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Anti-*Fusarium* Activity of Cyanobacteria and Actinomycetes in Soil and Rhizosphere

L. I. Domracheva, I. G. Shirokikh[1], and A. I. Fokina

Laboratory of Biomonitoring, Institute of Biology, Komi Scientific Center, Ural Division, Russian Academy of Sciences, and Vyatka State Humanitarian University, Republic of Komi, Russia

Abstract—The effect of cyanobacteria (*Nostoc linckia*, *N. commune*, and *Microchaeta tenera*) and streptomycetes on the pathogenic micromycete *Fusarium* was studied in laboratory simulation experiments. Interpopulational relationships in the rhizosphere of spring wheat (*Triticum aestivum* L.) and in soddy-podzolic soil were investigated.

Keywords: cyanobateria, streptomycetes, phytopathogenic fungi *Fusarium*, wheat rhizosphere.
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Among the phytopathogens causing mass diseases in cultivated plants, sometimes on the scale of epiphytoties, the fungi of the genus *Lusarium* play a special part. Due to their cosmopolitism, broad range of plant hosts, capacity for prolonged survival in a saprophytic phase, and progressing resistance to pesticides, fusaria may compete successfully for new ecological niches.

Similarly to other fungal diseases, chemical suppression of fusarioses in crops is limited by both emergence of resistant strains and unfavorable environmental consequences of pesticide use. Development of the biological techniques for plant protection and enhancing the suppressive effect of soils on fungal phytopathogens is therefore required [1]. The functional importance of a phytopathogen may be decreased by the introduction of certain microorganisms, including antagonists against a specific target and microbial preparations of complex composition [2]. Competitive microorganisms with high growth rates, capable of abundant spore formation and survival under nutrient limitation and other unfavorable conditions are the most promising antagonists [1].

Unlike heterotrophic microorganisms traditionally used in biopreparations, the rate of cyanobacterial propagation is comparable to that of phytopathogenic fungi. Cyanobacteria are known to form macroscopic growth (bloom spots) in soil, with a population density up to 4×10^7 cells/cm^2 [3]. Due to their capacity to fix both carbon and dinitrogen, they contribute significantly to soil fertility. Cyanobacterial exometabolites include a number of biologically active compounds, which activate the growth processes of higher plants [4]. Until recently, however, no information concerning the fungicidal activity of phototrophic microorganisms was available. Recent studies [5, 6] demonstrated that cyanobacteria are important natural antagonists of *Fusarium*. A lipopeptide with a pronounced fungicidal effect was recently isolated from the cyanobacterium *Nostoc commune* [7].

The presence of mucous sheaths makes cyanobacteria centers for microbial associations in nature. Actinomycetes are among the common satellites of cyanobacteria [8]. Since the capacity for synthesis of antibiotics and chitinases is widespread among mycelial prokaryotes, actinomycetes may act as natural plant protectors against phytopathogenic fungi. Being K-strategists, actinomycetes are incapable of rapid growth. Development of combined biopreparations based on cyanobacteria capable of active growth and actinomycetes, which are functionally active in algo–bacterial associations and exist there in a mycelial form, may be one of the approaches to overcome this contradiction [9].

Actinomycetes able to synthesize the agar-diffusing metabolites inhibiting or suppressing the growth of phytopathogenic *Fusarium* fungi have been reliably revealed in the rhizosphere populations of barley, red clover, and winter rye grown in soddy-podzolic soil [10, 11]. Few antagonistic actinomycetes, albeit with a pronounced antagonistic activity, were found in the topsoil of typical chernozem soil [12]. Since, however, stimulation of fungal growth was the main way of interaction between streptomycetes and micromycetes from the same habitat, the authors stressed the importance of caution in utilization of aboriginal streptomycetes for suppression of active phytopathogens and of meticulous selection of control agents. These issues still remain poorly investigated. For example, the interaction between streptomycetes, fungi, and cyanobacteria depending on specific species and strains remains unclear, as does the picture of antagonistic action in different soil microloci.

