Journal of International Scientific Publications: Agriculture and Food

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

PERSPECTIVES OF USE OF MICROORGANISMS AGAINST SOYBEAN PATHOGENS IN KAZAKHSTAN

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Abstract

In laboratory studies revealed a Bacillus isolates and strains of fungi of the genus Trichoderma, with high antagonistic activity to the agents of fungal diseases of soybean. Selected strains are promising as a basis for the creation of biological products for the protection of soybean pathogens and increase yields.

Key words: *Soybean, Fusarium, Alternaria, Sclerotinium, isolates, Bacillus, Trichoderma, antagonism*

Due to population growth in the world there is a shortage of high-protein foods and protein feeds, which can be replenished by producing legumes such as soybeans. Soy - one of the most important food crops in the world and its protein contains all essential amino acids, and that the most similar in composition to the protein of meat (Petibskaya, 2004; Tokbaev et al., 2006; Mordvintsev, 2008; Fadi et al, 2010).

Soy is one of the most sensitive crops to the pathogens, it may be affected simultaneously by 30 different pathogens. Soybean diseases lead to lower yields by 40-50% and more. The most aggressive disease pathogens are fungi of the genus *Fusarium, Alternaria and Sclerotinium* and other producing dangerous mycotoxins contaminating grain, causing severe disease in humans and animals.

Since the products of soybean used in dietetic food, medicine, food and confectionery industries, must decline in the use of chemical seed dressing, replacing them with biological products to prevent contamination of food products derived residual amounts of fertilizers and pesticides. For these purposes, the most suitable drugs based on live microbial cultures - bacteria and fungi capable of producing the complex of the antibiotic substances, of the lytic enzymes and causing antagonism broad spectrum of phytopathogenic fungi.

In recent years, promising targets for agricultural biotechnology are soil-fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus*, which showed its effectiveness as antagonists of a wide range of plant pathogens in many crops (Shcherbakova, 2013; Sadykova et. al., 2010; Sharikov, 2011; Mishra et al, 2012; Shternshis, 2012).

Bacteria of the genus *Bacillus* with artificial inoculation of seed capable long time to develop and dominate in the root zone of plants, maintaining competition from native and phytopathogenic microflora (Kalieva, 2001). Antagonistic activity of *Trichoderma* fungi caused production of plant hormones, antibiotics, and the ability to rapid colonization of the substrate (Kolombet, 2005, 2010; Gneusheva et al, 2010).

Using cultures of microorganisms and their metabolites as an alternative biological method of plant pathogens can reduce the number of chemical treatments, to minimize crop losses, keep commodity product quality and ecological balance in the biocenosis (Sazonov, 2007; Menkis et al, 2009; Michat, 2010; Hysek et al., 2005; Shoresh et al., 2010).

Currently, on the basis of microorganisms for biological control of plant pathogens produced a variety of drugs, but the ability to use and their effectiveness depends on the survival of strains belonging to the biologics, their relations with the native microflora and pathogens of plants, soil climate and other regional conditions. Today biologics maximum adapted to the conditions of Kazakhstan has not been developed, and existing import biologics due to their unsuitability to the natural conditions of ineffective.

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MATERIALS AND METHODS

Research objects were vegetable soybeans (beans, stems, pods) with signs of disease. Bacterial isolates of the genus *Bacillus* (101, 102, 103, 104, 105), and microscopic fungi of the genus *Trichoderma* (five strains) derived from soybean rhizosphere.

Isolation of plant pathogens from the samples were conventional by microbiological methods (Bilai, 1973; Egorov, 1983). Identification of microorganisms was performed using the respective identifiers (Pidoplichko, 1977; Bergey, 1997; Sutton et al, 2001). The study of the antagonistic activity was carried out by methods wells and agar blocks (Egorov, 2004).

RESULTS AND DISCUSSION

From the affected soybeans samples (beans, pods, stems) was allocated 6 isolates microfungi. Morphological studies have attributed them to the genus *Fusarium* (2 isolates), *Alternaria, Cladosporium, Bipolaris* and *Sclerotinia* (Figures 1,2).

