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Full Length Research Paper

Impact of Biocontrol Agent *Streptomyces fumanus* on Bacterial Communities in the Rhizosphere of Wheat and Soybean in Newly Cultivated Soil

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The quantity and diversity of a rhizosphere functional microbial community's ammonificators, oligotrophs and diazotrophs after processing of wheat(*Triticum aestivum)* and soybean *(Glycine max)* seeds in *Streptomyces fumanus* gn-2 suspension (10^4 spores\ ml) were examined before planting into the soil. The rhizosphere microflora of wheat was investigated in shoot, till ring, heading and maturation phases by classical microbiological and molecular biology methods. The soybean rhizosphere microflora in three phases of vegetation, formation of the first trifoliate leaf, flowering and maturation, was investigated by the same methods. Treatment of wheat and soybean seeds with *Streptomyces fumanus* product promoted a population of the most useful microorganisms, including nitrogen-fixing bacteria in the rhizosphere of plants. A 16S ribosomal RNA analysis revealed a rich biodiversity of bacteria in the rhizosphere of the wheat's maturation phase, which differs from the biodiversity of bacteria in the rhizosphere of soybean in the same phase. Bacteria of *Microbacterium* genus from *Actinobacteria phylum* dominated in the rhizosphere of wheat. The bacteria species of *Chryseobacterium* genus from *Flavobacteriia* phylum dominated in the rhizosphere of soybean, which proves the different chemical composition of these organic plant exudates that attracts the preferred species of microorganisms.

Keywords: wheat and soybean seeds, biocontrol agent, rhizosphere microflora in vegetation phases, impact of *Streptomyces fumanus* on biodiversity of rhizosphere.

INTRODUCTION

Plants live in close association with the microbes that inhabit the soil in which they grow. Terrestrial plants provide a habitat for a wide variety of microorganisms (Bais et al., 2006; Lugtenberg et al., 2002). Microbial colonization may cover the complete surface of the host (Lindow and Brandl, 2003), and is concentrated largely in the rhizosphere (Berg and Smalla, 2009; Costa et al., 2006), where it can produce beneficial, presumably neutral, or even harmful effects on the plant, depending on the microbe involved, the plant species and its nutritional status (Mercado-Blanco and Bakker, 2007). A strong influence on root colonization and soil ecology has been ascribed to organic components of root exudates (Badri and Vivanco, 2009; Bais et al.,2006), which can inhibit the growth of certain microorganisms, while stimulating the proliferation of others, thus making the rhizosphere a selective environment (Bais et al., 2006; Kiely et al., 2006). Although their identities are not well characterized, some of

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the major organic components of exudates have been described in a few plant species (Uren, 2000; Walker et al., 2003). Readily available carbon sources secreted by plant roots include sugars or simple polysaccharides, amino acids, and phenolic compounds (Bertin et al., 2003), some of which can also be found in root tissues (Tan et al., 2004).

In the rhizosphere, the microbial density is typically higher than in bulk soil and ranges from10^8 to10^9 bacteria per gram. According to some authors, the rhizosphere can contain up to10^{11}microbial cells per gram root (Egamberdieva et al., 2008) and more than 30,000 prokaryotic species (Mendes et al., 2011). The collective genome of this microbial community is much larger than that of the plant, and is also referred to as the plant's second genome (Roeland et al., 2012).

The balance between the rhizosphere microflora and plant pathogens and soil microflora is important in host-pathogenic relationships. The rhizosphere microbiome can prevent colonization by pathogens. Mechanisms through which rhizosphere microorganisms can affect a soil-borne pathogen have been identified, and include production of antibiotic compounds, consumption of pathogen stimulatory compounds, competition for(micro)nutrients and production of lytic enzymes (Doornbos et al., 2012; Lugtenberg and Kamilova, 2009). Many beneficial soil-borne microorganisms have been found to systemically boost the defensive capacity of the plant.

As is well known, plant growth-promoting bacteria (PGPB) occupy the rhizosphere of many plant species and have beneficial effects on the host plant. They may influence the plant in a direct or indirect manner. A direct mechanism would increase plant growth by supplying the plant with nutrients and hormones (auxin, gibberellins and cytokinin). Indirect mechanisms, on the other hand, include reducing susceptibility to diseases, and activating a form of defence referred to as induced systematic resistance (ISR) (Van der Ent et al., 2009).

This is observed world wide and has been associated with the build-up of antagonistic fluorescent *Pseudomonas spp*. that produce the antifungal compound 2,4-diacetylphloroglucinol (DAPG) (Weller et al.,002).Other microorganisms that can confer suppressiveness have been found among the proteobacteria and firmicutes and for fungi among the ascomycota (Raaijmakers et al., 2009).

