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EXPLORING STREPTOMYCES BIOAGENTS FOR IMPROVING OF SOIL FERTILITY AND PLANT PROTECTION FROM PATHOGENS

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Abstract. *Streptomyces species isolated from soil and rhizosphere of wild and crop plants were screened in vitro and in vivo experiments for antibacterial, antifungal, growth-promoting activities and for producing the volatile and phosphorus solubilizing metabolites.*

The tested Streptomyces strains were able to inhibit bacterial pathogens of vegetables and fruits like Erwinia carotovora, Erwinia amylovora, Pseudomonas syringae, Ralstonia salonosarum and fungal pathogens like Alternaria tenuissima, Fusarium graminearum,Venturia inaequalis and Monilia fructicola. Additionally, the tested isolates were able to colonize of rhizosphere and phyllosphere of plants and suppress disease symptoms on the leaves, flowers, stems, and roots. Selected active Streptomyces strains multiplied rapidly on the plant leaves after treatment and reached densities to suppress the pathogens, maintaining its persistence from 25 to 35 days depending on the type of plants.

Streptomyces diastochromogenes SK-6 and Streptomyces alfalfae, CI-4 were selected for improving the soil fertility, for the biological control of bacterial, fungal diseases of vegetables, fruits, and cereals in organic agricultural fields.

Keywords: *rhizosphere, Streptomyces bacteria, biocontrol agent, bioinoculant in organic production*

Introduction

The Kyrgyz Republic has significant potential for cultivating cereals, vegetables, and fruit crops. Since 2004 in Kyrgyzstan, the agriproducts produced ecologically start increasing. To successfully implement organic production, the farmers should use the practices and techniques specific to organic farming.

The principles of organic farming are based on preserving the ecological balance of the environment and human health, abandoning chemically synthesized fertilizers and pesticides, genetically modified seeds, and seedlings. Instead, organic fertilizers (compost, manure, siderites and biofertilizers) are proposed, and the use of biopesticides and biofungicides, which preserve biodiversity in the soil and the environment and create the conditions for performing their helpful service [Compant et al., 2005, Copping and Menn, 2000].

Microbial inoculants and technologies can be used for chemical-free agriculture, replacing harmful pesticides and fertilizers for crop protection and enhancing yields. There are more than 22,000 biologically active compounds of microbial origin registered, of which 7,600 are produced by actinomycetes of the Streptomyces genus [Demain and Sanchez, 2009; Bérdy , 2005; Lam, 2007]. In addition, 70% of all known antibiotics were found from *Streptomyces* strains [Demain, 2010, Labeda et al., 2012].

A good deal of Streptomyces is able to colonize the root hairs of plants and can penetrate them, thereby the rhizosphere for them is the main habitat and these bacteria are regularly found in the roots of plants [Tarkka, 2008; Bonaldi et al. 2015; Kortemaa et al. 1994]. Additionally,

Streptomyces are able to inhibit the growth of phytopathogens not only in vitro but also in vivo, for instance, soil-derived Streptomyces effectively reduced a root rot on alfalfa and soybean caused by *Phytophthora medicaginis and Phytophthora sojae* [Xiao et al., 2002], *Fusarium* wilt disease in chick pea and tomato [Gopalakrishnan et al., 2011, El-Tarabily and Alkhajeh, 2016.] , and also protect tomato seedlings against the phytopathogen *Rhizoctonia solani* [Cao et al., 2004]. Streptomyces can cause a state of the increased protective ability of plants against pathogens, thereby providing plants with induced systemic resistance, enhancing their ability to resist the disease [Conn et.al., 2008; Tarkka et.al.,2008; Kurth et.al.,2014]. Like other growth-stimulating rhizobacteria, actinomycetes produce various phytohormones such as auxins, cytokinins and gibberellins [Cassan et.al.,2001; Bottini et.al., 2004; Solans et. al., 2011].

They are numerous in the soil, they occupy a rhizosphere niche of plants , due to the ability to form spores at the branching filaments, they enter into a beneficial relationship with plants [Miguélez et al.,2010; Tyc et al., 2017].They can also be endophytes colonizing inner tissues of host plants [Sousa and Olivares, 2016; Carvalho and Oliveira , 2017].

Another useful property of actinomycetes, they play an important role in the self-cleaning of soils from pathogens. They are able to remain in the soil for the longest time, to have a suppressive effect on other groups of microorganisms, In the creation of soil fertility, they are able to solubilize phosphate in phosphate-deficient soils [ElTarabily et al. 2008; El-Tarabily and Sivasithamparam 2006].

