Antagonistic activity of *Pseudomonas chlororaphis* against some mold fungi

Sofia Akhapkina*, *Alexander* Seleznev, *Victoria* Efimova, *Nikita* Lyakhovchenko, and *Inna* Solyanikova

Belgorod State National Research University, BelSU, Belgorod, Russia

Abstract. The paper presents a study of the antagonistic potential of Pseudomonas chlororaphis VKM B-3546D, isolated from the soil, against mold fungi Alternaria brassicicola VKM F-1864 and Aspergillus unguis VKM F-1754. P. chlororaphis VKM BS-1393 was used as a control culture of bacteria. It was revealed that in the presence of bacteria, the growth rate of mold fungi decreases by 10,2 mm/day. in the presence of P. chlororaphis VKM BS-1393, and by 12,9 mm/day. with P. chlororaphis VKM B-3546D, while the average increase in the colony of Alternaria is 10% and 20%, and aspergillus is 30% and 40%, respectively. It was shown that the degree of inhibition of the average colony growth in the native strain of pseudomonas is 7% higher than the contro strain for A. brassicicola VKM F-1864, and 10% for A. unguis VKM F-1754. Thus, the strain of P. chlororaphis VKM B-3546D turned out to be more effective, than P. chlororaphis VKM BS-1393.

1 Introduction

In nature, there are different types of relationships between microorganisms. For example, antagonism, which is based on the ability of members of one population to form chemical compounds that are toxic to another. Various exoenzymes (e.g., lysozymes and proteases), organic and inorganic metabolic products (acids, reactive oxygen species and ammonia) can have an antagonistic effect. However, the most well-known category of chemical compounds with biocidal properties are antibiotics. In most cases, they are secondary metabolites formed in the processes of the main metabolic pathway [1-4].

Antagonist microorganisms can be pathogens - phytopathogens, for example, representatives of such genera as *Pseudomonas, Xantomonas, Erwinia, Agrobacterium, Ralstonia*, etc. Like animal pathogens, they have virulence that allows them to overcome the protective barriers of the plant. Standard factors are enzymes pectinase, cellulase, various phytotoxins, phytohormones and adhesives factors [5, 6]. Among phytopathogenic organisms, there are also pathogens of various *Alternaria* cabbage, rapeseed, mustard, etc. - representatives of the genus of mold fungi *Alternaria*, which can cause great damage to the agro-industrial sector [7, 8].

* Corresponding author: sofyaahapkina@mail.ru

Representatives of genera such as *Bacillus* and *Pseudomonas* are widely used as bases for biological plant protection products [9].

In countries with developed agriculture, the share of biological methods of plant protection is increasing [10-12]. This is due to the urgent need to obtain sufficient volume of full-fledged safe food and improve agrocenoses, which have been polluted with pesticide residues, heavy metals and nitrosamines for more than half a century. It is noticed that in the world there is a tendency to increase the volume of production and sales of biological products [13-16].

Thus, the search for microorganisms with high agrobiotechnological potential, which can be the basis for preparations of plant protection products of biological origin, becomes relevant.

The aim of this study was to identify the degree of antagonistic activity of the antagonist bacterium against mold fungi *Alternaria brassicicola* VKM F-1864 – a necrotrophic phytopathogen capable of infecting many types of crops, causing black spot disease in almost all plant species *Brassicaceae,* and *Aspergillus unguis* VKM F-1754 - the causative agent of plant diseases - aspergillosis.

2 Materials and methods of research

Cultures of *Pseudomonas chlororaphis* VKM B-3546D (isolated from the soil of the Belgorod region), *Pseudomonas chlororaphis* VKM BS-1393*, Alternaria brassicicola* VKM F-1864, *Aspergillus unguis* VKM F-1754 taken from the working collection of microorganisms were used for the work.

To assess the antagonistic potential, the method of agar wells was used [17-18]. This method is based on the ability of antibiotic substances to diffuse into a nutrient medium with a passed test culture. 20 ml of Saboureau nutrient medium was poured into each Petri dish (composition (g/l): glucose – 40.0; peptone – 10.0; yeast extract – 5; agar – 18.0). After it solidifies, In a dense nutrient medium, holes were made with a sterile cork drill with a diameter of 10 mm, "wells", which were then filled with a suspension of the bacterium under study (80 µl each). A disc impregnated with a suspension of mold spores was placed in the center of the cup.

