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# BIOLOGICAL METHOD PROTECTION OF THE SUGAR BEET AGAINST PATHOGENIC FUNGI CAUSING ROOT ROT

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**Abstract**

*From the rhizosphere of the healthy plants of sugar beet, 43 new strains of cellulolytic bacteria were isolated, 12 strains have antagonism against pathogenic fungi of the genus Fusarium, causing root rot and seedling of sugar beet. Two strains of bacteria with high antifungal and growth promoting activities were selected. Field tests have shown, that inoculation seeds of the cellulolytic bacteria infestation of fusariosus seedling decreased by 2.0-2.4 times, and the prevalence of root crops - 2.5-3.0 times as the control and increase sugar beet yield amounted to 30-34 c/ha. On the basis of these strains was developed a biological method for stimulating the growth and protection of sugar beet against root rot.*

**Key words:** *cellulolytic bacteria, pathogenic fungi, disease of plant, rot root, sugar beet*

## INTRODUCTION

At present in the Republic of Kazakhstan is an acute problem to improve the environmental situation and, in this regard, much attention is paid to agricultural biosafety**.** One of the most valuable commercial crops is sugar beet. At the same time, the sugar industry of Kazakhstan is working mainly on imported raw materials, a large number of finished product is imported into the country. Therefore, the decision of the Government plans to expand the area under the crop and increase its production in the Republic. Due to lack of resources of producers simplified technology of crops and, in this regard, the phytosanitary condition deteriorated arable land. Change in the economic structure of the country has resulted in technological violations of sugar beet. Especially noticeable loss of the influence of biotic stress factors on irrigated lands, where sugar beets are grown. For example, in 2010 the sugar beet crop losses from diseases caused by phytopathogenic microorganisms, reached 30%. On sugar beet in Kazakhstan recorded more than 20 species of pathogens, the most common and most dangerous disease of root crops, caused by fungi of the genus *Fusarium* (Dzhanuzakov, Agataev, 2001; Maui, 2003; Abugaliev, Kostin. 2007). According to several authors reduce root rot of sugar beet harvest by 50 percent or more (Kazenas, 2005; Agataev, 2002; Maui, Ismuhambetov root crops, 2012). Therefore, without addressing the protection of plants against pathogens can improve the efficiency and stability of the sugar beet.

The using of plant protection chemicals has several negative consequences: the formation of resistant strains pathogens, reducing the number of beneficial microorganisms in the microbial and accumulation of toxic substances in the soil (Reddy et al, 2009). An alternative approach involves the creation of biological methods of plant protection from diseases through the using of biological products. The basis of such biological products - highly antagonistic strains of bacteria pathogens (Novikova, 2003, Labutova, Suo et al., 2011).

Cellulose is degradated by many groups of microorganisms, but the basic role in degradation of cellulose belongs to fungi and bacteria. Fungi have more powerful fermentative systems for destruction of cellulose, but cellulolytic bacteria are characterized by a high growth rate and a high degree of population stability. In a course of evolution there has been competition between these by two groups of microorganisms for the source of carbon and energy - cellulose. And as a result of this competition bacterium have developed substances capable of suppress growth and development of fungi (Smirnova, 2004).

In this respect, the most promising is the use of cellulolytic bacteria, physiological and biochemical properties (high growth rate, simplicity to power sources, easy cultivation, etc.) provide them with high technology the production of biomass. In addition, due to their biological characteristics, such as population stability and ability to produce antifungal metabolites are active antagonists of fungi. In this regard, have suggested that some of them may be present antagonist activity of pathogenic fungi that cause root rot of sugar beet.

The objective of the study was the selection of the rhizosphere and rizoplana healthy sugar beet cellulolytic bacteria - antagonists and growth promoters, the selection of the most active strains. On the base of these strains

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a biological method for plant protection against root rot and stimulation of growth of sugar beet will be developed.

