

# Antimycotic activity of bacterial strains against the pathogen of grape necrotic leaf spotting *Alternaria* sp.

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**Abstract.** Species of the genera *Bacillus* Cohn and *Pseudomonas* Migula are successfully used as biocontrol agents for many mycopathogens, including *Alternaria* sp. To assess the bacteria antagonistic potential against *Alternaria* sp, we used the method of counter cultures on various nutrient media – universal and specialized. Of the 24 bacterial strains of the genus *Bacillus*, 18 strains were developed antagonistic activity to *Alternaria* sp. after 10 days of co-cultivation on the Tylona-3 medium. The maximum sterile zone (8.0 mm) was formed by bacterial strains (R-9, 5B-1, 01 cor f *Bacillus* sp.). 3 strains of 9 bacteria of the genus *Pseudomonas*, developed antagonistic activity to *Alternaria* sp. on Kinga B medium - 14-3 *Pseudomonas* sp., Oif 2-1 *Pseudomonas* sp., 14-4 *Pseudomonas* sp. The maximum antibiotic activity on potato-sucrose agar was established in strains 3-3 *Bacillus* sp., and K 1-1 *Bacillus* sp. (sterile zone 7.5 mm). All the tested bacteria most often caused one sign of antagonism – antibiosis.

## 1 Introduction

*Alternaria* Nees species are mainly saprotrophic fungi. Nevertheless, some species have acquired the ability to cause diseases of a wide range of host plants [1]. *Alternaria* fungi are pathogens of aboveground plant organs, their main harmfulness lies in the suppression of the photosynthetic apparatus of plants, the pathogenesis is quite slow, but with a wide distribution. The infection leads to the formation of necrotic lesions on the leaves in the form of spots, which are a reaction of the hypersensitivity of the plant tissues in response to pathogen invasion. The process of plant infection with *Alternaria* fungi includes the germination of conidia, penetration and colonization of the plant surface. Conidia usually produce several germ tubes. According to the results of studies by a number of authors, the penetration of the pathogen into plant tissues occurs either directly through the epidermis or microcracks in it, or through the stomata or lenticels [2-4]. The site of pathogen penetration is surrounded by necrotic and often chlorotic halos. Such tissue damage is caused by the diffusion of secondary fungal metabolites – toxins.

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*Alternaria* spp. It belongs to the kingdom *Fungi*, subclass *Eumycotera*, phylum *Fungi Imperfecti*, class *Hyphomycetes*, order *Moniliales*, family *Dematiaceae*, genus *Alternaria* [1, 5-6]. All known teleomorphs of *Alternaria* belong to the genus *Lewia* Barr & Simmons [7]. It is believed that the overwintering stock of the fungus is a seed infection, or infected plant remains, where the infection accumulates and stored for a long time in the form of mycelium and conidiospores. A seed-borne infection is dangerous because almost immediately the disease attacks the sprouts or seedlings of agricultural crops, leading to great damage.

Data on grape *Alternaria* blight are extremely limited. The authors of the published works provide signs of grape infection with *Alternaria* blight with reference to the only source F. Cavara (1888) [8]. The described signs of the disease are always grayish spots with a brownish coating, localized along the leaf veins. The plaque is a mycelium and bundles of conidiophores with conidia. D.P. Lawrence and other (2013) with reference to P. Joly (1964) states that the species *Alternaria vitis* is not a specialized pathogen of grape, but is actually a cosmopolitan species of *Alternaria tenuissima* [9].

When studying the *Alternaria* blight of grapes in the Krasnodar Krai, the species *A. tenuissima* was noted as the causative agent, while the signs of the disease were radically different from those listed above. The disease is described as leaf spotting, which begins with the appearance of dark dots first on the underside, which increase and become visible on the upper side in the form of small rounded spots, and as they grow, they cover almost the entire leaf surface [10].

There are more and more reports about the spread and harmful manifestation of *Alternaria* blight in various crops [11-16], in this connection, the study of the features of pathogenesis and the development of measures to combat these diseases is becoming more relevant. The world is actively searching for effective producers of biological products - strains of antagonistic bacteria. The global biotechnological trend is the species and strains from the genera *Bacillus* and *Pseudomonas*. Thus, according to the results of Aldib et al. *P. brassicacearum* and *P. jessenii* strains effectively suppressed the potato pathogen *Alternaria solani* [17]. In tests of antibiotic activity against *A. alternata*, six bacteria were tested (*Bacillus subtilis* F9-2, F9-8, and F9-12, *Pseudomonas arsenicoxydans* F9-7, *P. koreensis* F9-9, and *P. moraviensis* F9-11), the growth of fungal mycelium was inhibited by an average of 27% [18]. Chinese researchers (Dai et.al., 2021) isolated a strain *Bacillus velezensis* C16 with high antifungal activity. Antagonistic mechanisms (LPs) and volatile organic compounds (VOCs) produced by the C16 strain against *A. solani* were studied. The results showed that VOCs and unstable lipopeptides showed significant antagonistic activity against *A. solani*, with a decrease in colony diameter and significant inhibition of conidia germination [19]. In another study, an endophytic strain of *B. velezensis* SEB1 isolated from *Vigna mungo* (L.) Hepper was tested for the effectiveness of biocontrol of the pathogenic potato isolate *A. alternata*. SEB1 was accepted to be effective for inhibiting pathogen growth in double culture *in vitro*. SEB1 secretes thermally stable antimycotic metabolites [20].