Soil bacteria belonging to the *Streptomycetes* are regarded as promising biocontrol organisms due to their potential to produce a vast array of secondary substances such as vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors (Lehr et al., 2008; Abd and El-Mehalawy, 2002; Rothrock and Gottlieb,1984). They are capable of exhibiting beneficial as well as detrimental effects on plants, including promotion of symbiosis, improved growth, abiotic stress resistance and enhanced resistance to fungi and bacterial diseases. Studies even show that use of *Streptomyces* enhances growth of crop plants (Compant et al., 2005).

Our previous studies have shown that *Streptomycetes fumanus* gn-2 is effective in biocontrol of root rot and damping–off diseases, and promotes growth of vegetable and field crops (Doolotkeldieva, 2007; Doolotkeldieva, 2010). *Streptomyces fumanus* has a rhizosphere origin and was isolated from the rhizosphere of mustard (*Sinapis alba*) and recommended as a biofertilizer for seed and soil application to increase plant growth and protect against pathogens.

We aim to investigate the role of *Streptomyces fumanus* gn-2 in the regulation of the functional diversity of rhizosphere bacteria of wheat and soybean and, at the same time, the influence of the rhizosphere microbial community on the efficiency of the biological agent.

MATERIALS AND METHODS

Bacterial strains and culture

Streptomyces fumanus gn-2 was obtained from the rhizosphere of mustard *(Sinapis alba*) in Issuk-Kul region of Kyrgyzstan (Doolotkeldieva, 2010; 2012).YEPG Medium, ISP4 Medium, starch-ammonia agar (SAA) and oatmeal agar were used to study and maintain actinomycete cultures under laboratory conditions (Gauze et al., 1983). Molecular techniques were used to analyse the diversity of actinomycete isolates (Dunbar et al., 1999; Wawrik et al., 2005; Yuan and Crawford, 2005). The 16S rRNA genes were PCR amplified with 27f and 1522r primers, polyketide synthase (PKS) genes were screened by polyketide synthetase primers.

Fermentation of *Streptomyces fumanus* gn-2 to obtain a liquid sample of biological product

Production of liquid samples of biological product based on *Streptomyces fumanus*gn-2 was conducted in submerged cultivation in a bioreactor (Applicon, USA, 3l), with automatic control of oxygen, pH, temperature and other relevant technical indicators. The composition of the medium contained carbohydrate sources, nitrogen, phosphorous and magnesium salts (pH 7.0-7.2), which had been pre-optimized for maximum biomass yield of the resulting product.

Experimental plots

In 2008,the experimental field of the Agricultural Faculty was established on the territory of the Jal Campus of Kyrgyz-Turkish Manas University, with 80% sand, 9%silt and 11 %clay (in top soil) and containing significant different metals - Mn, Ni, Co, Ti, Cr, Mo, W, Zr, Nb, Cu, Pb,

Ag, Sb, Z, Cd, Sn, In, Ga, Yb, Y, La, Ce, P, Sr, Be, Ba, Li, Sc, Hf, Ta, Th, U, Pt and Au. These soils have not previously been used as farmland. A continuous application of composted farmyard cattle manure with black soil in a 5cm layer without mineral fertilizer was conducted for three years (2008-2011).The farmyard cattle manure was composted for six months before application. The pH of the soil was 6.3 and its organic carbon content was 1-2%.

The rhizosphere microflora of two plants, wheat (*Triticum aestivum*) and soybean (*Glycine max*) in different phases of vegetation was studied. The seeds of wheat and soybeans were treated in liquid samples of biological product based on *Streptomyces fumanus*gn-2 for three hours before planting into soil.

The rhizosphere microflora of wheat grown from treated seeds and seeds without a treatment was investigated in shoot, till ring, heading and maturation phases by classical microbiological and molecular biology methods. The soybean rhizosphere microflora in three phases of vegetation, formation of the first trifoliate leaf, flowering and maturation, was investigated by the same methods in samples treated with *Streptomyces fumanus* gn-2 and those not treated with the bioproduct. In addition, the micro diversity of unplanted soil was investigated for comparison to the rhizosphere microflora of cropped soil.

The study of the rhizosphere of plants by culture-dependent methods

To analyse uncropped soil and the rhizosphere of plants, the whole volume of soil with plants was taken out of the experimental plots. In the case of plants, the bulk soil was shaken off from the plant roots taken from depths of approximately 15-25 cm, and the roots with the remaining adhering soil (thickness of no more than 2–3 mm) were used for analysis. The sample of root with attached rhizosphere soil was placed into a flask with 100 ml of sterile tap-water, and was shaken for 30 min. Then the roots were taken out and the suspension was left to let the soil particles settle, after which a range of dilutions was prepared for isolation of rhizosphere microorganisms. The sampling was made in three replicates per experimental plot.

