strains isolated from the ecological farming and 37% from the conventional farming tested on the medium with pH 7. On the medium with pH 6 less strains operated very strongly – 30% from ecological system and 23% from conventional system.

Of the strains coming from potato rhizosphere the isolates coming from the ecological system appeared stronger mycoantagonistic (Fig. 2). Only one strain from this system inhibited the growth of *R. solani*. As many as 80% of isolates very strongly inhibited the growth of fungal spawn on the medium with pH 7, and 60% – on the medium with pH 6. From the conventional system 10% strains operated with low and medium intensity, but only on the medium with pH 6. On the medium with pH 7 all strains from this system showed strong (40% of isolates) and very strong (60% of isolates) inhibitory properties.
Actinomycetes living in potato rhizoplane were also very active. There were no strains detected inhibiting the growth of the spawn of *R. solani* (Fig. 3). Only one strain coming from the conventional cropping system operated weekly in tests on the medium with pH 7. The other isolates from the conventional system on the medium with pH 7 were characterized with the effect: medium – 20%, strong – 53%, and very strong – 20% of cultures; while on the medium with pH 6 – 7%, 66%, and 27% of strains, respectively. Actinomycetes isolated from ecological farming inhibited the growth of *R. solani* on the medium with pH 7 more actively, because most of isolates (53%) inhibited the pathogen growth very strongly.

Fig. 1. Influence of *Streptomyces* spp. strains isolated from non-rhizosphere soil of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of *Rhizoctonia solani*
Due to the greatest inhibition zone in relation to *R. solani* the following strains are worth noticing: ZER-9, ZER-10, ZER-12, ZER-30, ZERp-3, ZERp-11, ZTRp-8 – inhibiting growth to 36.0 mm; ZER-15, ZER-25, ZTR-7 – inhibition area was 35.3 mm.

Fig. 2. Influence of *Streptomyces* spp. strains isolated from rhizosphere of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of *Rhizoctonia solani*
Discussion

The contribution and count of antagonistic microorganisms in relation to the pathogens causing potato diseases, as well as biotic interaction between them are very important factors for the occurrence and increase of these pathogens in soil (Choroszewski 1989). The phenomena of antagonisms, irrespective of their mechanism, regulate coexistence of various species of microorganisms, both pathogens and saprophytes (Balicka 1983).

Fig. 3. Influence of Streptomyces spp. strains isolated from rhizoplane of potato cultivated in ecological (A) and conventional (B) farming, on growth inhibition of Rhizoctonia solani
Of 150 actinomycetes strains tested, 37% indicated very strong antagonistic properties in relation to *R. solani* on the medium with pH 6, and 51% on the medium with pH 7. No strain not inhibiting the growth of the spawn of *R. solani* has been detected. The weak effect was shown by 1–4% of actinomycetes only from the conventional system (Fig. 4).

Marcinowska and Bis (1997) report that fungi show much more resistance to antibiotics in comparison to bacteria. We did not confirm this in our studies, where fungi, and especially *R. solani*, were strongly inhibited by the tested population of *Streptomyces* spp. According to Broadbent et al. (1971) 80% of strains of *Streptomyces* spp. exerted mycoantagonistic influence on such pathogens as: *R.*
solani, Fusarium oxysporum Schlecht., Pythium debaryanum Hesse and Phytophthora sp. through antibiotic production, and the other 20% through competition for nutritive substances. Bogucka et al. (1989) observed the impact of Streptomyces scabies (Thaxt.) Waksman et Henrici on 21 species of fungi most frequently occurring on potato. They noticed the reduction in growth of most species tested. In some cases, like in relation to R. solani, the stimulating effect of the actinomycetes culture was indicated. Results of our research has not confirmed the stimulating influence of actinomycetes tested on the growth of R. solani in any case investigated.

Antagonistic effect plays a very important role in inhibition and development of pathogenic microorganisms, as well as in reduction of their vitality. The results obtained in our studies and shown by other presented authors, indicate the high mycoantagonistic activity of strains Streptomyces spp. and their enormous ability on protection of plants against fungal potato diseases. Rhizoctonia solani are also known to be pathogenic factors for many cultivated plants and vegetables.

The results obtained suggest that the more favourable conditions for the development of antibiotic actinomycetes can be found in the ecological farming. More strains of Streptomyces spp. inhibiting growth of the pathogenic fungi came from the ecological potato cultivation than from the conventional one. Besides, the inhibition area of the spawn of fungi was more limited by strains from ecological system. There are no data concerning the comparison between the activity of actinomycetes isolated from the ecological and conventional farming in available literature.