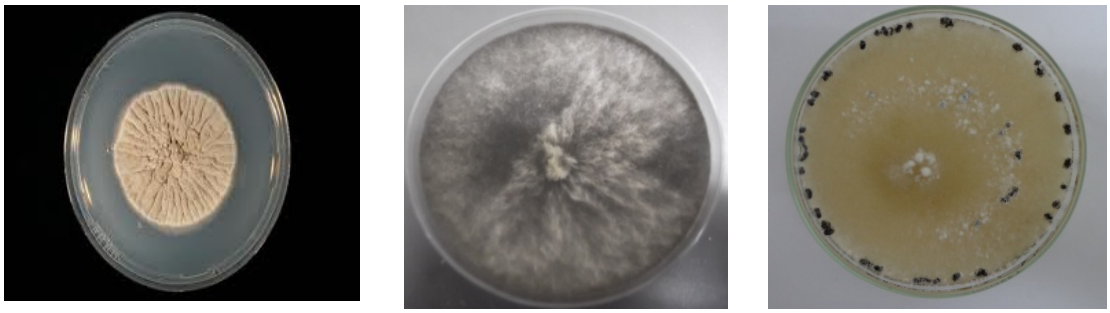


beans pods stems

Figure 1 - Isolation of pathogenic fungi from the soybeans samples on culture medium in Petri dishes



Fusarium sp. №1 *Fusarium sp.* №2 *Alternaria, sp.*



Cladosporium sp. *Bipolaris sp.* *Sclerotinia sp.*

Figure 2 - Pathogenic fungi isolated from the soybeans samples

From the rhizosphere of soybean plants with signs of disease (Figure 3), allocated five bacterial isolates of the genus *Bacillus* (101, 102, 103, 104, 105) and five fungal isolates of the genus *Trichoderma* (F-1, 175, 30, 1M, 10) as potential biocontrol agents for soybean plant pathogens.



leaves stem root system soil

Figure 3 - Soybean plants affected by fungal diseases

The study of the antagonistic activity of bacterial isolates of bacteria of the genus *Bacillus* revealed that the two strains (101 and 103) acted on all isolates of the phytopathogens. The strains 102 and 104 had no effect on *Alternaria sp.* and *Bipolaris sp.* All bacterial strains were active on the growth and development of fungi of the genus *Sclerotinia* and *Cladasporium*. The most inhibitory effect had the strains 101 and 103 (zone of inhibition growth of the *Sclerotinia sp*. and *Cladosporium sp.* were 5.0 x 8.0 x 6.0 cm and 6.0 x 6.0 cm respectively). Bacterial strain 105 had no effect on isolates *Fusarium sp.* 1 and 2 and isolate *Alternaria sp.* While on fungi *Bipolaris sp*. and *Cladasporium sp*. growth inhibition zone were 2.8 x 2.9 cm and 3.0 x 3.5 cm respectively (Table 1).

Table 1 - The antagonistic effect of the *Bacillus* bacteria on the growth of soybean plant pathogens

Isolated of the phytopathogens	Zones of inhibition of growth, cm.				
	101	102	103	104	105
Fusarium sp.№1	3.5 x 3.0	2.0 x 2.5	5.0 x 5.0	0	0
Fusarium sp. №2	4.0 x 4. 5	3.0 x 2.0	5.5 x 5.0	0.5 x 0.7	0
Alternaria sp.	0.1 x 0.2	0	3.0 x 5.5	0	0
Cladosporium sp.	3.0 x 3.0	4.0 x 3.0	6.0 x 6.0	2.0 x 3.0	2.8 x 2.9

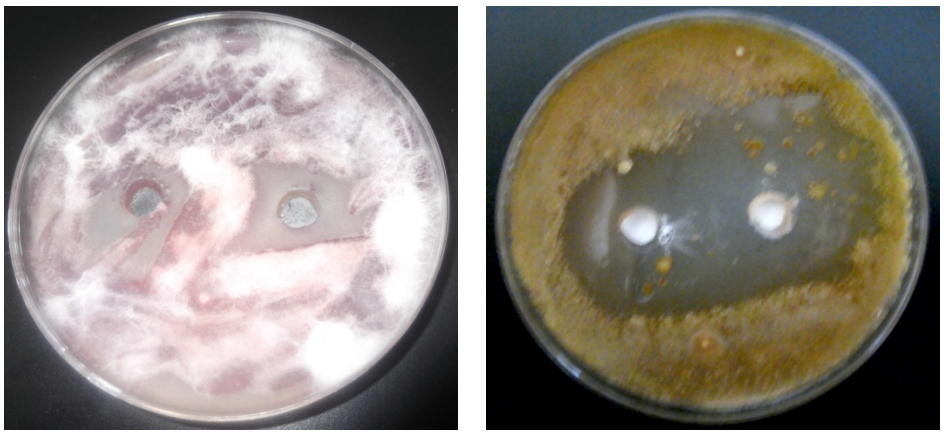
Bipolaris sp.	3.0 x 3, 0	0	2.0 x 2.5	0	3.0 x 3.5
Sclerotinia sp.	5.0 x 8.0	2.0 x 2.5	5.0 x 5.5	3.0 x 4.0	0.3 x 0.5

It has been established that the bacterial isolates are not only growth inhibition of the pathogen, also they affect the morphological signs.