## MATERIALS AND METHODS

From rhizosphere and rizoplana healthy plants of sugar beet from Almaty region of Kazakhstan cellulolytic bacteria have been isolated and selected. The isolation cellulolytic bacteria from various natural substrates containing cellulose such as dead roots, leafs, bit of steams and soil. The pure cultures of cellulolytic bacteria were select on the Hutchinson medium with sources of carbon native cellulose - wheat straw.

As phytopathogenic test cultures using fungi *Fusarium: Fusarium oxysporum M2* and three strains of *F. solani*: *F. solani (ros.), F. solani M3, F. solani M4.*

These phytopathogens have been isolated from diseased seedlings and sugar beet in 2010 - 2011, in Almaty and Zhambyl region of Kazakhstan and identified by the authors (Figure 1).



healthy seedling diseased seedling root rot of root crops

Figure 1. The defeat of the roots of seedlings and root crops of sugar beet by the root rot

Cultivation of bacteria was carried out on the liquid and solid medium Hutchinson. As a source of cellulose was used wheat straw. Fungi grown on mediums Chapek-7 and Saburo.

Antagonist activity was determined by growth inhibition zones of phytopathogenic fungi (Egorov, 2001). For that cellulolytic bacteria were grown in a liquid medium Hutchinson. Test culture inoculated with pathogenic fungi profound way in melted and cooled to $40^0$C medium and filled into Petri dishes. After solidification of the agar medium cut blocks. In flooded wells received cell suspension of bacteria with a $1\times10^6$ cells/ml per 0.2 ml per well. The plates were incubated at $28^0$-$30^0$C in an incubator for 10 days or more. Accounting results were largest areas of growth suppression test cultures.

To study the growth promoting activity of bacterial strains were grown on medium Hutchinson for 5-7 days. Sugar beet seeds before planting were inoculated bacterial suspension at $1\times10^6$ cells per 1 g of seeds for 6-8 hours at room temperature. In the control of seeds soaked in sterile water. After 7 days, germination was determined, and after 10 days was carried out measurement of the aerial parts and roots.

All results were statistically processed (Rokitsky, 2007).

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## RESULTS AND DISCUSSION

From the rhizosphere and rizoplana healthy sugar beet plants were isolated 43 cultures of cellulolytic bacteria belonging to the genera *Cellulomonas*, *Bacillus*, *Brevibacterium* and *Flavobacterium*. From them 18 were selected strains with antagonism against phytopathogenic fungi of the genus *Fusarium*, causing root rot of sugar beet. In a detailed study, were selected 12 strains with increased antifungal activity. With these strains was continued further work.

Data for the study of cellulolytic bacteria antagonistic activity against phytopathogenic fungi causing root rot of sugar beet presented in Table 1.

Table 1. The antifungal activity of cellulolytic bacteria

| Strains of the bacteria | The average diameter of fungal growth suppression zones, mm | | | |
|---|---|---|---|---|
| | *F. oxysporum M2* | *F. solani (ros.)* | *F. solani M3* | *F. solani M4* |
| 21(8) | 26±1.3 | 25±1.3 | i.g. | 20±0.8 |
| 21N | 0 | 15±0.7 | 0 | 0 |
| 22T | 27±1.1 | 25±1.2 | 20±0.8 | i.g. |
| 60(5)4 | 27±0.8 | 28±0.6 | i.g. | 16±0.5 |
| 80 | 0 | 23±1.0 | i.m. | i.m. |
| 82 | 34±2.1 | 27±1.4 | 22±1.0 | i.g. |
| 95 | 0 | i.m. | i.m. | 0 |
| 150 | i.m. | i.m. | i.m. | 18±0.7 |
| 158 | 12±0.3 | i.m. | i.m. | 20±0.8 |
| 177 | 25±0.5 | i.g. | i.g. | 22±1.1 |
| 212(м) | 12±0.2 | i.g. | 0 | 0 |
| 604 | 28±1.5 | i.g. | i.g. | 28±1.6 |

Note: i.m. - complete suppression of formation of aerial mycelium throughout Petri dishes; i.g. - complete suppression of fungal growth throughout Petri dishes

From Table 1, it follows that all the investigated strains have antifungal activity against fungal test - organisms. Shown that the activity varies greatly depending on the strain of bacteria and fungal phytopathogen investigated. Found that the antagonistic activity of bacteria against phytopathogenic fungi manifest as a fungicidal or fungistatic activity. Fungistatic activity was expressed in the partial or complete suppression of the formation of aerial mycelium and sporulation of fungi or rarefaction growth in all Petri dishes, fungicide as a complete or partial inhibition of fungal growth (Figure 2). A number variants was also discoloration pigmentation fungi.