The control culture was the *P. chlororaphis* strain VKM BS-1393.

The crops were incubated at 25ºC for 2 and 4 days. Every 24 hours, the diameter of the fungal colony was measured and averaged according to the formula:

$$S = \sqrt{\frac{\sum V^2}{n}}, \quad (1)$$

where S – is the mean square, V – is the value, n – is the volume [7].

Confidence intervals (Δ*)* were calculated using formula [19]:

$$\Delta = t_{st} \cdot m, \quad (2)$$

where is t_{st} – the standard value of the Student's confidence criterion, m is the error of representativeness of the mean square and is calculated by formula [19]:

$$m = \frac{\sigma}{\sqrt{n}}, \quad (3)$$

where σ is the standard deviation and is calculated by formula [19]:

$$\sigma = \sqrt{\frac{\sum(V-S)^2}{n-1}}, \quad (4)$$

where *V* is the date, *S is* the mean square diameter of the colony, *n* is the sample size.

The significance of the difference in averaged values was calculated statistically using the difference method [20].

The average increase in the diameter of the colonies was calculated by formula:

$$X = (Lg\ G_{v+1} - 1) \cdot 100\%, \quad (5)$$

where is

$$Lg\ G_{v+1} = \frac{\sum Lg\ (V+1)}{n}. \quad (6)$$

The growth rate of colonies (*K*, mm/day) was found by formula:

$$K = \frac{S - S_0}{t - t_0}, \quad (7)$$

where S_0 is the mean square diameter of the colony at the first measurement, S is the mean square diameter of the colony at the last measurement, t_0 is the incubation time at the time of the first measurement, t is the incubation time at the time of the last measurement [18].

To calculate the degree of inhibition (*IR),* formula [21] was used:

$$IR = \left(\frac{K_K - K_O}{K_K}\right) \cdot 100\%, \quad (8)$$

where K_k is the growth rate of the crop in the control variant at the end of incubation, and K_o is the growth rate in the experimental variant.

3 Results of the study and discussion

The growth of the colony of *A. brassicicola* VKM F-1864 and *A. unguis* VKM F-1754 in the control and experimental groups differed statistically insignificantly during the first day, and in the second mold fungi without the studied microorganisms increased significantly in diameter (Fig. 1 A, B).

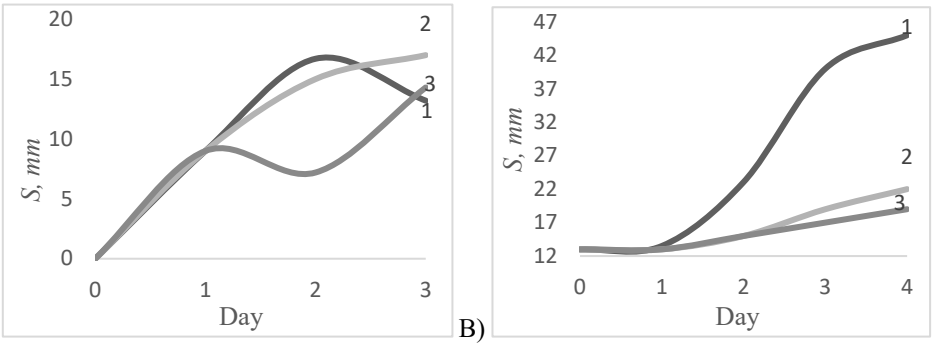

Fig.1. Growth of colonies of mold fungus *A. brassicicola* VKM F-1864 (A) and *A. unguis* VKM F-1754 (B) without bacterial strains (1) and in the presence of strains of *P. chlororaphis* VKM BS-1393 (2) and *P. chlororaphis* VKM B-3546D (3).

Based on the data obtained, the bacterium has a mycostatic effect when *A. brassicicola* VKM F-1864 and A. *unguis* fungi are co-cultivated with *P. chlororaphis* VKM B-3546D. This statement is supported by the fact that the average increase in the mycelium of *A. brassicicola* VKM F-1864 in the control group is 10% higher than in the presence of *P. chlororaphis* VKM BS-1393, and 20% higher than with *P. chlororaphis* VKM B-3546D. And the average increase in the mycelium of *A. unguis* VKM F-1754 shows that in the

control group the growth of mycelium is higher by 30%, and by 40%, than with the content of strains of *Pseudomonas chlororaphis* VKM BS-1393 and *Pseudomonas chlororaphis* VKM B-3546D (Fig. 2 A, B).