Fungi of the genus *Sclerotinia* in normal form sclerotia. The isolate of the fungus *Sclerotinia sp*. at action the bacterial strains 101 and 103 forming sclerotia were not observed.

Lysis of mycelium of the isolates *Bipolaris sp.* and *Cladasporium sp.* was observed when the bacterial strain 105 inhibited them. Also in the microscopic study revealed no full reproductive organs. Discoloration of the mycelium from pink to the beige brown of the isolate *Fusarium sp.* 2 under the effect of strains 101 and 103 was marked.

For comparison, 102 isolates showed an inhibitory effect, but did not cause discoloration (Figure 3).



Isolate 102 Isolate 103

Рисунок 3 - Ингибирующее действие изолятов *Bacillus sp.*102 *и* 103 на *Fusarium sp.* №2

Studies of antagonistic activity of isolates of the fungus *Trichoderma*, isolated from the rhizosphere of soybean showed their high activity. All isolates inhibited the growth of pathogenic fungi, the zones of inhibition of growth ranged from 1.0 x 1.0 cm to 6.0 x 6.3 cm

Table 2 - The antagonistic effect of *Trichoderma* isolates microfungi on the growth of soybean plant pathogens

Isolated of the phytopathogens	Zones of inhibition of growth, cm.				
	F-1	175	30	1M	10
Fusarium sp.№1	1.5 x 2.0	5.0 x 5.5	4.0 x3.5	2.5 x 3.0	3.2 x 3.5
Fusarium sp. №2	6.0 x 6.3	4.1 x 3.5	2.0 x 2.0	4.5 x 4.7	3.0 x3.3
Alternaria sp.	3.6 x3.8	3.0 x 3.0	3.2 x 3.1	2.2 x1.2	1.5 x 1.3
Cladosporium sp.	1.5 x 1.8	2.0 x 2.0	1.2 x 1.5	3.4 x 3.2	3.2 x 3.2
Bipolaris sp.	1.0 x 1.0	2.5 x 2.0	1.1 x 1.0	2.0 x 2.0	1.0 x 1.0

Sclerotinia sp.	3.4 x3.4	2.0 x 2.0	1.7 x 1.5	3.2 x 3.5	1.9 x 1.5

The strain number 175 of the fungus *Trichoderma* had the highest activity in suppressing of the growth of the *Fusarium sp.* № 1 (zone of inhibition was 5.0 x 5.5 cm) - Figure 4.

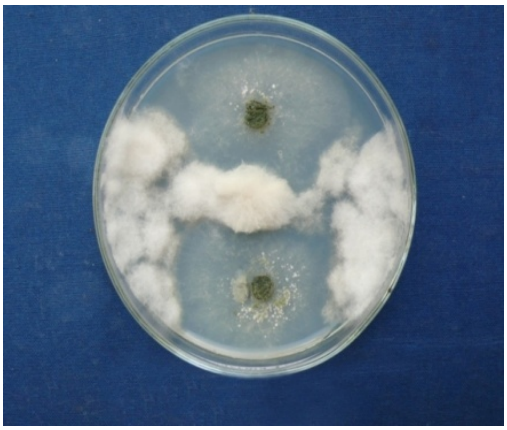


Figure 4 - The antagonistic activity of the fungus *Trichoderma* strain number 175 against isolate *Fusarium sp.* № 1

The most antagonistic activity of strain *Trichoderma F-1* noted against of the isolate *Fusarium sp. F-2* (zone of growth inhibition was 6.0 x 6.3 cm). Strains 175 and 30 were effective against *Alternaria sp.* (zones of inhibition of growth were 3.0 x 3.0 and 3.2 x 3.1 cm, respectively) - Figures 5, 6.