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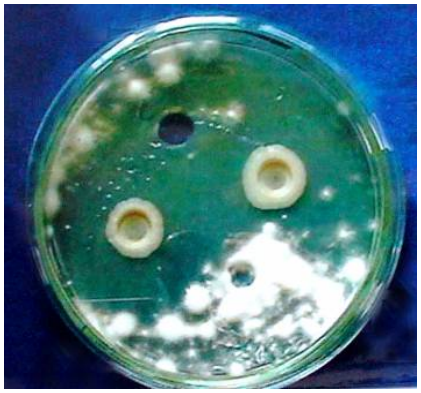
*F. oxysporum M2*



*F. solani M3*



*F. solani (ros.)*



*F. solani M4*

Figure 2. Effect of strains of cellulolytic bacteria on phytopathogenic fungi of the genus *Fusarium*

Established that the eight of the 12 cultures of cellulolytic bacteria strains inhibited the growth and development of the fungus *F. oxysporum M2*, three strains did not have antagonistic effect against this fungus. All investigated strains possessed antifungal activity against fungus *F. solani (ros.).* Also shown that almost all the strains of bacteria inhibited the growth of pathogenic fungi F. *solani M3* and *F. solani M4*, with the exceptions of the three strains 21N, 212 (m), and 95 had no effect on these phytopathogens. According to the results obtained by studies, the 6 strains, characterized by a high antagonistic activity to test cultures of pathogenic fungi: 21(8), 22T, 82, 177, 60(5)4 and 604.

To study the ability of strains of cellulolytic bacteria to stimulate germination and growth of sugar beet plants in the laboratory experiments were conducted. As cultures have used 12 strains of cellulolytic bacteria. For the tests were taken seeds of different varieties of sugar beet "Rielta", "Yaptushkovskaya 30" and "Kaz-Sib-14", recommended for use in Kazakhstan. Studies on the growth promoting activity of the strains was carried out pots 250 ml, the amount of soil -150 g (Figure 2).



Figure 3. Experiments on the growth promoting activity of cellulolytic bacteria on sugar beet seedlings

The results showed that the pre-treatment of seeds cellulolytic bacteria are stimulated germination and seedling growth in all studied varieties of sugar beet. It was established that the effect of the strains on different varieties of sugar beet is not identical. Thus, in variants the experience with beet cultivar "Rielta" found that bacterial cultures from seven effectively enhance germination six plants (on average 30-49% compared to control). In these variants the same experiment showed a significant increase in the length of the germ (10-12%), the average root length (25-30%). It should be noted that this kind of beet has a low germination rate (51%). Also, it was found that the variety is weakly resistant to diseases caused by phytopathogenic fungi. In control variant more than half of Fusarium seedling noted lesion (Figure 4). When processing cellulolytic bacteria even in variants where there was no effective stimulation of seed germination and seedling growth, Fusarium seedling loss not established.




Figure 4. Effect of treatment beet seed kind "Rielta" cellulolytic bacteria on seed germination and seedling Fusarium damage

In variants experience with beet varieties "Yaptushkovskaya 30" of the seven cultures of cellulolytic bacteria identified five bacterial cultures increase seed germination (an average of 20-25% compared as control), and stimulating further growth of seedlings (Figure 5). In the control variant of the 30-35% seedlings were damaged

by Fusarium rot. In variants with treatments was performed seeds cellulolytic bacteria damage seedlings were founded.