The total number of microorganisms in unplanted soil and the rhizosphere of *Triticum aestivum* and soybean *Glycine max* was determined by the fluorescence microscopy method and the plating dilution technique using a nutrient agar medium (Difco). For isolation and enumeration of the total number of cultivable ammonificator, oligotroph and diazotrophs microorganisms, the plating dilution technique was used. Plates were incubated at 30°C for 3–7 days, and colony-forming units (CFU) were counted and calculated. Bacto-actinomycetes isolation agar (Difco) and starch ammonia agar were used to estimate the number of actinomycetes. The selective agar media Fedorov's medium was used for the isolation of nitrogen-fixing microorganisms, consisting of (in grams per litre of distilled water): mannitol, 20.0; K_2HPO_4, 0.3; $CaHPO_4$, 0.2; K_2SO_4, 0.2; $MgSO_4$, 0.3; NaCL, 0.5; $FeCl_3$, 0.01; $CaCO_3$, 5.0; micronutrient solution, 1.0; agar-agar, 20.0(Zvyagintsev, 1991).The micronutrient solution (g l–1) consisted of: H_3BO_3, 5.0; $(NH_4)_2MoO_4$, 5.0; KI, 0.5; NaBr, 0.5; $ZnSO_4$, 0.2; $Al_2(SO_4)_3$, 0.3. After 3–5 days of incubation, the colonies grown on nitrogen-free agar plates were counted.

PCR analysis of rhizosphere bacteria

DNA was extracted from soil and pure cultures maintained in MPM medium at 25 °C during the active phase of microbial growth from enrichment cultures using Ultra Clean™ Soil DNA Isolation Kit and an alternative protocol from MO BIO Company. Polymerase chain reaction (PCR) amplification was performed with a Multigene Thermal Cycler (TC9600-G/TC, Labnet International). The 16S rRNA genes were PCR amplified with 27f and 1522r primers. Sequence analysis was performed by Macrogen Company (Seoul, Korea), and sequences were edited with Applied Biosystems 3730XL sequencers; only sequences with > 700 nucleotides were used for diversity analyses.

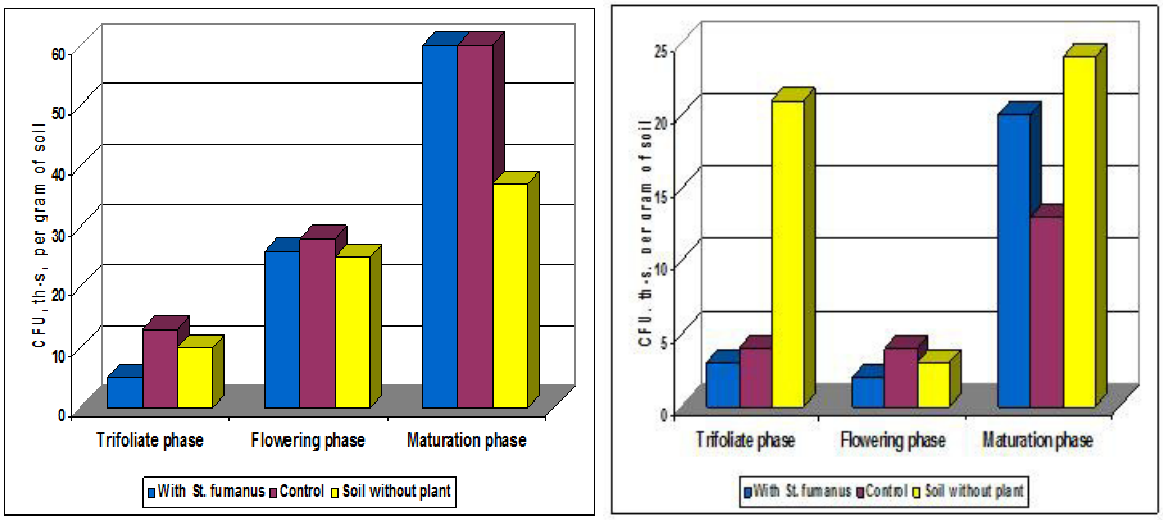
Statistical analysis

Statistical analysis for the total count of different group bacteria in plant rhizosphere was performed by standard methods with the use of programs EXCEL and STATGRAPHICS Plus. ANOVA analysis of variance was used for determination of the F-distribution (probability distribution) function, and information about the variances of each population (within) and grouping of populations (between). A P value below 0.05 was deemed significant.

RESULTS AND DISCUSSION

Dynamics of rhizosphere microflora of wheat in the vegetation phases under application of *Streptomyces fumanus*

The rhizosphere microflora of wheat in shoot, till ring and maturation phases was investigated. Population density of species and biodiversity in three physiological groups, ammonificators, oligotrophs and diazotrophs were studied. To identify the impact of *Streptomyces fumanus* on the formation of wheat rhizosphere microflora, we compared these organisms in the experimental, control variants and bulk soil. While the seeds are soaking in the suspension of biological product for three hours, the spores and mycelia of *Streptomyces fumanus* will be continuing the adhesion to and penetration of the cell wall of seeds; thus, the



A B

Figure1. A. The number of ammonificators in the rhizosphere of wheat at different vegetation phases; B. The number of oligotrophs in the rhizosphere of wheat at different stages of vegetation

mycelium of the biological agent penetrates further into the root system of growing plants.