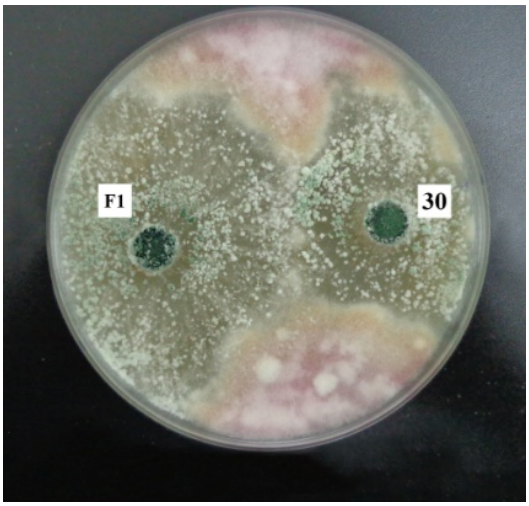


Figure 5 - The antagonistic activity of *Trichoderma* fungi strains F-1and 30 against phytopathogen *Fusarium sp.* № 2

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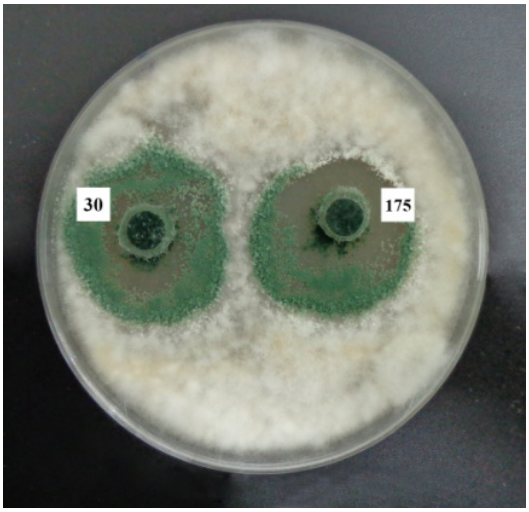


Figure 6 - The antagonistic activity of *Trichoderma* fungi strains 30 and 175 against phytopathogen *Alternaria sp*.

The *Trichoderma* strains 1M and 10 showed the greatest antagonistic activity against isolates of *Fusarium sp.* № 1,2 and *Cladosporium sp.* Phytopathogen *Sclerotinia sp*. was inhibited of the all strains of fungi of the genus *Trichoderma*, and the most inhibitory effects have strains 1 and F-1M (growth inhibition zones were 3.4 x 3.4 and 3.2 x 3.5 cm respectively).

Inhibitory effect of the *Trichoderma* may be due to the growth on colony of the pathogen and formation sporulation on it surface, that is manifested biparasitic activity. A similar effect was observed in the strain number 10 against Sclerotinia sp. (Figure 7).

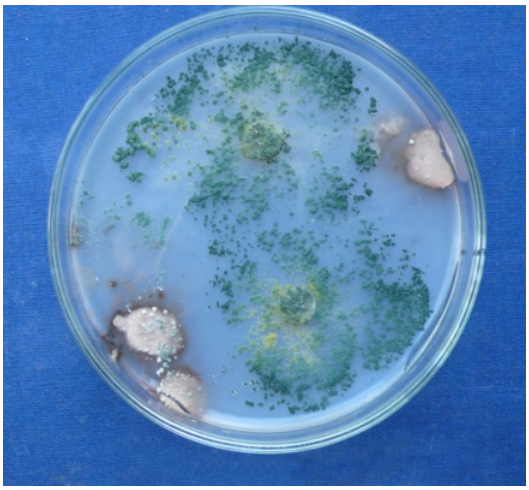


Figure 7 - Biparasitism of the strain of the fungus *Trichoderma* number 10 against *Sclerotinia sp.*

It should be noted that unlike from the bacterial isolates 101 and 103 fungal strains of the genus *Trichoderma* not cause of morphological changes of phytopathogenic fungi.

Thus the bacterial strains of the genus *Bacillus*, and microfungi of the genus *Trichoderma* in varying degrees have an inhibitory effect on soybean phytopathogens.

Screening microorganisms allowed to select the most active strains of bacteria of the genus *Bacillus* (101 and 103), and fungi from the genus *Trichoderma* (F-1, 30 and 1 M) having the highest antagonistic activity, which can be used subsequently as the basis for creation of biologics to combat against soybean diseases such as *Fusarium, Alternaria, Sclerotinia*, etc. Use of microbial antagonists lead to a reduction of infectious diseases of soybean caused by phytopathogenic fungi this will increase soybean yields and keep it commercial quality.

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