Antifungal Activity of Gram-Negative Pigment-Forming Bacteria Against *Aspergillus Unguis*

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Abstract. The paper presents a study of the antifungal activity of the pigment-forming strains *Janthinobacterium lividum* VKM B-3515, *Pseudomonas chlororaphis* VKM B-3546D and their mixture against the test culture *Aspergillus unguis* VKM F-1754. The growth rate of the colony and the average growth of *A. unguis* VKM F-1754 in the presence of *J. lividum* VKM B-3515 were 46.3% lower than the control variant. The growth rate of fungi in the presence of a mixture of two bacteria was 32% lower than in the control, and in the variant with a pure culture of *Pseudomonas*, it was 21%. The average increase in the diameter of the colonies of *A. unguis* VKM F-1754 cultivated with (*P. chlororaphis* VKM B-3546D-*J. lividum* VKM B-3515) was 33% lower than the control variant with *Pseudomonas*. While the mixture (*P. chlororaphis* VKM B-3546D-*J. lividum* VKM B-3515) showed less activity compared to the pure culture of *J. lividum* VKM B-3515. Thus, the use of two strains together may not be appropriate.

1 Introduction

Representatives of the genus *Aspergillus* are among the most common plant pathogens. There is evidence that *A. flavus* causes kiwi fruit rot [1]. In addition to direct damage to plants, aspergillus poses a threat to the human body due to the formation of mycotoxins that accumulate in plant foods. Thus, the negative effect of these secondary metabolites on grain quality has been described [2]. The group of such secondary metabolites includes about 20 chemically related metabolites, some of them are identified as B1, B2, G1 and G2 [2, 3].

Thus, the use of antifungals is essential to ensure food security and reduce the risk of crop spoilage. At the same time, modern world trends in the development of plant protection products are aimed at biologization, namely, the replacement of existing chemical pesticides with biological ones (for example, antagonist microorganisms and their secondary metabolites). This direction requires the search for new effective producers of antibiotic substances, their combinations, and the development of a set of optimal parameters for obtaining biologically active substances. However, it should be taken into account that microorganisms in nature are in constant interaction with other organisms [4]. Previously, it was shown that *Janthinobacterium lividum*, isolated from the Vezelka River

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in the city of Belgorod, and the crude extract of the pigment, violacein, which it forms, inhibits the growth of the mold fungus *Alternaria brassiciccola* [5]. Thus, the purpose of this study was to evaluate the antifungal activity of the native strain *J. lividum* VKM B-3515 against *A. unguis* VKM F-1754 and to study changes in the activity of *J. lividum* VKM B-3515 in the presence of another antagonist, *P. chlororaphis* VKM B-3546D, since in nature microorganisms are in a state of constant interaction and this can affect the properties of individual strains.

2 Materials and methods

To assess the antagonistic potential of the native strain *J. lividum* VKM B-3515 against fungus *A. unguis* VKM F-1754, the agar well method was used [6]. This method is based on the ability of antibiotic substances to diffuse into a nutrient medium with a passivated test culture. Each Petri dish was filled with 20 ml of Sabouraud nutrient medium (composition (g/l): glucose - 40.0; peptone - 10.0; yeast extract - 5; agar - 18.0). After it solidified, holes, “wells” were made in a dense nutrient medium with a sterile cork drill with a diameter of 13 mm, which were then filled with a suspension of the daily studied bacterium (30 µl each). A disc impregnated with a suspension of mold spores was placed in the center of the cup. The repetition for each variant was 4 cups. Inoculations of *A. unguis* VKM F-1754 without the studied strains served as controls. Cups were incubated at 25ºC for 7 days. Every 24 hours, the diameter of the fungus colony was measured and averaged according to the formula (1):

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

where S is the root mean square (RMS), V is the value, and n is the sample size [7].

The growth rate of colonies (K) was found by formula (2):

$$\mathrm{K} = \frac{S - S_0}{t - t_0}, \quad (2)$$

where K is the colony growth rate, S_0 is the root mean square diameter of the colony at the first measurement, S is the root mean square diameter of the colony at the last measurement, t_0 is the incubation time at the moment of the first measurement, and t is the incubation time at the moment of the last measurement [7].

Confidence intervals (Δ) were calculated using formula (3) [7]:

$$\Delta = t_{st} \cdot m, \quad (3)$$

where t_{st} is the standard value of the Student's test of reliability, m is the mean square representativeness error and is calculated by formula (4) [7]:

$$\mathrm{m} = \frac{\sigma}{\sqrt{\mathrm{n}}}, \quad (4)$$

where σ is the standard deviation and is calculated as [7]:

$$\sigma = \sqrt{\frac{\sum (V - S)^2}{n - 1}}, \quad (5)$$

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

The average increase in the diameter of colonies was calculated by formula (6) [7]:

$$X = (Lg\ G_{v+1} - 1) \cdot 100\%, \quad (6)$$

where

$$Lg\ G_{v+1} = \frac{\sum Lg\ (V+1)}{n} \quad (7)$$

The significance of the difference between the averaged values was calculated by the statistical difference method [8].

Formula (8) [9] was used to calculate the degree of inhibition:

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

where K_c is the growth rate of the culture in the control variant at the end of incubation, and K_e is the growth rate in the experimental variant.