At the same time, they secrete enzymes that can transform complex organic substances into mineral forms accessible to plants, thereby improving plant nutrition. They contribute to the increase in the number of nitrogen-fixing and ammonifying microorganisms in the rhizosphere [Doolotkeldieva,et.al., 2015;2016].

The present study was focused on evaluating the *in vitro and in vivo* potential of *Streptomyces* strains toward various bacterial and fungal phytopathogens of cereals, vegetables and fruit crops, for improving soil fertility, for combating the problems related to pollution and global warming.

2. Material and methods

2.1. Sources of Biocontrol microorganisms (*Streptomyces)* from laboratory collection.

The *Streptomyces* strains from Plant Protection Department laboratory collection having broad spectrum antimicrobial activity against fungal and bacterial phytopathogens were used.

2.2. Plant Pathogenic microorganisms

Pathogenic bacteria (*Erwinia carotovora, Erwinia amylovora, Pseudomonas syringae, Ralstonia solanacearum*) isolated from diseased plant organs were stored in sterilized nutrient broth at - 20^0 C. Pathogenic fungi (*Alternaria tenuissima, Fusarium graminearum,Venturia inaequalis and Monilia fructicola, Aspergillus sp.*) isolated from diseased plant organs preserved on potato dextrose agar (PDA) slopes at 4◦C.

2.2. Production of bioactive metabolites by *Streptomyces* strains.

The liquid samples of a biological product based on Streptomyces sp. were carried out by submerged cultivation in a bioreactor (LAMBDA Laboratory Instruments, CZ, 7l), with a working volume of 6.0 l with automatic regulation of oxygen supply, pH, temperature and other relevant technical indicators. After 72 h. of incubation, filtered sterile cell-free supernatant or cells were used for further experiments.

2.3. Determination of phosphate soluble activity of *Streptomyces* strains.

Phosphate soluble activity of *Streptomyces* strains was determined in a nutrient medium containing $Ca_3(PO_4)_2$ (NBRIP) in its composition as the sole source of phosphorus.

The wells of 1 cm^2 in size were made on this medium and 1 ml of *Streptomyces* strains supernatant was poured into each well and incubated at 30 ° C for 2 weeks. The formation of a hydrolysis zone around the colonies indicated the solubility of phosphate.

2.5. Determination of the presence of volatile compounds in *Streptomyces strains*

A sealed system in Petri dishes with a Parafilm thin membrane was used to determine the presence of volatile metabolites of *Streptomyces* and its effect on the development of the mycelium of phytopathogenic fungi without contact. On one side of the membrane, colonies of Streptomyces strain were grown; on the other side, phytopathogenic fungi—*Alternaria tenuissima, Fusarium graminearum and Aspergillus spp.*

2.6. Determination of antibiotic activity of *Streptomyces* strains against bacterial pathogens.

The sprayed method of bacterial pathogens was used.

In the Petri dishes, tested *Streptomyces* strains were planted in the centre on the surface of the medium. *Erwinia carotovora, Erwinia amylovora, Pseudomonas syringae and Ralstonia solanacearum* were used as a test phytopathogenic bacteria. 48 h culture suspensions of these phytopathogens were sprayed onto the grown colonies of biocontrol *Streptomyces* strains on the 7th day of the growth when antibiotic substances of actinomycetes were pronounced. An activity of tested *Streptomyces* strains was evaluated by antagonism and hyperparasitism effects.

2.7. Evaluation of antagonistic activity of biocontrol agents in liquid media

The antagonistic activity of biocontrol agents against the bacterial pathogens was studied also by co-cultivation of the antagonist and the test culture in a liquid medium. The *Erwinia amylovora, Pseudomonas syringae and Ralstonia solanacearum's* cultures were incubated in 5 ml tubes in a meat-peptone broth for 48 hours. Then 1 ml of an antagonist culture was added to each tube: After incubation at 28˚C for 24 hours, tube contents were analyzed the activity of the biocontrol agents was evaluated by microscopy and the cell titer was determined using a UV/VIS spectrophotometer (Jenway, Stone, UK) at 550 nm.

2.6.3. *In vitro* determination of antibiotic activity of *Streptomyces* strains against fungal pathogens.

2.6.3.1. Agar wells method was used. The dilutions (1:10, 10: 100, 10: 1000) from *Streptomyces* biological product supernatant were prepared. In 100 ml of Czapek's medium, cooled to 50-55 ° C, a pathogen fungal mycelium was introduced with a microbiological loop, mixed thoroughly and poured into Petri dishes. After agar has hardened, holes are made from 4 sides and each of them is marked (I, II, III, C), each of the holes corresponds to its own test organism.