1 Corresponding author; e-mail: irgenal@mail.ru

Table 1. Antagonistic activity of actinomycetes against *Fusarium* phytopathogenic fungi

Strain	Growth inhibition zone, mm					
	F. sporotrichiella K-8999-K	*F. oxysporum* C-099-K	*F. avenaceum* 7/2	*F. avenaceum* 10/2	*F. sporotrichiella* KK-794-24	*Fusarium* sp. 21n-1
Actinomyces griseocastaneus A-24	30 ± 1.3	30 ± 0.8	26 ± 2.0	20 ± 0.3	29 ± 1.6	30 ± 2.7
S. felleus A-3	21 ± 0.5	20 ± 0.5	31 ± 1.0	20 ± 0	0	21 ± 2.0
S. hygroscopicus A-4	33 ± 1.2	29 ± 1.5	50 ± 1.3	35 ± 1.8	31 ± 1.8	30 ± 0.5
S. hygroscopicus A-9	35 ± 1.2	30 ± 1.2	46 ± 0.9	39 ± 1.0	20 ± 0.2	28 ± 0.5
S. hygroscopicus A-22	36 ± 1.0	18 ± 2.1	42 ± 1.9	42 ± 2.2	30 ± 1.6	26 ± 0.3
S. luteogriseus A-23	40 ± 0.4	24 ± 1.7	26 ± 1.5	25 ± 0	36 ± 1.0	41 ± 0.9
S. hygroscopicus 1-6-1	29 ± 0	24 ± 0.3	46 ± 1.4	49 ± 2.0	45 ± 1.7	34 ± 1.2
S. omiyansis 12-1	30 ± 2.3	22 ± 1.0	38 ± 1.5	40 ± 1.3	36 ± 1.5	30 ± 0.6

The goal of the present work was investigation of anti-*Fusarium* activity of actinomycetes and cyanobacteria under model conditions of soil and rhizosphere and determination of the cultures that may be used for control of the phytopathogenic fungal populations.

MATERIALS AND METHODS

The strains used in the present work included environmental *Fusarium* isolates from the Kirov oblast, cyanobacterial strains from the collection of the Department of Botany, Plant Physiology, and Microbiology, Vyatka State Agricultural Academy, and streptomycete strains from the collection of the Laboratory of Genetics, Rudnitskii Research Institute of Agriculture of the Northeast, Russian Academy of Agricultural Sciences.

Cyanobacteria were grown in nitrogen-free liquid Gromov no. 6 medium [13]. Streptomycetes were grown in mineral Gauze medium [14]. The mixed culture was obtained by combining cyanobacteria *Nostoc linckia* no. 273 and *Streptomyces luteogriseus* A-23 in liquid medium.

The antifungal activity of actinomycetes was preliminarily determined by the agar block method [15]. To determine the antifungal activity of cyanobacteria and mixed cultures, they were plated upon a lawn of *Fusarium* grown on Czapek medium. Antagonistic activity was determined as the diameter of growth inhibition zones. Each test was carried out in triplicate.

The suppressive effect of soil after introduction of various cyanobacterial species was determined in a simulation experiment. Sterile soddy-podzolic soil in petri dishes was inoculated with a suspension of *F. culmorum* macroconidia (1.8×10^5 cells/ml). The soil was simultaneously inoculated with one of the three cyanobacterial species (2.5×10^5 cells/ml).

The effect of pure cultures of *N. linckia, S. luteogriseus*, and their mixed culture on development of *Fusarium oxysporum* in the root zone of plants was determined as follows. The grains of spring wheat (Iren' variety) were infected with *Fusarium* macroconidia (9×10^5 conidia per grain). Five infected grains were placed into a petri dish with sod–podzolic soil. In the variants with microbial antagonists, infected grains were incubated for 1 h in liquid suspensions of the relevant cultures prior to plating. Each experimental variant was carried out in five repeats. After 7 days of sprouting, the seedlings were removed from soil, the roots and rhizosphere soil were sampled, and the number of spore structures and the fragments of fungal mycelium was determined by direct microscopy in smears [16]. Nine preparations were made for each sample.

The data were treated using the standard statistical techniques [17]. Average values of the parameters and their standard deviations are presented in the tables and figures.

RESULTS AND DISCUSSION

For initial determination of the anti-*Fusarium* activity of cyanobacteria and actinomycetes and selection of active strains, their effect on phytopathogenic fungi was studied in pure cultures. The experiments with actinomycetes demonstrated drying of the fungal mycelium and decreased level of conidia formation. Depending on the fungal test culture and actinomycete strain, the zones of suppressed growth varied from 18 to 50 mm (Table 1).

Placing of the films of cyanobacteria *N. paludosum* no. 18, *N. linckia* no. 273, and *Microchaeta tenera* no. 263 on the lawns of *F. oxysporum*, *F. nivale*, and *F. culmorum* resulted in growth delay, drying, and lysis of the fungal mycelium. Microscopy of inoculated plates demonstrated that the action of cyanobacteria resulted, apart from lysis of the fungal mycelium, in transition of the fungus from the active stage of development to sporulation, specifically, to chlamydospore accumulation.