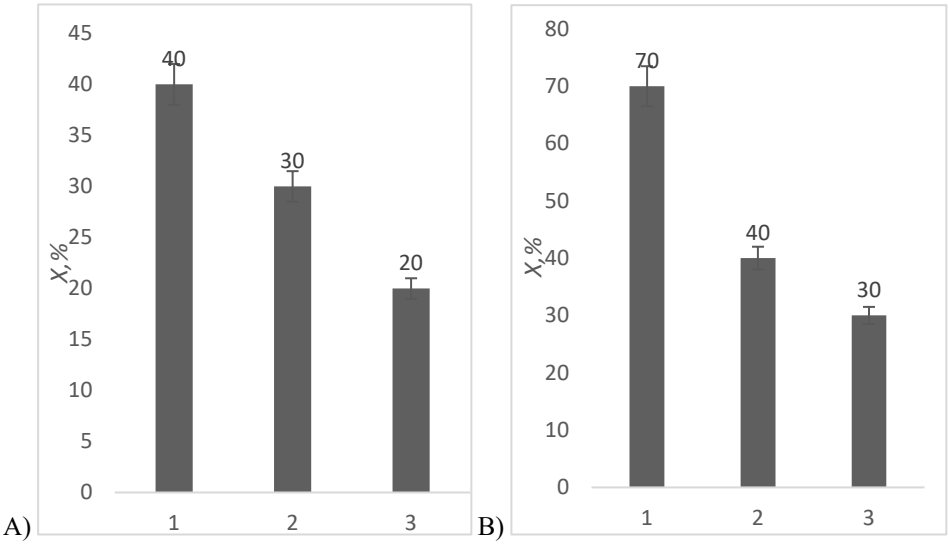

Fig.2. Average increase in colonies of mold fungus *A. brassicicola* VKM F-1864 (A) A. *unguis* VKM F-1754 (B) without bacterial strains (1) and in the presence of strains and *P. chlororaphis* VKM BS-1393 (2) and *P. chlororaphis* VKM B-3546D *(3).*

Calculation of the colony growth rate shows that with the co-cultivation of *A. brassicicola* VKM F-1864 and P. *chlororaphis* VKM BS-1393, colony growth decreased by 10.2 mm/day. relative to the control, and when cultivating the fungus with P. *chlororaphis* VKM B-3546D by 12.9 mm/day. And the growth rate of *A. unguis* VKM F-1754 by 7.7 mm/day and 8.7 mm/day. respectively (Fig. 3 A, B).

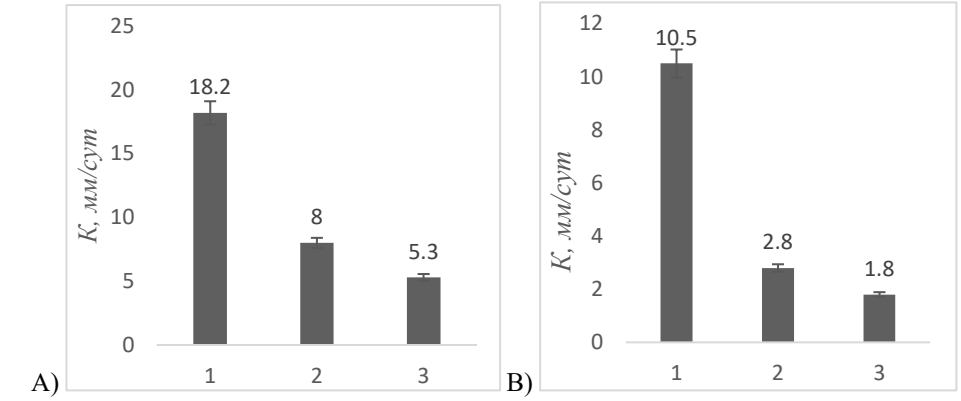

Fig.3. Growth rate of A. *brassicicola* VKM F-1864 colonies (A), *A. unguis* VKM F-1754 (B) without bacterial strains (1) and in the presence of strains and *P. chlororaphis* VKM BS-1393 (2) and *P. chlororaphis* VKM B-3546D *(3).*

According to the obtained data on the degree of inhibition *of P. chlororaphis* VKM B-3546D on the fungus (Fig. 3), a significant difference was found between the effects of the

bacteria *P. chlororaphis* VKM BS-1393 and *P. chlororaphis* VKM B-3546D on the fungus. The difference between the strains was 10% and 7% (Fig. 4A, B).