Number and biodiversity of ammonificators by phases of wheat

In the shoot phase, the number of ammonificators in the wheat rhizosphere microflora was low, whereas in bulk soil the number of these bacteria was 1.5 times higher. In the till ring phase, the number of ammonificators in the rhizosphere was almost 1.2-1.5 times higher than in the shoot phase. In this phase, in the control variant the number of these bacteria was higher than in the variant treated with *Streptomyces fumanus* and in bulk soil.

The density of the ammonificators population increased with the plants' ripening. In the maturation phase, the number of bacteria in bulk soil was 47.6 ± 0.97 × 10^3 CFU/g soil (P≥00.5), while in the control and the variant with *Streptomyces fumanus* the number was equal, and consisted of 64 ± 1.23 x 10^3 CFU/g soil(P≥00.5) (Figure 1A).

Usually, the ammonificators get nutrients and energy from the decomposition of fresh organic matter in the root system of plants. Low numbers of these bacteria in the shoot phase, apparently, can be related to the fact that the growing, young root hairs produce a small number of exudates, which are a source of nutrients for the bacteria living in the rhizosphere. With the development of lateral roots, the amount of organic secretions is increased to attract microorganisms.

Thus, the biological agent containing *Streptomyces fumanus* does not stimulate the growth of ammonificators in the rhizosphere. But this agent does affect the species composition of the rhizosphere, thus causing a shift in functional groups of rhizobacteria.

16S ribosomal RNA analysis revealed a rich biodiversity of rhizosphere bacteria of wheat in the maturation phase. The ordinary saprophytic bacteria from *Actinobacteria* phylum were dominant: *Microbacterium hydrocarbonoxydans*, *Microbacterium oxydans*, *Microbacterium* sp. VKM Ac-2051 and *Curtobacterium* sp. VKM Ac-2061*, Brevibacterium* sp.*, Uncultured bacterium,* and *Actinomycetes*P032 (Figure. 2).

Number and biodiversity of oligotrophs by phases of wheat vegetation

In the shoot phase, their number in the variant containing *Streptomyces fumanus* was 2.6 ± 0.75 × 10^3 CFU/g of soil, and in the till ring it was even less, only 1.8 0 ± 0.56 × 10^3CFU/g soil, and by the end of the growing period, their number increased dramatically, reaching 21.6± 1.23 x 10^3 CFU/g of soil. In the control, the number of actinomycetes in the shoot and in the till ring phases was the same, and in the maturation phase it doubled and amounted to 12.4 ± 0.89 × 10^3 CFU/g soil. In bulk soil, were veiled a high number of colony-forming units in the shoot and maturation phases; their number reached23.6 ± 0.79 × 10^3CFU/g of soil, and in the till ring stage it decreased by four times (Figure 1B).

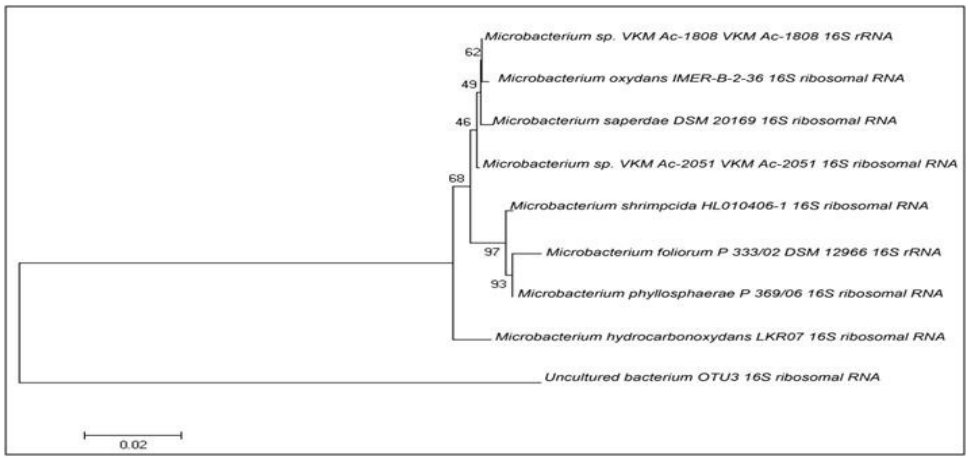


Figure 2. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from wheat rhizosphere.