Table 2. Dynamics of *F. culmorum* total mycelium length (mm/cm^2) in soil depending on the introduced cyanobacterial species

Days of experiment	Control	*Nostoc paludosum*	*Nostoc linckia*	*Microchaeta tenera*
1	0.65 ± 0.075	0.48 ± 0.023	0.62 ± 0.689	0
2	20.2 ± 4.100	0.14 ± 0.031	0.14 ± 0.027	0
3	>5000	0	0	0

Anti-*Fusarium* activity of cyanobacteria was also observed in soil in the simulation experiment without plants. Introduction of *F. culmorum* spores into the soil, addition of cyanobacterial cultures always resulted in suppression of the fungus manifested in a significant decrease in mycelial length compared to the cyanobacteria-free control (Table 2). The antifungal effect in soil developed gradually, increasing from the first to the seventh day. Introduction of *M. tenera* had an especially strong effect: even during the first day, fungal mycelium was not detected, while in the control variant, the fungus developed actively, with a total mycelium length of over 5 m/cm^2 of the seventh day.

In the tests of *F. oxysporum* lawns, the antifungal effect of *N. linckia* mixed with the streptomycete was preserved. The number of colony-forming units of the actinomycete *S. luteogriseus* in mixed culture (0.85×10^6 CFU/ml) was close to the value for the pure culture (1.35×10^7 CFU/ml). From the practical point of view, the changes in the anti-*Fusarium* properties of the mixed culture, compared to those of its individual components, in the plant rhizosphere, i.e., during the parasitic stage of fungal development. Significant differences in the population dynamics of *F. oxysporum* in soil without plants and in the rhizosphere have been reported [18], with the antifungal effect of antagonistic *B. subtilis* and *S. felleus* being more pronounced in the root zone of host plants than in greenhouse soil without plants.

Artificial infection of the seeds with *F. oxysporum* resulted in a 16% decrease in their germinating ability compared to the control. Treating of the infected seeds with a *N. linckia* culture not only eliminated the negative effect of the fungus, but increased the germinating ability by 12% compared to the control. Treatment with the pure culture of *S. luteogriseus* or its combination with *N. linckia* had no significant effect on the germination ability of infected seeds.

Unlike root-free soil, in the presence of an additional nutrient source in soil (root deposit of the seedlings), antagonists did not cause complete death of the fungus, although they suppressed its growth significantly (Table 2). The contact with antagonistic microorganisms resulted in a decrease of the vegetative fungal growth: increase in mycelial length on the roots and in the rhizosphere soils was significantly lower than in the control (Fig. 1). *N. linckia* had an especially pronounced inhibitory effect on mycelial growth: mycelial length on the seedling roots decreased 33-fold. In the case of the streptomycete and a mixed cu, a 3.3-fold and twofold decrease occurred, respectively. In the rhizosphere soil, inoculation with the mixed culture was the most efficient, causing a 20-fold decrease in the length of mycelium, compared to two- to threefold decrease in the case of the individual components. The density of mycelium is believed to indicate conditions favorable or unfavorable for fungal growth [19]. Decreased density of *F. culmorum* mycelium in soil and on the roots in the presence of microbial antagonists, including *Pseudomonas fluorescens*, was reported previously, with competition for nutrients suggested as the mechanism of the antagonistic effect [20].

The intensity of the infection process caused by phytopathogenic fungi is determined to a significant degree by the number of individual mycelial fragments, rather than by the total mycelial length. The higher the number of these fragments, the more intensely the colonization of space (and therefore infection of plants) occurs [6]. Direct microscopic count demonstrated that although the number of mycelial fragments in the experimental variants fluctuated within a broad range, it decreased in the variants with treatment with antagonistic cultures. This decrease was more pronounced for the roots and less for the rhizosphere soil (Fig. 2). *N. linckia* was the leader in this respect. *S. luteogriseus* was significantly

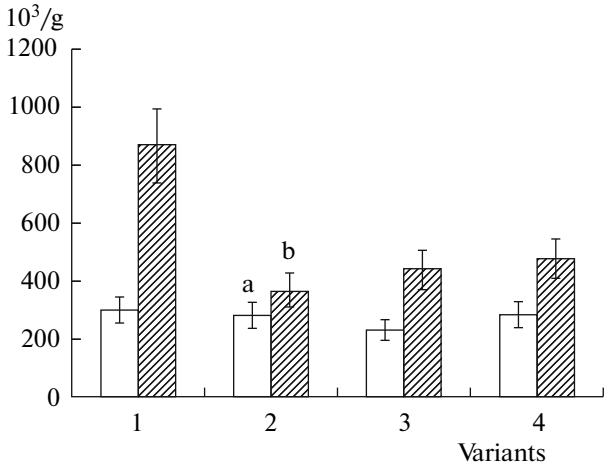

Fig. 1. Effect of antagonistic microorganisms on mycelial growth of *F. oxysporum* in the rhizosphere (a) and rhizoplane (b) of wheat seedlings: control (*1*), *S. luteogriseus* (*2*), *N. linckia* (*3*), and *N. linckia* + *S. luteogriseus* (*4*).