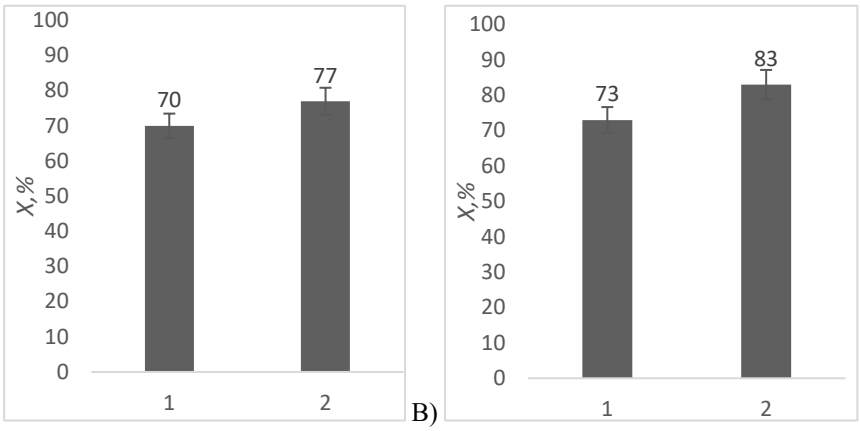

Fig. 4. Degree of colony growth rate inhibition of *P. chlororaphis* VKM BS-1393 (1) and *P. chlororaphis* VKM B-3546D (2) against the mold fungus *A. brassicicola* VKM F-1864 (A) *A. unguis* VKM F-1754 (B).

The study revealed that the strain of *P. chlororaphis* VKM B-3546D has antagonistic properties against the mold fungus *A. brassicicola* VKM F-1864, and against *A. unguis* VKM F-1754, which are common phytopathogens.

Bacteria of the genus *Pseudomonas* are part of such antifungal agents as Pseudobacterin-2, Zh (Biotechagro LLC, Russia, Krasnodar Territory, Timashevsk), Pseudobacterin-2, Pseudobacterin-3, Zh ("Organic Park"). There are federal programs that specify the tasks of increasing the production of biotechnological products, increasing the level of introduction of biotechnology in agriculture. The Belgorod region is one of the leading regions of agro-industrial activity in the Russian Federation and almost 78% of the territory is occupied by places for sowing various crops. Presumably, a narrow range of biocides leads to the fact that the cost of drugs increases in price, and this is not profitable with a large production of agricultural products. In addition, the continued use of pesticides in such an area can increase the likelihood of pathogens that are resistant to plant defenses of both chemical and biological origin. Therefore, it is important to find ways to increase the range of various fertilizers and antifungal agents in order to reduce the risk of adaptation of phytopathogens and ensure a level of competitiveness for a lower price for the product.

4 Conclusion

The study found that *P. chlororaphis* VKM B-3546D has antagonistic potential against A. unguis VKM F-1754 and *A. brassicicola* VKM F-1864. This is confirmed by the fact that the average increase in the mycelium of *A. brassicicola* VKM F-1864 in the control group is 20% higher than in the experimental group. And the average increase in the mycelium of *A. unguis* VKM F-1754 shows that the control group is 40% higher than with *P. chlororaphis* VKM B-3546D. The growth rate of colonies shows that in the presence of the *P. chlororaphis* VKM B-3546D strain with *A. brassicicola* VKM F-1864, growth decreases by 12,9 mm/day, and with *A. unguis* VKM F-1754 by 7.7 mm/day, in the presence of the same strain *P. chlororaphis* VKM BS-1393 with *A. brassicicola* VKM F-1864 height

decreased by 10,2 mm/day, with *A. unguis* VKM F-1754 by 8,7 mm/day. respectively, as well as, during inhibition, a difference was found between the effect of the native strain of the bacterium *P. chlororaphis* VKM B-3546D and the collection *P. chlororaphis* VKM BS-1393 on the mushroom, and the difference was 10% and 7%.