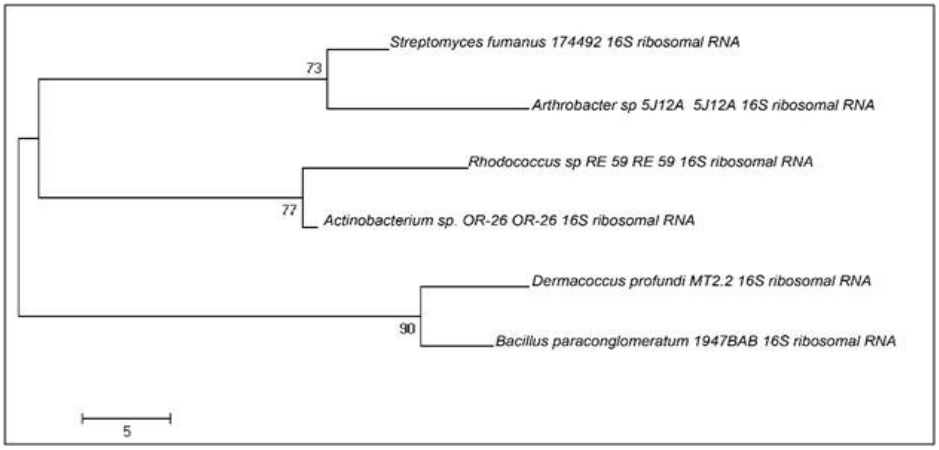


Figure 3. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from wheat rhizosphere (with a biologic product).

Biodiversity of actinomycetes was represented by species from the *Actinobacteria* phylum: *Streptomyces fumanus*, *Arthrobacter* sp., *Dermacoccus profundi*, *Rhodococcus* sp., *Actinobacterium* OR-26, and others. In the variant in which the biological product was used, *Streptomyces fumanus* was isolated during the whole growing season. This indicates he survival ability of this culture in the rhizosphere of wheat.

The number and biodiversity of nitrogen-fixing bacteria in growth stages of wheat

In contrast to other microorganisms in the rhizosphere of wheat, nitrogen-fixing bacteria have shown a significant number at all phases of plant development, particularly where a *Streptomyces fumanus* product was applied. So, the presence of the biocontrol microorganism *Streptomyces fumanus* in the rhizosphere not only plays an important role in the suppression of pathogenic species, but also enhances growth and development of useful groups, such as nitrogen-fixing bacteria. The population densities and the diversity of the root microflora affected the number and activity of *Azotobacter* bacteria, which may contribute to additional nitrogen inputs to the soil (Figure 4).

Dynamics of the rhizosphere microflora of soybean in phases of vegetation

We have explored the rhizosphere microflora of soybean in three phases: in the formation of first trifoliate leaf, flowering and maturation.

Number and biodiversity of ammonificators by phases of soybean

In all phases of the growing season the ammonificators bacteria, in the presence of a biological agent consisting of

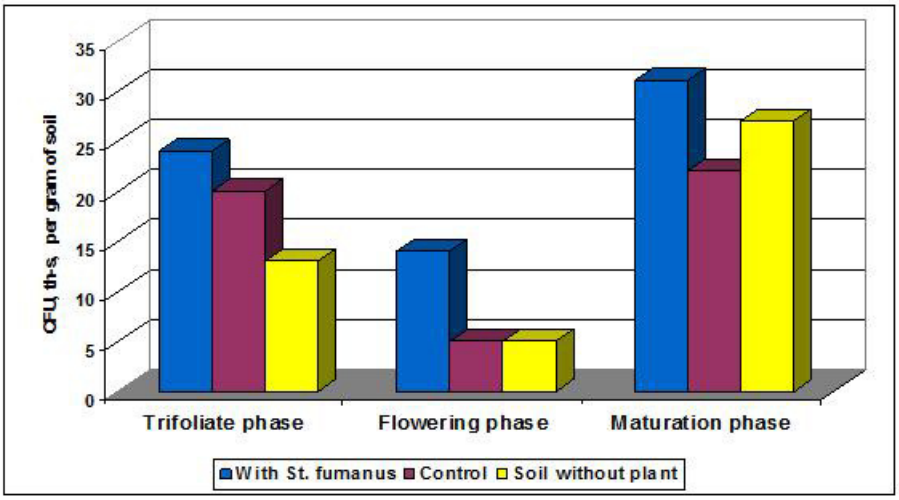


Figure 4. The number of nitrogen-fixing bacteria in the rhizosphere of wheat at different stages of vegetation, in %