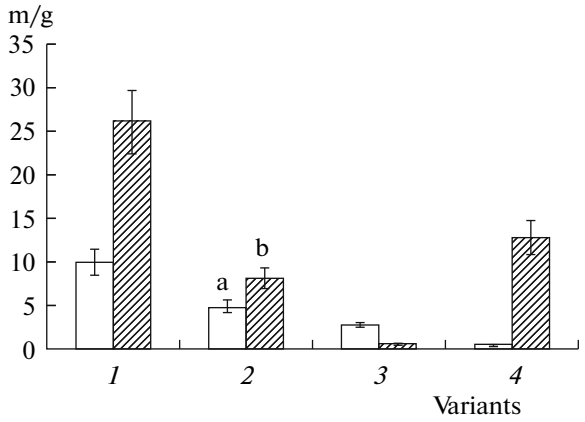

Fig. 2. Number of the fungal mycelial fragments in the presence of antagonistic microorganisms in the rhizosphere (a) and rhizoplane (b) of wheat seedlings: control (*1*), *S. luteogriseus* (*2*), *N. linckia* (*3*), and *N. linckia* + *S. luteogriseus* (*4*).

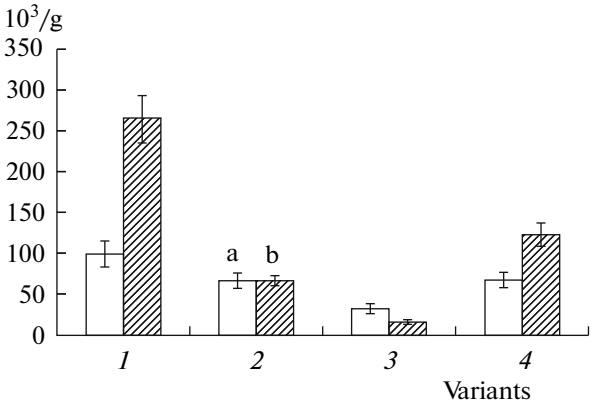

Fig. 3. Number of *Fusarium* propagule in the rhizosphere (a) and rhizoplane (b) of wheat seedlings: control (*1*), *S. luteogriseus* (*2*), *N. linckia* (*3*), and *N. linckia* + *S. luteogriseus* (*4*).

less efficient both in pure and mixed cultures. Comparison of the average length of mycelial fragments in the rhizoplane revealed that it was the lowest for *N. linckia* culture (47.9 ± 10.2 μm) compared to 97.7 ± 10.4 μm in the control, 119.9 ± 10.5 μm for *S. luteogriseus*, and 119.9 ± 21.0 μm for *N. linckia* + *S. luteogriseus*.

Sporulation ensures survival and propagation of the fungi with cessation of vegetative growth. Inoculation with antagonistic cultures resulted in a significant decrease in the number of spore structure on the root surface, but not in the rhizosphere soil, where the differences between the variants were not reliable (Fig. 3). According to the literature data [21], sporulation intensity is determined by mycelium density in a given type of soil. In our case, a decrease in mycelium density in the rhizosphere soil within an order of magnitude did not affect spore production by *F. oxysporum*.

However, the ratio of different spore types formed in the rhizoplane and in the rhizosphere soil was shown to vary depending on the variant of inoculation. For example, in the control rhizosphere soil, the number of macroconidia, microconidia, and chlamydospores was almost equal, while microconidia predominated on the roots (78%) (Table 3). In the presence of *N. linckia*, which was most efficient in suppression of mycelial growth, formation of microconidia in the rhizosphere soil was stimulated. Their ratio there increased from 41 to 50%, while remaining almost unchanged on the roots (82%). In the rhizoplane of this variant, macroconidia were not detected, while the ratio of chlamydospores was similar to the control. In this case the fungal strategy was probably oriented to space colonization by mass production of the lightest and smallest spores, which may be transferred via great distances.