Acknowledgements

The work was carried out within the framework of the grant FZWG-2023-0007 "Adaptive responses of microorganisms: theoretical and applied aspects"

References

1. M.I. Hutchings, A.W. Truman, B. Wilkinson, Current Opinion in Microbiology, **51**, 72-80 (2019) https://doi.org/10.1016/j.mib.2019.10.008.
2. Philippe Vandenkoornhuyse, Achim Quaiser, Marie Duhamel, Amandine Le Van, Alexis Dufresne, New Phytologist, 206(4), 1196–1206 (2015) https://doi.org/10.1111/nph.13312.
3. I.V. Maksimov et al., Plant Physiology **62 (6)**, 763-775 (2015) https://doi.org/10.7868/S0015330315060111.
4. A.V. Golovchenko, A. L. Kharlak, T. V. Glukhova, Bulletin of Tomsk State University. Biology, **43**, 25-43 (2018) https://doi.org/10.17223/19988591/43/2.
5. M.R. de Faria, L.S.A.S. Costa, J.B. Chiaramonte, et al. Trop. plant pathol., **46**, 13–25 (2021) https://doi.org/10.1007/s40858-020-00390-5.
6. A.O. Berestetsky, Applied biochemistry and microbiology, **44 (5)**, 501-514 (2008)
7. P.D. Meena, R.P. Awasthi, C. Chattopadhyay, S.J. Kolte, A. Kumar, Journal of Oilseed Brassica, **1 (1)**, 1-11 (2016)
8. R. R. Azizbekyan, Biotechnology, **34 (5)**, 37-47 (2018) https://doi.org/10.21519/0234-2758-2018-34-5-37-47
9. A. Gebremariam, Y. Chekol, F. Assefa, Egypt J Biol Pest Control **31**, 28 (2021). https://doi.org/10.1186/s41938-021-00375-9.
10. O. Petrova et al., Phytopathology, **111 (10)**, 1811-1817 (2021) https://doi.org/10.1094/PHYTO-11-20-0510-R.
11. N.N. Klimenko, Magarach. Viticulture and winemaking, **22 (3)**, 221-224 (2020) DOI: 10.35547/IM.2020.22.3.007,
12. N.A. Provorov, I.A. Tikhonovich, Ecological genetics, **17 (1)**, 5-10 (2019) https://doi.org/10.17816/ecogen1715-10.
13. S. S. Sanin, Protection and quarantine of plants, **4**, 9-16 (2020)
14. Sh. B. Bayrambekov, O. G. Korneva, Protection and quarantine of plants, **8**, 30-31 (2009)
15. O. A. Monastyrsky, Agrochemistry, **11**, 86-90 (2019) https://doi.org/10.1134/S0002188119110085.
16. I. A. Degtyareva, D. S. Dmitricheva, Scientific notes of the Kazan State Academy of Veterinary Medicine named after NE Bauman, **212 (4)**, 29-34 (2012)
17. T.Z. Esikova, T.O. Anokhina, T.N. Abashina, N.E. Suzina, I.P. Solyanikova, Microorganisms, **9**, 755 (2021) https://doi.org/10.3390/microorganisms9040755

18. N.S. Lyakhovchenko, I.A. Nikishin, E.D. Gubina, D.A. Pribylov, V.Y. Senchenkov, A.A. Sirotin, I.P. Solyanikova, *Assessment of the antifungal activity of the violacein-forming strain Janthinobacterium sp. B-3515 against the mould fungus Alternaria brassicicola F-1864,* IOP Conference Series: Earth and Environmental Science, 908, IV All-Russian Conference with International Participation "Diversity of Soils and Biota of Northern and Central Asia", Ulan-Ude, Russia (2021) https://doi.org/10.1088/1755-1315/908/1/012006.
19. E.A. Snegin, *Practicum on biometrics: a textbook*, (Belgorod: Publishing House "Belgorod" NRU "BelSU", 2016) 56 p.
20. V.F. Moiseichenko, M.F. Trifonova, A.X. Zaveryukha, V.E. Yeshchenko, Fundamentals of scientific research in agronomy, (M., Ear, 1996) 336 p.
21. W.-H. Choi, J.-H. Yun, J.-P. Chu, and K.-B. Chu, Entomological Research, **42**, 219-226 (2012) https://doi.org/10.1111/j.1748-5967.2012.00465.x.