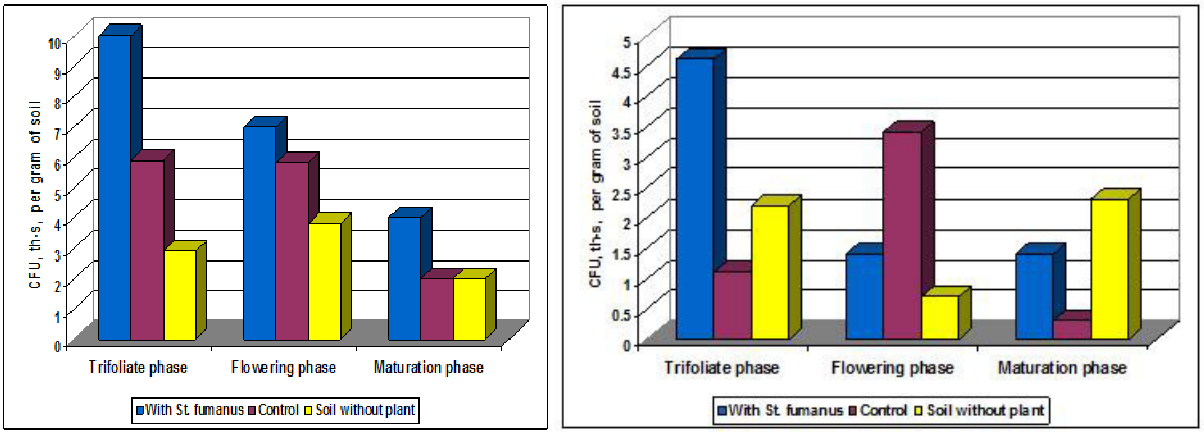


Figure 5. A.The number of ammonificators in the rhizosphere of soybean at different stages of vegetation; B. The number of actinomycetes in the rhizosphere of soybean at different vegetation phases.

Streptomyces, developed rapidly and were constantly at significant density (Figure 5A).This suggests that a balance is created between the rhizosphere's inhabitants due to the *Streptomyces* culture which produced the growth stimulating compounds, thus enhancing cell division and differentiation of root hairs. This results in increased number of lateral root hairs and in growth of the nutritional and respiratory surface root system. Its how once more that *Streptomyces* cells and mycelium take root in the rhizosphere; they do not exhibit an inhibitory effect on the development of saprophytic bacteria.

16S ribosomal RNA analysis revealed the biodiversity of bacteria that make up the rhizosphere microflora of the soybean in the till ring phase in the control variant. It was dominated by the true rhizosphere species of *Flavobacteriia phylum*, such as *Chryseobacterium* sp. RBA2, *Chryseobacterium* sp. QT2, *Elizabethkingia* sp., *Candidatus Chryseobacterium massiliae, Trichloroacetic* acid-degrading bacterium, uncultured bacterium, and others (Figure 6).

PCR assay found completely different species of ammonificators in the variant with biological product. Bacteria from *Bacteroidetes* phylum dominated: *Pedobacter borealis*, *Pedobacter kribbensis*, *Pedobacter agri*, *Pedobacter terrae*, *Bacteroidetes bacterium* 7-11, *Sphingobacteriaceae bacterium* and *Flavobacterium* sp. (Figure.7).

16S rRNA analyses from bulk soil detected mainly spore-forming bacteria, mostly representatives of *Firmicutes* phylum: *Bacillus aryabhattai*, *Bacillus subtilis*, *Bacillus horikoshii, Bacillus pumilus*, *Bacillus flexus*, *Bacillus megaterium* and *Bacterium* LWA-2-2 (Figure 8).

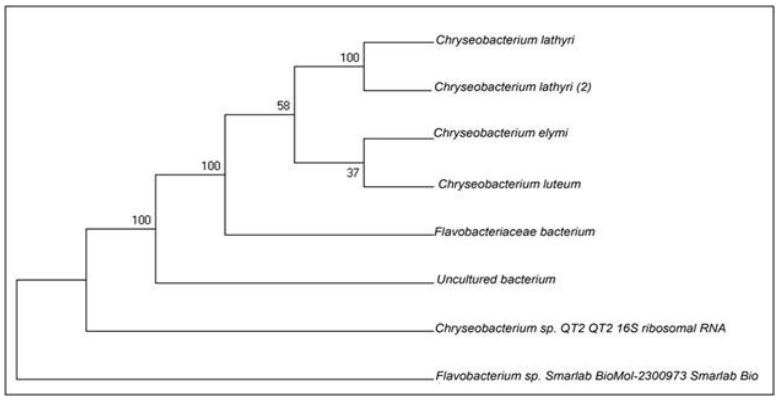


Figure 6. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from soybean rhizosphere in control variant

The number and biodiversity of oligotrophs by phases of soybean vegetation

The number of colony-forming units of actinomycetes was high in the tillering phase, and at the end of the growing season remained significant when the biological control microorganism was used. These figure indicate that the culture of *Streptomyces fumanus*, which is the basis of the biological product, survives perfectly among other species of *Streptomyces* in the root zone of soybean, reproducing intensively so that it reaches significant quantities there (Figure 5B).

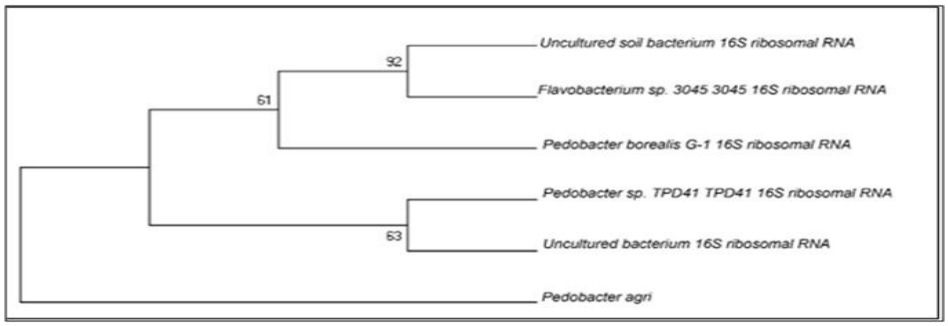


Figure 7. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from soybean rhizosphere in experimental variant.