The study of the influence of the companion strain *Pseudomonas chlororaphis* VKM B-3546D on the antifungal activity of *J. lividum* VKM B-3515 was carried out by the method of co-cultivation of variants and their mixture, designated as *P. chlororaphis* VKM B-3546D-*J. lividum* VKM B-3515, using the test culture of *A. unguis* VKM F-1754 on Sabouraud's agar nutrient medium. To do this, the mold fungus was passaged in the center of the Petri dish using the imprint method, and along the edges, along the circumference, the dish was introduced with a suspension of the studied cultures into the wells (diameter 13 mm) in a volume of 30 µl. *A. unguis* VKM F-1754 cultures without bacteria served as a control variant. Antifungal activity was judged by the absence of a statistically significant difference in the average diameter of the fungal colony in the presence of strains and their mixture using the parameter of the degree of inhibition of the growth rate of the colony.

3 Results and Discussion

When determining the antagonistic properties of bacteria by the method of agar wells, it was found that the growth rate of the fungus in the presence of the native strain of the bacterium *J. lividum* VKM B-3515 was 2.9 mm/h, and in the variant containing only A. *unguis* VKM F-1754 (control) – 5.4 mm/h. This indicates the inhibitory effect of the bacterium *J. lividum* VKM B-3515 on growth, since in its presence the growth rate is reduced by 46.3%.

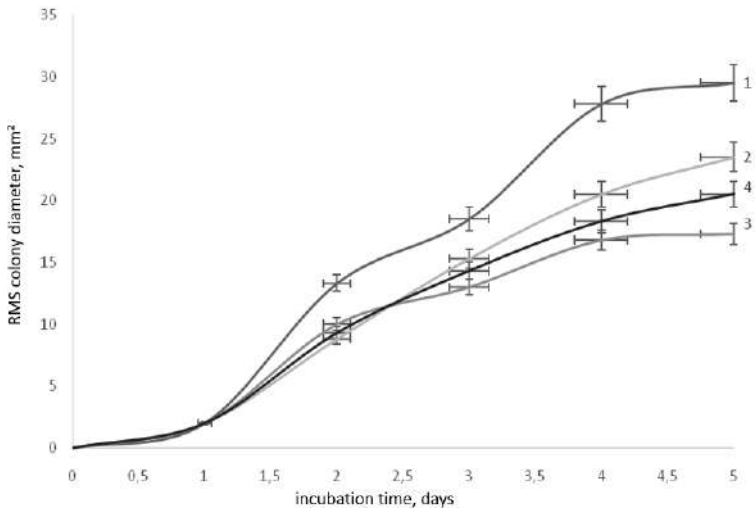

Fig. 1. Change in the root mean square diameter of *Aspergillus unguis* VKM F-1754 colonies as an indicator of fungal growth where: 1 - control (without the studied cultures); 2 - in the presence of *Pseudomonas chlororaphis* VKM B-3546D; 3 - in the presence of *Janthinobacterium lividum* VKM

B-3515; 4 - in the presence of a mixture of "*P. chlororaphis* VKM B-3546D - *J. lividum* VKM B-3515".

In the course of evaluating the antifungal activity of *J. lividum* VKM B-3515 in the presence of *P. chlororaphis* VKM B-3546D (a mixture of *P. chlororaphis* VKM B-3546D-*J. lividum* VKM B-3515), it was found that the antagonistic effect of Pseudomonas was better with co-cultivation than in pure culture, and in *J. lividum* VKM B-3515 it is worse (Fig. 1).

The growth rate (*K*) of the mold fungus in the presence of the mixture of two bacteria turned out to be 32% lower than that of the control, while in the variant with a pure culture of *P. chlororaphis* VKM B-3546D it was 21% lower (Fig. 1). Average growth (*X*) of a colony of *A. unguis* VKM F-1754 cultivated with *P. chlororaphis* VKM B-3546D-*J. lividum* VKM B-3515 was 33%, which is 17% lower than the variant with Pseudomonas relative to the control (Fig. 1). Whereas *P. chlororaphis* VKM B-3546D - *J. lividum* VKM B-3515 showed less activity in comparison with the pure culture of *J. lividum* VKM B-3515.

Previously, it was shown that *J. lividum* VKM B-3515 is characterized by activity against *A. brassiciccola* VKM F-1864, statistically significantly inhibiting the growth rate by 10% [5]. In turn, the strain suppressed the growth of the mycelium of the mold fungus *A. unguis* VKM F-1754 by 46.3%, which is 36.3% higher than for alternaria.

4 Conclusions

Thus, during the study of the antifungal activity of *J. lividum* VKM B-3515 against *A. unguis* VKM F-1754 both in pure culture and in the presence of *P. chlororaphis* VKM B-3546D, it was found that the culture of *J. lividum* VKM B-3515 was the most effective, since it suppressed the growth of mold mycelium by 46.3%, and the pseudomonas strain by only 21%, while in the presence of *J. lividum* VKM B-3515, the degree of suppression by the mixture increased to 32%. It can be assumed that competing interactions occur between bacteria, as a result of which the overall antagonistic activity changes.

The work shows for the first time the antifungal activity of *J. lividum* VKM B-3515 against *A. unguis* BKM F-1754. In addition, information on the cooperative efficiency of Pseudomonas and *J. lividum* VKM B-3515 has not been found in the scientific literature, which requires further study.

So, when developing combined preparations of plant protection products, it is necessary to take into account the mode of of the interaction between the active components and their total effectiveness.

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