## 2 Materials and methods

The causative agent of necrotic leaf spotting *Alternaria* sp. was isolated from the affected grape leaves in the vineyard of the Riton variety in the Krasnodar Krai. Bacterial strains for their antimycotic activity determination were taken from the collection of Dr. L.V. Maslienko (FSBSI FSC "VNIIMK", city of Krasnodar, Russia).

From seven-day cultures on potato sucrose agar (PSA) *Alternaria* sp. and each of thirty-three species of bacteria (*Bacillus* sp. Cohn, *Pseudomonas* sp. Migula), medium-sized discs (5 mm<sup>3</sup>) were cut out and used for inoculation on Petri dishes 9 cm in diameter containing three agar culture media - PSA, Tylona-3 and Kinga B. Disc with *Alternaria* sp. culture and discs with cultures of bacteria were placed at a distance of 2 cm from opposite edges of the

Petri dish (4.5 cm from each other), along the same diameter. Five control dishes with a single microorganism were also prepared. All the dishes were incubated in the dark at room temperature for ten days.

The degree of bacteria antagonistic activity was determined on the 10th day of cultivation by the area occupied by the antagonist and pathogen, as well as by the presence and size of the antibiotic zone; cultures of antagonists and pathogens sown on different Petri dishes (PD) were taken as controls [21].

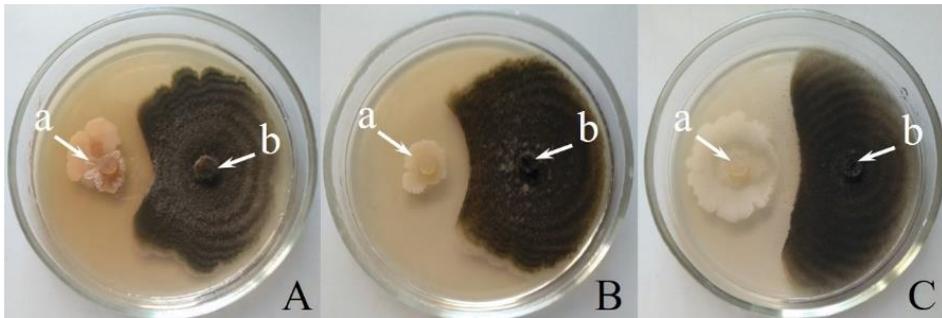
### 3 Results and discussions

Analysis of screening results of 24 strains of *Bacillus sp.* Cohn on Tylona-3 medium, specialized for them, showed that 18 of them had antimycotic activity against the causative agent of necrotic leaf spotting for the type of antibiosis (Table 1).

**Table 1.** Antimycotic activity of bacteria-antagonist strains from the genus *Bacillus* to the pathogen of necrotic leaf spotting of grape *Alternaria sp.*, at a temperature of 25 °C on the Tylona-3 medium, on the 10th day of cultivation

Strain of <i>Bacillus sp.</i>	Area of the nutrient medium surface colonization				Size of antibiotic zone, mm	Area of antagonist suppression by pathogen, cm <sup>2</sup>
	Antagonist		Pathogen			
	cm <sup>2</sup>	%	cm <sup>2</sup>	%		
R-9	6.7	8.3	30.6	37.8	8.0	-
5B-1	2.9	3.6	34.7	42.8	8.0	-
01 cor f	15.2	18.8	39.6	48.9	8.0	-
3-3	10.4	12.8	42.3	52.2	7.0	-
D-10	17.0	21.0	41.4	51.1	6.5	-
D 1-3	4.2	5.2	39.1	48.3	6.5	-
5-3	3.4	4.2	38.8	47.9	6.5	-
B (2-1)	17.9	22.1	22.7	28.0	6.0	-
D 7-1	6.0	7.4	36.0	44.4	6.0	-
K 1-2	30.2	37.3	22.3	27.5	5.0	-
K 1-1	10.4	12.8	38.4	47.4	5.0	-
B-5	4.8	5.9	37.2	45.9	5.0	-
D 1-1	10.0	12.3	40.6	50.1	3.8	-
11-2	20.8	25.7	20.2	24.9	4.0	-
11-1	16.1	19.9	25.2	31.1	4.0	-
B-12	17.8	22.0	32.0	39.5	3.5	-
Fa 4-1	16.1	19.9	35.5	43.8	3.5	-
Fz-9	17.1	21.1	30.0	37.0	2.5	-
D 7-3	14.3	17.7	53.6	66.2	-	-
Fa 4-2	6.8	8.4	42.6	52.5	-	-
B-2	2.6	3.2	57.1	70.4	-	-
11-3	1.1	1.4	59.8	73.8	-	1.1
1a	1.6	2.0	72.9	90.0	-	1.6
BB(C)	2.4	3.0	59.7	73.7	-	2.2
<i>Alternaria sp.</i> (control)	-	-	72.3	89.5	-	-