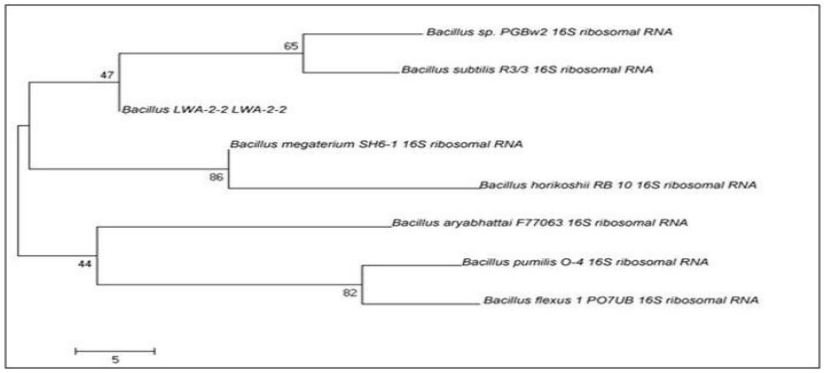


Figure 8. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the position of isolated bacteria strains from soybean rhizosphere in bulk soil

The number and biodiversity of nitrogen-fixing bacteria in growth stages of soybean

The influence of *Streptomyces fumanus* on the dynamics of nitrogen-fixing bacteria was evident. So, in the flowering phase, the number of nitrogen-fixers increased two-fold compared to bulk soil. In the maturation phase, the density of nitrogen-fixing bacteria also remained stable and relatively high (Figure 9).

Thus, as the results have shown, the effects of *Streptomyces fumanus* on the rhizosphere microflora of wheat (*Triticum aestivum*) differed from that of soybean (*Glycine max*) in relation to phases of plant development. In the shoot phase of wheat, we observed a marked restraining effect of biological agents on the development of ammonifiers in the rhizosphere. Since only at the maturation phase did their amount equal the control, it seems that a balance among bacteria-forming rhizosphere communities of wheat was established in the maturation phase. By contrast, the ammonificators in the presence of a biological agent have developed rapidly, and remained constantly in significant numbers in all phases of soybean.

16S ribosomal RNA analysis revealed a rich biodiversity of bacteria in the rhizosphere of wheat in the maturation phase, which differs from the biodiversity of bacteria in the rhizosphere of soybean in the same phase. Bacteria of *Microbacterium* genus from the *Actinobacteria* phylum dominated in the rhizosphere of wheat. The bacteria species of *Chryseobacterium* genus from the *Flavobacteriia* phylum dominated in the rhizosphere of soybean, which proves a different chemical composition of

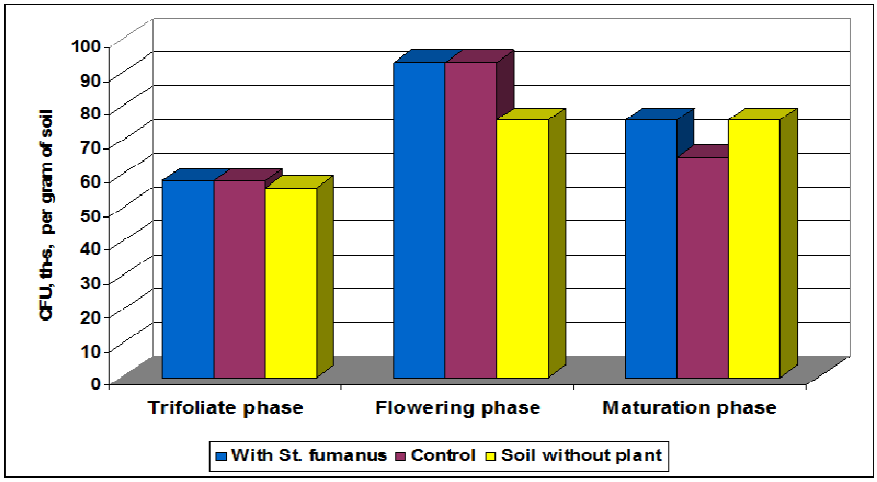


Figure 9. The number of colony-forming units of nitrogen-fixing bacteria in the rhizosphere of soybean at different vegetation phases.

Table1. The growth stimulating effect of *Streptomyces fumanus* biological product on seeds and seedlings of wheat and bean crops in newly cultivated soil

Dosage of biological product, cells/ml	Seed germination in%	Length of stem, mm	Root length, mm	Total biomass in grams
Wheat				
Strep.fumanus, gn-2, 10^4	100	164.0±0.37	142.0±0.15	23.32±0.47
Control, water	82.0	93.0± 0.65	65.0±0.54	15.25±0.53
Soybeans				
Strep.fumanus, gn-2, 10^4	100	95.0±0.38	150.0±0.19	29.86±0.43
Control, water	82.0	26.0±0.26	90.0±0.15	27.42±0.27

plant exudates attracting the preferred species of microorganisms.