Figure 5. Effect of treatment beet seed kind "Yaptushkovskaya 30" cellulolytic bacteria on seed germination and seedling Fusarium damage

In the variants with wheat kind "Kaz-Sib-14" shows that this kind is characterized by high germination (90%) and resistance to phytopathogenic fungi (only 5-7% of the seedlings were damaged by Fusarium rot). According to the results of research were selected five strains, increase seed germination to 98-99%, and characterized by high stimulating activity of the growth and development of seedlings. On average length of seedlings was increased by 17-20% compared with the control, root length - 25-30% (Figure 6).




Figure 6. Effect of treatment beet seed kind "Kaz-Sib-14" cellulolytic bacteria on seed germination and seedling Fusarium damage

Thus, the effect of bacterial cultures on germination and growth of seedlings of different varieties of sugar beet was studied. It was established that the effect of strain of the cellulolytic bacteria on various kinds of beet not identical. Six strains of the cellulolytic bacteria with high active stimulants and positive effect on the plants of the different kinds of sugar beet were selected.

Study of growth promoting ability of selected strains of bacteria on the beet seed kind "Yaptushkovskaya 30" is presented in Table 2.

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Table 2. Effect of cellulolytic bacteria strains on the germination and growth of sugar beet kind "Yaptushkovskaya 30"

| Strains of the bacteria | Length of the stem, cm | Root length, cm | Germination, % |
|---|---|---|---|
| Control | 12.8±1.2 | 8.8±0.2 | 82.6±2.4 |
| 21(8) | 17.1±1.3 | 10.2±1.0 | 89.7±2.3 |
| 22T | 15.1±1.2 | 9.7±0.9 | 85.6±2.2 |
| 60(5)4 | 18.3±1.4 | 15.9±1.1 | 89.9±2.7 |
| 82 | 16.1±1.0 | 14.2±1.1 | 86.9±2.1 |
| 177 | 14.1±1.3 | 11.2±0.9 | 89.1±2.4 |
| 604 | 19,1±1.5 | 17.9±1.1 | 92.7±2.6 |

On the received data were selected two strains of cellulolytic bacteria, 604 and 60(5)4, characterized by both a high antifungal activity and the ability the growth promotion of sugar beet.

Testing the effectiveness of selected strains in the field, jointly with the Kazakh Scientific Research Institute of Plant Protection and Quarantine Ministry of Agriculture of the Republic of Kazakhstan. Seeds of sugar beet kind "Yaptushkovskaya 30", recommended for cultivation in the Almaty region of Kazakhstan, inoculated bacteria suspension and plated in the fields. Control seeds were treated with chemicals TMTD and Scor registered in Kazakhstan, served as a control untreated seeds. Acreage of sugar beet was 10 hectares.The results are presented in Table 3.

Table 3. Effect of inoculation of sugar beet seeds cellulolytic bacteria on germination, seedling infestation and yield

| Variants | Density of germination, pieces/$m^2$ | Disease, % | | Yields, c/ha |
|---|---|---|---|---|
| | | black leg | fusariusus root rot | |
| 604 | 74.0 | 17.2 | 7.1 | 304.0 |
| 60(5)4 | 73,1 | 17.8 | 8.3 | 300.0 |
| Standard (TMTD, 80% (6,0 kg/t)+ Scor (0,4 l/ha) | 72.7 | 18.5 | 10.0 | 280.0 |
| Control | 68.9 | 39.0 | 21.0 | 270.0 |

On the results of field experience shows high potential of cellulolytic bacteria to process sugar beet seeds. In the experiments, where the seeds were inoculated with cellulolytic bacteria infestation of fusariosus seedling in 2,0 - 2,4 times was lower than in controls, and root crops disease decreased by 2.1 - 3.1 times as compared with the control. Using bacteria significantly increased the resistance of plants to disease, and sugar beet yield increase amounted to 30-34 c/ha.

Thus, from the rhizosphere of the healthy plants of sugar beet new strains of cellulolytic bacteria were isolated. Two strains of bacteria (604 and 60(5)4) with high antifungal activity and growth promoting for plant of sugar beet were selected. On the basis of these strains was developed a biological method for stimulating the growth and protection of sugar beet against root rot.

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