Strains R-9 *Bacillus* sp., 5B-1 *Bacillus* sp. and 01 cor f *Bacillus* sp. had the greatest antimycotic activity against *Alternaria* sp. on the Tylona-3 medium, a sterile zone was 8.0 mm when co-cultured (Fig. 1).



**Fig. 1.** Antimycotic activity of bacterial strains from the genus *Bacillus* to the causative agent of necrotic leaf spotting, after 10 days of cultivation. A – R-9 *Bacillus* sp.; B – 5B-1 *Bacillus* sp.; C – 01 cor f *Bacillus* sp. a – antagonist, b – pathogen.

The remaining 15 strains of *Bacillus* sp. formed a sterile zone of 2.5-7.0 mm with medium surface colonization by 4.2-37.3 %.

On the PSA strains of *Bacillus* sp. together with the causative agent of necrotic leaf spotting showed slightly less antimycotic activity. The maximum sterile zone in the double culture was shown by strains 3-3 and K 1-1, which was 7.5 mm (Table 2).

**Table 2.** Antimycotic activity of bacteria-antagonist strains from the genus *Bacillus* to the pathogen agent of necrotic leaf spotting of grape *Alternaria* sp., at a temperature of 25 °C on the potato-sucrose agar, on the 10th day of cultivation

Strain of <i>Bacillus</i> sp.	Area of the nutrient medium surface colonization				Size of antibiotic zone, mm	Area of antagonist suppression by pathogen, cm <sup>2</sup>
	Antagonist		Pathogen			
	cm <sup>2</sup>	%	cm <sup>2</sup>	%		
3-3	8.2	10.1	43.2	53.3	7.5	-
K 1-1	5.5	6.8	41.7	51.6	7.5	-
D 7-1	2.5	3.1	39.2	48.4	7.0	-
B-12	17.8	22.0	32.0	39.5	6.0	-
11-1	3.0	3.7	41.7	51.5	6.0	-
K 1-2	2.0	2.5	43.3	53.5	6.0	-
5B-1	3.5	4.3	28.7	35.4	5.5	-
R-9	4.0	4.9	43.4	53.6	4.0	-
B-5	3.1	3.8	43.3	53.5	4.0	-
B (2-1)	8.3	10.2	41.7	51.7	2.5	-
5-3	7.4	9.1	45.9	56.7	2.5	-
11-2	8.7	10.7	45.0	55.6	2.0	-
01 cor f	8.3	10.2	38.0	46.9	2.0	-
D 1-1	30.6	37.8	40.1	49.5	1.0	-
D-10	22.5	27.8	32.0	39.5	-	-
Fa 4-1	19.0	23.5	36.1	44.6	-	-
Fz-9	11.3	14.0	42.1	52.0	-	-
D 1-3	6.8	8.4	42.0	51.9	-	-
Fa 4-2	6.3	7.8	49.5	61.1	-	-
D 7-3	1.2	1.5	65.3	80.6	-	-

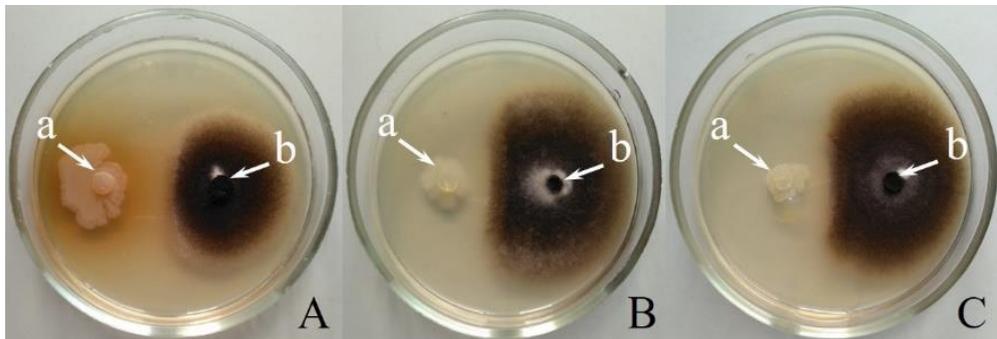
BB(C)	2.4	3.0	47.2	58.3	-	1.1
B-2	3.7	4.6	54.0	66.7	-	1.8
1a	14.8	18.3	41.1	51.1	-	4.5