Table 3. Ratio of *F. oxysporum* reproductive structures in the rhizosphere and rhizoplane of wheat seedlings

Variant	Locus	Reproductive structures, %		
		Macroconidia	Microconidia	Chlamydospores
Control	1	3 ± 0.5	78 ± 4.5	19 ± 0.5
	2	25 ± 7.1	41 ± 2.9	34 ± 1.2
S. luteogriseus	1	28 ± 1.5	39 ± 5.7	33 ± 2.6
	2	23 ± 1.1	46 ± 2.6	31 ± 1.5
N. linckia	1	0	82 ± 5.2	18 ± 1.5
	2	42 ± 2.1	50 ± 1.7	8 ± 0.6
N. linckia + *S. luteogriseus*	1	13 ± 1.0	65 ± 8.6	22 ± 3.8
	2	54 ± 2.5	15 ± 1.5	31 ± 5.7

Note: 1 stands for the rhizoplane and 2 for the rhizosphere.

Almost no effect of the streptomycete on the reproductive characteristics of *F. oxysporum* was observed in the rhizosphere soil. The ratio of all spore types was the same as in the control. In the same variant, the highest ratio of macroconidia (28%) and chlamydospores (33%) occurred. Due to their rigidity, resistance, and ability to survive in soil for long periods, they are considered structures used to survive unfavorable conditions. The stimulation of formation of thick-walled chlamydospores probably resulted from the ability of *S. luteogriseus* to produce toxic metabolites with antifungal effect. Increased chlamydospore production by *F. oxysporum* was previously found in the acid-treated rhizosphere of red clover [22]. Inoculation of the seeds with a mixed culture of *N. linckia* and *S. luteogriseus* resulted in an increase in the ratio of the spores assuring survival under unfavorable conditions. While in the rhizosphere, soil of the control microconidia prevailed (41%), inoculation with mixed culture resulted in predominance of macroconidia (54%), which are more inert and robust structures capable of both survival and propagation. On the seedling roots inoculated with the *N. linckia* + *S. luteogriseus* mixed culture, the ratio of macroconidia increased from 3% in the control to 13%.

One of the indices of suppression of fungal development caused by microbial antagonists is the level of specific spore production (number of spores per 1 m of mycelium), which reflects the mobilization of internal reserves of the fungus for reproduction as a response to an unfavorable biotic factor. The highest level of specific production for *Fusarium* propagule (in this case, the sum total of macroconidia, microconidia, and chlamydospores) was observed in the variant treated with *N. linckia* (Table 4). In other experimental variants, specific production of *F. oxysporum* propagule was an order of magnitude lower and did not differ reliably from the control.

Thus, laboratory testing demonstrated that both antagonists exerted a significant effect on the structure of the *F. oxysporum* population, to a greater (*N. linckia*) or smaller degree (*S. luteogriseus*) inhibiting the growth of fungal mycelium in the root zone of wheat seedlings. While in the rhizoplane the antagonistic activity of *N. linckia* was responsible for the inhibition of mycelial growth, which has the highest infective potential, the highest inhibition of mycelial growth in the rhizosphere occurred when the mixed *N. linckia* + *S. luteogriseus* culture was used.

In the soil saturated with microbial antagonists, the number of *F. oxysporum* mycelial fragments in the rhizoplane and rhizosphere decreased drastically. Suppression of mycelial growth by the cyanobacterium was accompanied by an increase in the fungal reproductive function, i.e., increased specific production of spores, which are more tolerant to antagonistic action. The stimulating effect of the streptomycete on specific production of fungal propagule was not revealed. However, the ratio of inert and robust reproductive structures (macroconidia and chlamydospores) increased in the presence of the actinomycete.

Table 4. Specific production of *F. oxysporum* propagule in the rhizosphere and rhizoplane of wheat (10^3/m mycelium)

Variant	Rhizoplane	Rhizosphere
Control	23.1 ± 4.0	33.2 ± 3.0
S. luteogriseus	37.4 ± 3.6	45.8 ± 4.3
N. linckia	531.7 ± 54.6	552.5 ± 49.8
N. linckia + *S. luteogriseus*	27.6 ± 7.4	37.3 ± 3.2

The changes in the population structure of the phytopathogenic fungus *F. oxysporum* caused by treatment of the seeds by microbial antagonists indicate the possibility of successful development of artificial phototrophic–heterotrophic associations. In the future, binary preparations combining microorganisms differing in life strategies and nutrient requirements may well be useful for enhancement of suppressive activity of soils and for biocontrol of phytopathogens in the plant rhizosphere.

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