C- control option 1% solution of nystatin;

I - 1ml supernatant of *Streptomyces* at a dilution of 1: 10;

II - 1ml supernatant of *Streptomyces* at a dilution of 1: 100;

III - 1ml supernatant of *Streptomyces* at a dilution of 1: 1000;

The antagonistic activity of the strain was determined by the zones of inhibition around the holes.

3.RESULTS

Determination of phosphate soluble activity of *Streptomyces* strains. Among all tested strains, two - *Streptomyces alfalfae* C1-4 and *Strep. lividans* TR-59 have demonstrated the highest activity of phosphate solubilization, showing a clear, transparent halo around the holes. The procedure was repeated three times, and each time the resulting transparent halos were measured and displayed arithmetic mean calculations. These results indicate that *Streptomyces* strains are able to produce organic gluconic acid in the environment. Gluconic acid chelates the antiactylones (Ca2 +) of negatively charged insoluble phosphate through its carboxyl group, turning it into soluble forms [Rajput et al., 2013; Ro ´zycki, and Strzelczyk, 1986; El-Tarabily et al., 2008].

Determination of volatile compounds that damage the cell wall of fungal pathogens

During a 92-h incubation, all tested *Streptomyces* strains suppressed the growth of *Fusarium graminearum* relative to the control variant, with the pathogen showing high sensitivity to volatile *Streptomyces* metabolites following non-contact exposure. *Streptomyces lividans* Tr-59 and Streptomyces *medioloni* Pat- 3 strains have shown a high degree (90%–95%) of antifungal activity against phytopathogenic fungi like *Alternaria. tenuissima, Rhisoctonia solani and Aspergillus* spp. by inhibiting the growth of fungal colonies during non-contact co-cultivation, although these fungi can form multiple mycotoxins.

Determination of antagonistic effects of *Streptomyces* strains to bacterial pathogen by *sprayed* method

Streptomyces lividans TR-59 showed an antagonistic effect after 24 h to *Erwinia carotovora* bacterium, the growth of the actinomycete colonies increased and spread over the colonies of the causative agent of wet rot. In 48 h. an hiperparasitism action of this *Streptomyces* strain to the *Erwinia carotovora* was evident. As the results of screening have shown, the *Streptomyces alfalfae*, CI-4, strain has shown a solid antagonistic effect against the causative agent of bacterial canker (Fig. 8). After spraying with a suspension, the phytopathogenic bacterium (*Pseudomonas syringae*), aggressive growth of the *Streptomyces* was observed already in 18 h around the phytopathogen colony. Moreover, the strain *Streptomyces alfalfae*, CI-4 has shown the same antibacterial solid activity as the causative agent of the fire blight of the Rosaceae family plants, the bacterium *Erwinia amylovora*.

Streptomyces alfalfae C1-4, isolated from the rhizosphere as a biofertilizer, was intended for seed and soil application to increase plant growth and protect from pathogens in this study. In vitro experiments, such vital properties as phosphate solubilization and producing volatile compounds, inhibiting the pathogens of this strain, were adequately evaluated, and a biofertilizer was developed based on this active strain. In the first stage of our research, we used this active phosphate-dissolving strain in newly developed, low-fertile soils in order to find out whether it could be working in unfavorable field conditions. Used as a bioinoculant *Streptomyces alfalfae* C1-4 by soaking the seeds for 2 hours before planting in the soil, this application mechanism has shown encouraging results on wheat and soybeans. The biofertilizers have to have success criteria for wide application: they have to be effective in actual field conditions, in a range of soils and different host cultivars. Despite the low soil fertility and lack of irrigation water in the summer, treatment of seeds by *Streptomyces alfalfae* C1-4 product has shown a growth stimulatory effect on all phases of soybean than in wheat. It ultimately has increased biomass and grain yield overall. In all phases of vegetation, the ammonifying bacteria in the presence of an antagonist (a biological agent) developed rapidly and were constantly present in significant numbers in the rhizosphere.

This indicates a balance between the rhizosphere inhabitants and the *Streptomyces alfalfae* C1-4 biological agent.

Thus, using *Streptomyces* bioinoculants that promote plant growth, improve nutrient availability, control phytopathogens, and reduce abiotic stress in plants is essential for sustainable agriculture and an excellent alternative to environmentally hazardous chemical fertilizers and pesticides. Volatile compounds produced by this group of bacteria have yet to be used as metabolites; their usefulness for agriculture still needs to be studied, and strains with such compounds could undoubtedly be widely used as fumigants of seeds and fruits during storage instead of chemicals. In future studies, using advanced technologies and methods, it is necessary to study the chemical composition of all metabolites produced by these *Streptomyces* local strains to study the level of expression of genes responsible for the biocontrol properties in these strains. Also, to develop molecular markers to identify the clusters present in these strains.

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