One of the work stages was the screening of bacterial strains of antagonists from the genus *Pseudomonas*. The tests were carried out on 2 different media – PSA and Kinga B. The Kinga B medium was selected as a specialized medium for *Pseudomonas* bacteria (Table 3).

**Table 3.** Antimycotic activity of bacteria-antagonist strains from the genus *Pseudomonas* to the pathogen of necrotic leaf spotting of grape *Alternaria* sp., at a temperature of 25 °C on the Kinga-B medium, on the 10th day of cultivation

Strain of <i>Pseudomonas</i> sp.	Area of the nutrient medium surface colonization				Antibiotic zone area, cm <sup>2</sup>	Area of antagonist suppression by pathogen, cm <sup>2</sup>
	Antagonist		Pathogen			
	cm <sup>2</sup>	%	cm <sup>2</sup>	%		
14-3	4,3	5,3	29,5	36,5	9,0	-
Oif 2-1	1,3	1,6	36,8	45,4	8,0	-
14-4	7,0	8,6	36,3	44,8	3,5	-
15-1	4,5	5,6	29,3	36,2	-	-
Sgc-1	4,3	5,3	24,8	30,6	-	-
13-2	3,2	4,0	40,6	50,1	-	-
16-2	2,8	3,4	35,3	43,6	-	-
12-2	2,5	3,1	36,4	44,9	-	-
Sgrc-1	1,6	2,0	45,3	55,9	-	-
<i>Alternaria</i> sp. (control)	-	-	48,3	39,6	-	-

Of the nine collection bacteria of the genus *Pseudomonas*, only three bacterial strains – 14-3, 14-4, Oif 2-1 - showed antibiotic activity against the pathogen agent of necrotic leaf spotting (Figure 2).



**Fig. 2.** Antimycotic activity of fungal strains to the pathogen agent of necrotic leaf spotting on grape after 10 days of cultivation on Kinga B medium. A – 14-3 *Pseudomonas* sp.; B - 14-4 *Pseudomonas* sp.; C - Oif 2-1 *Pseudomonas* sp. a - antagonist, b - pathogen.

It was found that on potato-sucrose agar, as well as on Kinga B medium, the maximum sterile zone in the double culture was formed by the strain 14-3 *Pseudomonas* sp. – 9.0 mm. The suppression zone of *Alternaria* sp. growth on the PSA by strains Oif 2-1 and 14-4 was 6.5 and 5.0 mm, respectively (Table 4).

**Table 4.** Antimycotic activity of bacterial strains-antagonists from the genus *Pseudomonas* to the causative agent of necrotic leaf spotting of grape *Alternaria* sp., at a temperature of 25 °C on potato-sucrose agar, on the 10th day of cultivation

Strain of <i>Pseudomonas</i> sp.	Area of the nutrient medium surface colonization				Size of antibiotic zone, mm	Area of antagonist suppression by pathogen, cm <sup>2</sup>
	Antagonist		Pathogen			
	cm <sup>2</sup>	%	cm <sup>2</sup>	%		
14-3	4,5	5,6	42,0	51,9	9,0	-
Oif 2-1	3,0	3,7	41,9	51,7	6,5	-
14-4	2,8	3,5	43,2	53,3	5,0	-
16-2	21,1	26,0	29,5	36,4	-	-
15-1	4,0	4,9	48,7	60,1	-	-
Sgc-1	3,6	4,4	39,7	49,0	-	-
12-2	3,1	3,8	46,0	56,8	-	-
Sgrc-1	1,2	1,5	74,7	92,2	-	1,2
13-2	2,0	2,5	67,5	83,4	-	2,0

## 4 Conclusion

Thus, the conducted screening showed that the most promising strains of antagonist bacteria for further biotechnological research are the following:

- from genus *Bacillus*: P-9 *Bacillus* sp., 5B-1 *Bacillus* sp., 01 cor f *Bacillus* sp.;
- from genus *Pseudomonas*: 14-3 *Pseudomonas* sp., 14-4 *Pseudomonas* sp., Oif 2-1 *Pseudomonas* sp.

It was found that the size of the antibiotic zone was larger on the media specialized for each bacteria genus than on the universal culture medium.

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