The degree of *Streptomyces fumanus* survival in the rhizosphere of soybean was stable and higher than in the rhizosphere of wheat. Thus, at the end of vegetation in the variant treated with biological agent, the number of actinomycetes in the soybean rhizosphere was much higher than in the control and bulk soil.

The results allowed us to confirm that *Streptomyces fumanus* is an ideal biological agent for use against soil infections, due to its high colonization of the root system of soybeans and significant colonization of wheat. S*treptomyces fumanus* introduced into non-sterile soil entered into competition with the local soil microflora and had the ability to colonize the rhizosphere system of plants. Using *Streptomyces fumanus* through the seeds has improved the composition of the soil microflora, attracting saprophytic rhizosphere microorganisms

DISCUSSION

Streptomyces fumanus isolated from the rhizosphere was intended for seed and soil application as a biofertilizer to increase plant growth and to protect against pathogens in our study.

Biofertilizers have success criteria for wide applications: they have to be effective in actual field conditions, in a range of soils and in different host cultivars. Despite the low soil fertility and lack of irrigation water in the summer period, a treatment of seeds by a *Strep. fumanus* product showed a growth stimulatory effect on all phases of soybean and wheat, and ultimately increased biomass and grain yield overall (Table 1).

As shown in Table 1 above, the germination rate of the wheat seeds for eight days was 100%;the soybean seeds' germination rate for six days was 100%.The length of wheat shoots at eight days was 1.76 times higher than in controls. The length of soybean shoots was 3.65 times higher compared to the control in six days. The growth stimulatory effect on the root system of the seedling crop is expressed in the following terms: root length of wheat seedlings is 1.66 times higher than in the control; in soybeans it was 1.13 times greater than in the control. The biomass of wheat seedlings in eight days was1.52 times greater than in the controls; for soybean it was 1.08 times greater in six days.

It can be assumed that growth stimulating compounds produced by *Streptomyces fumanus* like auxin and cytokinins could enhance cell division and differentiation of root system and increase the number of lateral root hairs, consequently increasing the nutritional and respiratory surfaces of the root system as a whole. The healthier the root hairs, the more intensely they emit exudates, which are food for saprophytic bacteria (ammonificators). Cells and mycelium of *Streptomyces fumanus* have taken root in the rhizosphere, showing no inhibitory effect on the development of saprophytic bacteria. In all phases of vegetation, the ammonificators in the presence of an antagonist, the biological agent, developed rapidly and were constantly in significant numbers in the rhizosphere of soybean. This indicates there is some balance created between the rhizosphere inhabitants and the *Streptomyces* biological agent.

In general, there was no marked fungal infection in the root system of plants, and there was no single case of plant diseases on the crop area, despite the low content of organic matter in the soil. Root exudates of soybean provide a nutritional base for the growth of antagonistic organisms, which not only play an important role in controlling the soil phytopathogens, but also contribute to the active functioning of a very important group - nitrogen-fixing bacteria in the rhizosphere.

The results confirmed that the introduction of *Streptomyces fumanus* as a biological agent in soil alongside the seeds stimulated the growth and reproduction of microorganisms that are useful and important for the soil environment. It entered into competition with the local soil microflora and had the ability to colonize the rhizosphere system of the plants. The use of a formulation of *Streptomyces* gn-2 has improved the composition of rhizosphere microflora, attracting saprophytic microorganisms: ammonificators and oligotrophs. The presence of the biocontrol microorganism *Streptomyces fumanus* in the rhizosphere plays an important role in enhancing the growth and development of useful groups, such as nitrogen-fixing bacteria. The population densities and diversity of the root microflora affected the number and activity of *Azotobacter* bacteria, which may contribute to additional nitrogen inputs to the soil. So, the rhizosphere can be considered a microbiological buffer zone in which the microflora serves to protect the plants from attack by pathogens and to improve soil fertility.

CONCLUSIONS

Thus, the results obtained from experimental and that was not processed, cultivated or previously used as farmland leads us to the following conclusions:

- The use of *Streptomyces fumanus* as a biological agent has improved soil microflora composition, attracting saprophytic rhizosphere microorganisms.
- The use of *Streptomyces fumanus* as a biological agent hastened the phenophases of plants by 3-5 days, increasing the height of the plants and the size of the leaf blade, creating a hostile environment for the development of weeds and phytopathogens.
- *Streptomyces fumanus* is an ideal biological agent for use against soil infections, due to its high colonization

of the root system of soybeans and significant colonization of wheat.

- Treatment of seeds by biological fertilizer such as *Streptomyces* gn-2 is an important prerequisite for profitable crop production and ensuring a full and environmentally healthy crop.
- Treatment of seeds by biological fertilizer protects the culture, ranging from seed to plant, and provides the optimal stand density and minimum consumption of seeds (one seed - one plant).
- Treatment allows significant reduction of the burden on the environment.

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