The contribution of antagonistic strains and the power of their impact depended also on environment (soil beyond rhizosphere, rhizosphere, and rhizoplane), from which actinomycetes were isolated. The biggest areas of inhibited growth were obtained in strains coming from rhizosphere. It testifies to favourable influence of potato roots excretions on antibiotic activity of Streptomyces spp. (Pięta and Patkowska 2001).

Studies on the activity of antagonistic actinomycetes were carried out experimentally, and proper conditions for growth and development of isolated strains were created. On the ground of reports in literature we can draw a conclusion, that the antagonism of the strains of Streptomyces spp. in vitro is not always confirmed in natural environment (Pięta and Patkowska 1997). Most antibiotics excreting is unsteady in soil and (is subject to) inactivation as a result of purely physicochemical reactions or biological decay. Antibiotic substances produced by actinomycetes can maintain their biological activity for a long time, although they are strongly adsorbed on soil particles (Singleton 1999). Instability of antibiotics can be also caused by changeable pH of soil. In our studies the change of pH did not influenced significantly the antagonistic activity of Streptomyces spp. However, in the case of R. solani higher abilities of inhibiting the fungal spawn growth were obtained on the medium with pH 6 than on the medium with pH 7. In order to confirm the results of studies carried out experimentally, concerning the protective influence of antagonistic actinomycetes against the phytopathogens of potato, it is necessary to carry on with analyses in field conditions.
Conclusions

1. The investigated collection of actinomycetes of the genus *Streptomyces* showed strong antagonistic features in relation to the pathogenic fungus *Rhizoctonia solani*. The most active strains were: ZEG-1, ZEG-13, ZEG-18, ZER-5, ZER-9, ZERp-3, ZTRp-7, and ZTRp-8.

2. Comparing the inhibition areas on the medium King B with pH 6 and those with pH 7, we can find, that the bigger inhibition areas were obtained on the medium with lower pH.

3. The population of *Streptomyces* strains isolated from potato cultivation in the ecological farm was characterized by stronger mycoantagonistic features in comparison to the isolates from the conventional farming.

Summary

The aim of the study was to estimate the antagonistic activity of actinomycetes of the genus *Streptomyces* to the pathogenic fungus *Rhizoctonia solani*. We compared the impact of the strains of *Streptomyces* spp. coming from two potato plantations with different farming systems. Analyses were conducted experimentally *in vitro* on King B medium with pH 6 and 7. The results obtained showed that the investigated strains of *Streptomyces* spp. are strong antagonists of the fungus *R. solani*. All strains from the collection of 150 isolates inhibited the growth of the pathogen tested. The strains from the ecological system predominated in the group of very strong antagonists. The results obtained indicate that ecological system of potato farming was more favourable for the development of antagonistic actinomycetes in comparison with conventional farming system. Actinomycetes can be used in biological plant protection.

Streszczenie

ODDZIAŁYWANIE PROMIENIOWCÓW Z RODZAJU *STREPTOMYCES* WYIZOLOWANYCH Z UPRAWY ZIEMNIACA NA PATOGENICZNY GRZYB *RHIZOCTONIA SOLANI*

Celem badań było określenie antagonistycznych właściwości promieniowców z rodzaju *Streptomyces* w stosunku do patogenicznego grzyba *Rhizoctonia solani*. W pracy porównano oddziaływanie szczepów *Streptomyces* spp. pochodzących z dwóch różnych się systemem uprawy plantacji ziemniaka. Analizy przeprowadzono w warunkach laboratoryjnych *in vitro* na podłożu King B o dwóch poziomach pH: 6 i 7. Na podstawie uzyskanych wyników okazało się, że badane szczepy *Streptomyces* spp. są silnymi antagonistami grzyba *R. solani*. Z kolekcji 150 izolatów wszystkie szczepy hamowały rozwój testowanego patogenu. W grupie bardzo sil-
nie działających antagonistów przeważały szczepy z uprawy ekologicznej ziemniaka. Z uzyskanych badań wynika, że uprawa ziemniaka systemem ekologicznym bardziej sprzyja rozwijowi antagonistycznych promieniowców niż uprawa systemem konwencjonalnym. Promieniowce z rodzaju *Streptomyces* z powodzeniem mogą być wykorzystane w biologicznej ochronie roślin.

**Literature**


Effect of actinomycetes of Streptomyces genus...